PERGAMON

European Journal of Cancer 39 (2003) 1835–1841

European Journal of Cancer

www.ejconline.com

Review

# New non-angiogenesis dependent pathways for tumour growth

D. Ribatti[a,*], A. Vacca[b], F. Dammacco[b]

[a]Department of Human Anatomy and Histology, University of Bari Medical School, Piazza Giulio Cesare, 11, Policlinico, 70124, Bari, Italy
[b]Department of Biomedical Sciences and Human Oncology, University of Bari Medical School, Bari, Italy

Received 8 October 2002; received in revised form 17 January 2003; accepted 12 March 2003

**Abstract**

The current wisdom is that tumours are endowed with an angiogenic capability and that their growth, invasion and metastasis are angiogenesis-dependent. This article summarises the literature concerning recent histomorphological studies that indicate that some tumours may be vascularised without significant angiogenesis, probably by using existing vessels, a process later described as vascular co-option, or even by forming vascular channels on their own through a non-endothelial cell process called ''vascular mimicry''. Moreover, the possibility that bone marrow-derived stem cells may also be a source of endothelial precursor cells recruited for tumour-induced neovascularisation, is reviewed. In fact, it has been assumed that the additional endothelial cells required to construct new tumour vessels come from the division and proliferation of local endothelial cells and that endothelial cells incorporated into sites of neovascularisation, including tumour-induced new blood vessels, may be derived from these precursor cells. Finally, lymphoangiogenesis as a mechanism of *de novo* formation of lymphatics, favouring the metastatic dissemination of tumour cells, is summarised. Potential therapeutic applications are also discussed.


*Keywords:* Angiogenesis; Anti-angiogenesis; Tumour progression

## 1. Current thinking

The current wisdom is that tumours are endowed with an angiogenic capability and that their growth, invasion and metastasis are angiogenesis-dependent [1]. In 1984 Judah Folkman, a highly respected pioneer and researcher in this field wrote: ''Once tumor take has occurred, early increase in tumor cell population must be preceded by an increase in new capillaries that converge upon the tumor'' [2].

This implies that neoplastic cell populations can only form a clinically observable tumour if the host produces a vascular network sufficient to sustain their growth and provide them with a gateway through which they enter the circulation and metastasise to distant sites. Tumour angiogenesis is essentially mediated by angiogenic molecules produced by tumour cells [3].

This view enjoys the support of extensive experimental and genetic evidence. Moreover, angiogenesis is initiated by a switch that is tripped when the balance shifts in favour of the angiogenic inducers [4]. The mechanism of this switch was classified in 1985 by Hanahan [5], who developed transgenic mice in which the large ''T'' oncogene is hybridised to the insulin promoter. In this model for [-islet cell tumorigenesis (RIP-Tag model), all islet cells in a transgenic mouse line express the large T antigen at birth. By 12 weeks, 75% of islets progress to small foci of proliferating cells, but only 4% are angiogenic and their number is closely correlated with the incidence of tumour formation [6]. Angiogenic islets are detected both morphologically by their red colour and evident blood islands consequent to microhaemorrhaging, and functionally by their ability to elicit endothelial cell migration, proliferation and tube formation in an *in vitro* collagen bioassay involving coculture of dispersed capillary endothelial cells and isolated pancreatic islets [6].

The switch depends on increased production of one or more of the positive regulators of angiogenesis, such as the vascular endothelial growth factor (VEGF), fibroblast growth factor-2 (FGF-2), interleukin-8 (IL-8), placental growth factor (PlGF), transforming growth

* Corresponding author. Tel.: +39-080-5478240; fax: +39-080-5478310.
*E-mail address:* ribatti@anatomia.uniba.it (D. Ribatti).

0959-8049/03/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/S0959-8049(03)00267-3

factor-β (TGF-β), platelet-derived endothelial growth factor (PDEGF), pleiotrophins and others. These can be exported from tumour cells, mobilised from the extracellullar matrix, or released from host cells recruited to the tumour. The switch clearly involves more than simple upregulation of angiogenic activity and has thus been regarded as the result of a net balance of positive and negative regulators.

Among the positive regulators, VEGF plays a critical role in the angiogenic switch. In fact, the amount of VEGF (measured in different studies by immunohistochemistry, *in situ* hybridisation, quantitative immunoassay, Western blotting, or reverse-transcriptase polymerase chain reaction) expressed by cancer cells has been found to correlate with tumour sise, metastases and poor prognosis in many types of solid and haematological tumours [7].

Among the host cells recruited to the tumour, macrophages respond to microenvironmental factors in tumours, such as hypoxia, by producing angiogenic cytokines, including VEGF, FGF-2, IL-8 and tumour necrosis factor-α (TNF-α), and extracellular matrix-degrading enzymes, that, in turn, stimulate tumour angiogenesis [8].

## 2. Increases in microvessel density do not necessarily coincide with the onset of angiogenesis

The majority of reports published in the past 30 years have confirmed that microvascular density is a powerful, and often an independent, prognostic indicator of tumour progression and of the risk of future metastases for many different types of human cancer [3]. In fact, newly formed blood vessels convey oxygen and metabolites, whereas endothelial cells secrete growth factors for tumour cells and a variety of proteinases which facilitate invasion and increase opportunities for tumour cells to enter the circulation [3]. By contrast, low or no angiogenesis led to a dormant phase of the tumour, in which very few cells proliferate overlapping those entering apoptosis, and no chance of metastases occurred [3].

However, the amount of angiogenesis in human tumours is considerably lower than that in a physiological condition such as the granulation tissue of the reproductive organs; in fact, the endothelial cell proliferation index value is 0.15% for the human prostate or breast cancer compared with 6.7% in granulation tissue and 36% in the corpus luteum [9]. Moreover, the microvessel densities for human lung, mammary, renal cell and colon carcinomas, glioblastoma and pituitary adenomas are lower than those for their normal counterparts [10,11]. This apparent paradox is partially explained by the lower oxygen consumption rate of tumour cells [12], which are also known to tolerate oxygen deprivation [13]. As a result, the intercapillary distance in tumours is greater than in their normal tissue counterparts.

Nevertheless, the mechanisms responsible for vascularisation of *in situ* tumours and their remodelling are still poorly understood. Recent histomorphological studies indicate that some tumours may be vascularised without significant angiogenesis, probably by using existing vessels [14], a process later described as vascular co-option [15,16], or even by forming vascular channels on their own through a non-endothelial cell process called "vascular mimicry" [17].

## 3. A tumour can obtain an efficient blood supply from a pre-existing vascular bed: the vascular co-option

Pezzella and colleagues [14] were the first to describe a non-small-lung carcinoma growing with no morphological evidence of neoangiogenesis by exploiting normal tissue vessels. They reported that lung carcinomas without angiogenesis are characterised by lack of parenchymal destruction and absence of new vessels and tumour-associated stroma. They also questioned whether the neoplastic cells were truly non-angiogenic, as suggested by the apparent lack of new vessel formation. Clinical–pathological study has shown that patients with a putative non-angiogenic carcinoma have more aggressive disease [18]. A pattern of non-angiogenic growth has also been described in glioblastoma multiforme [19].

It has also been suggested, looking at the microvessel density in the primary tumour and in synchronous nodal metastases, that the lymph node is another site in which tumours grow independently of their angiogenic ability [20–22]. Naresh and colleagues reported that the percentage of endothelial cells in the cell cycle is higher in primary tumours than in metastases and suggested that while in the primary tumours a high vascular proliferating fraction is due to angiogenesis, in the nodal metastases, a low proliferating fraction in the endothelium indicates a reduced angiogenesis.

Holash and colleagues [15] reported that tumour cells migrate towards existing host organ blood vessels in sites of metastases, or in vascularised organs such as the brain, to initiate blood vessel-dependent tumour growth as opposed to classic angiogenesis. These vessels then regress owing to apoptosis of the constituent endothelial cells, apparently mediated by angiopoietin-2 (Ang-2). Ang-2, a ligand for the endothelial tyrosine kinase-receptor Tie-2, antagonises the activity of the other Tie-2 ligand, Ang-1, that keeps the vessel in a quiescent state by maintaining high pericyte coverage [15]. Finally, at the periphery of the growing tumour mass, angiogenesis occurs by co-operative interaction of VEGF and Ang-2.

Tumour cells often appear to have immediate access to blood vessels, such as when they metastasise to or are implanted within a vascularised tissue [15,23]. They

immediately co-opt and often grow as cuffs around adjacent existing vessels. A robust host defence mechanism is activated, in which the co-opted vessels initiate an apoptotic cascade, probably by autocrine induction of Ang-2, followed by vessel regression. This is of the co-opted vessels that carries off much of the dependent tumour and results in massive tumour death. However, successful tumours overcome this vessel regression by initiating neoangiogenesis.

Shortly after regression, a tumour upregulates its expression of VEGF, presumably because it is becoming hypoxic due to the loss of vascular support. As in normal vascular remodelling, the destabilising signal provided by Ang-2, which leads to vessel regression in the absence of VEGF, potentiates the angiogenic response in combination with VEGF. Many solid tumours may fail to form a well-differentiated and stable vasculature because their newly formed tumour vessels continue to overexpress Ang-2. Ang-2 induction in host vessels in the periphery of experimental C6 glioma precedes VEGF upregulation on tumour cells, and causes regression of co-opted vessels [15,16,24].

Vajkoczy and colleagues [25] have demonstrated parallel induction of Ang-2 and VEGFR-2 in quiescent host endothelial cells, suggesting that their simultaneous activity is critical for the induction of tumour angiogenesis during vascular initiation of microtumours. Consequently, the simultaneous expression of VEGFR-2 and Ang-2, rather than the expression of Ang-2 alone, may indicate the angiogenic phenotype of endothelial cells and thus provide an early marker of activated host vasculature. The VEGF/Ang-2 balance may determine whether the new tumour vessels continue to expand when the ratio of VEGF to Ang-2 is high, or regress when it is low during remodelling of the tumour microvasculature.

## 4. Vasculogenic mimicry

Maniotis and colleagues [17] described a new model of formation of vascular channels by human melanoma cells and called it ''vasculogenic mimicry'' to emphasise 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 the tumour uses cell pathways for transporting fluid in tissues that were clearly not blood vessels.

Maniotis and colleagues showed that highly invasive uveal and cutaneous melanoma cells formed looping patterns that stained positive with the periodic acid-Schiff (PAS) stain in three-dimensional cultures on type I collagen and Matrigel, independently of endothelial cells and fibroblasts. These PAS-positive patterns were thus throught to contribute to a microcirculation in human melanoma and that pattern-generating aggressive melanoma cells may contribute to a limited local extravascular circulation.

Microarray gene chip analysis of a highly aggressive compared with a poorly aggressive human cutaneous melanoma cell line revealed a significant increase in the expression of laminin 5 and matrix metalloproteinases (MMP)-1,-2, and -9 and Membrane Type 1 (MT1)-MMP in the highly aggressive cells [26], suggesting that they interact with and alter their extracellular environment differently than the poorly aggressive cells, and that increased expression of MMP-2 and MT1-MMP along with matrix deposition of laminin 5 are required for their ''vasculogenic mimicry''.

These data have been vigorously disputed by Mc Donald and colleagues [27], who consider the evidence presented neither persuasive nor novel. In their opinion, the data are not convincing because three key questions were not addressed: (a) if erythrocytes are used as markers, are they located inside or outside blood vessels?; (b) where is the interface between endothelial cells and tumour cells in the blood vessel wall?; (c) how extensive is the presumptive contribution of tumour cells to the lining of blood vessels?

Moreover, the possibility that cancer cells participate in the formation of blood vessels in tumours has been recognised for many years. Tumour cells in some uveal melanomas line cavernous spaces or cyst-like blood lakes that communicate with the microvasculature [28–30]. Warren [31], Prause and Jensen [32] and Hammersen and colleagues [33] subsequently added ultrastructural evidence of the contribution of cancer cells to the wall of tumour vessels. Tubes lined by tumour cells have been demonstrated histologically in melanomas [34], ovarian carcinomas [35] and inflammatory breast cancers [36].

Another possibility is that the endothelial cell lining is replaced by tumour cells, resulting in the so-called ''mosaic vessels'', where both endothelial and tumour cells contribute to the formation of the vascular tube [37]. These authors used CD31 and CD105 to identify endothelial cells and endogenous green fluorescent protein [GFP] labelling of tumour cells, and showed that approximately 15% of perfused vessels of a colon carcinoma xenografted at two sites in mice were mosaic with focal regions where no CD31/CD105 immunoreactivity was detected and tumour cells were in contact with the vessel lumen. This formation of mosaic vessels is distinct from vasculogenic mimicry, as described by Maniotis and colleagues [17].

## 5. The role of bone marrow-derived stem cells in tumour angiogenesis

Emerging data suggest that adult bone marrow is a major reservoir for organ-specific stem cells, including

haematopoietic stem cells, endothelial progenitors, neuronal and muscle stem cells.

Endothelial precursor cells (EPC) in the peripheral blood may derive from the bone marrow may not yet and have been incorporated into the vessel wall. Several lines of evidence suggest that EPC constitute the preponderance of circulating bone marrow-derived endothelial lineage cells.

Bone marrow-derived stem cells may be a source of EPC recruited for tumour-induced neovascularisation. It has been assumed that the additional endothelial cells required to construct new tumour vessels come from the division and proliferation of local endothelial cells. Endothelial cells incorporated into sites of neovascularisation, including tumour-induced new blood vessels, may be derived from these precursor cells.

High levels of VEGF produced by tumours may result in the mobilisation of bone marrow-derived stem cells in the peripheral circulation and enhance their recruitment into the tumour vasculature [38,39]. Moreover, Hattori and colleagues [39] showed that combined elevation of VEGF and Ang-1 resulted in remodelling of the bone architecture, with depletion of the sinusoidal spaces of haematopoietic cells and a parallel increase in bone marrow vascularisation and splenomegaly.

Hypoxia can also mobilise endothelial precursor cells from the bone marrow in the same way, as haematopoietic cytokines, such as granulocyte–macrophage colony stimulating factor (GM–CSF) [40]. Malignant tumour growth results in neoplastic tissue hypoxia, and may mobilise bone marrow-derived endothelial cells in a paracrine fashion and thus contribute to the sprouting of new tumour vessels.

Lyden and colleagues [41] demonstrated that transplantation and engraftment of β-galactosidase-positive wild-type bone marrow or VEGF-mobilised stem cells into lethally irradiated Id-mutant mice is sufficient to reconstitute tumour angiogenesis. In contrast to wild-type mice, Id-mutants fail to support the growth of tumours because of impaired angiogenesis. Tumour analysis demonstrates uptake of bone marrow-derived VEGFR-2-positive endothelial precursor cells into vessels surrounded by VEGFR1-positive myeloid cells. Defective angiogenesis in Id-mutant mice is associated with impaired VEGF-induced mobilisation and proliferation of the bone marrow precursor cells. Inhibition of both VEGFR-1 aand VEGFR-2 signalling is needed to block tumour angiogenesis and induce necrosis.

Reyes and colleagues [42] found that *in vitro*-generated multipotent adult progenitor cells (MAPC) respond to angiogenic stimuli by migrating to tumour sites and contributing to tumour vascularisation. They also found that *in vivo* angiogenic stimuli in a tumour microenvironment are sufficient to recruit MAPC to the tumour bed and induce their differentiation into endothelial cells that contribute to the tumour vasculature.

## 6. Tumour lymphoangiogenesis

Cancer cells escape a tumour by two primary routes, blood and lymphatic vessels, to establish distant metastases. Although the metastatic dissemination of tumour cells to regional lymph nodes is a common feature of many human cancers, it is not clear the exact mechanism whereby tumour cells enter the lymphatic system and whether tumours utilise existing lymphatic channels or tumour dissemination requires the *de novo* formation of lymphatics (lymphangiogenesis) [43,44].

VEGF-C and VEGF-D have been identified as specific lymphangiogenic factors, which bind to and induce tyrosine phosphorylation of VEGFR-3 [45]. A correlation between VEGF-C and VEGF-D expression, tumour lymphangiogenesis and the formation of metastases in regional lymph nodes has been described in a range of human tumours, including malignant melanoma and lung, breast, colorectal and gastric carcinomas [46]. Although these findings provide no information on the mechanism of tumour cell dissemination, they raise the possibility that VEGF-C and VEGF-D may increase metastasis by increasing the number and size of lymphatic vessels, or, alternatively, by altering the functional properties of existing lymphatics.

## 7. Therapeutic implications

The key role of VEGF in tumour angiogenesis has identified it as a prominent target in the therapeutic control of angiogenesis. Two approaches have been adopted: first, the use of antibodies to either VEGF or VEGFR; and, second, the development of specific inhibitors of VEGFR kinase.

Inflammatory breast cancer (IBC) xenografts exhibit invasive ductal carcinoma with a hypervascular structure in the tumour margin and vasculogenic mimicry without endothelial cells, central necrosis, or fibrosis in the tumour centre [47]. To explore the therapeutic potential of blocking VEGF and Ang pathways in IBC, established adenovirus vector encoding soluble VEGFR-1/Flt-1 (sFlt-1) and soluble Tie-2 (sTie-2) have been injected directly into IBC xenografts [36]. Both vectors produced growth inhibition ratios of the injected tumours that were significantly higher that those of non-IBC xenografts. Moreover, both vectors suppressed lung metastases into IBC xenografts.

Some MMP, such as MMP-2, may have effects on the early stages of angiogenesis when the breakdown of the basement membrane is required. Numerous MMP inhibitors (MMPI) are being evaluated as anti-angiogenic agents in a variety of cancers and at various stages of clinical development [48]. Seftor and colleagues [26] demonstrated that a highly aggressive human cutaneous melanoma characterised by the formation of vascular

channels through "vasculogenic mimicry" is inhibited by antibodies to MMP-2 or MT1-MMP.

One of the most compelling theoretical advantages of the use of conventional chemotherapeutic drugs as anti-angiogenic agents for the treatment of cancer is the possibility that they may not readily be susceptible to mechanisms of acquired drug resistance [49]. Xenografts of neuroblastoma cell lines were subjected to a continuous treatment with the chemotherapeutic drug, vinblastine, in combination with a monoclonal neutralising anti-VEGFR-2 antibody [50]. The combination therapy resulted in full and sustained regression of large established tumours, without an ensuing increase in host toxicity or acquired drug resistance, while both anti-VEGR-2 and vinblastine treatment individually resulted in significant, but transient, xenograft regression [50]. Chang and colleagues [37] emphasise that "antivascular effects of some conventional anticancer therapies could be explained by mosaic vessels, because killing exposed cancer cells could impair blood flow in 14% of the vessels causing significant antivascular effects".

The finding that circulating bone marrow-derived EPC are recruited to the tumour neovasculature provides an additional rationale for using bone marrow stem cells as the target in an anti-angiogenic gene therapy-mediated anticancer strategy. Because these gene-modified EPC are recruited factors produced by the tumours, a higher local milieu of angiogenesis inhibitors is probably established at the target sites.

Davidoff and colleagues [51] modified murine bone marrow-derived cells with a gene encoding an angiogenesis inhibitor, a soluble, truncated form of the VEGFR-2 (Flk-1) (tsFlk-1) together with GFP or GFP alone. Tumour growth in mice challenged 3 months after transplantation with tsFlk-1 expressing bone marrow cells was significantly inhibited compared with transplanted controls. Immunohistochemical analysis of tumours in each group demonstrated co-localisation of GFP expression in cells stained with endothelial cell markers, suggesting that endothelial cells of the tumour-induced neovasculature were derived, at least in part, from bone marrow precursors.

These data suggest that chemotherapeutical destruction of the bone marrow may partially slow tumour development by inhibiting angiogenesis. We thus need to know whether bone marrow replacement in cancer patients, redelivers cells that can support the tumour vasculature.

With regard to tumour lymphangiogenesis, it seems reasonable to hypothesise that blocking the growth of new lymphatic vessels will inhibit lymphogenic metastases. It has recently been demonstrated that inhibition of VEGFR-3 signalling with a soluble fusion protein, VEGFR-3-Ig, can suppress tumour lymphoangiogenesis and lymphatic metastases, but not lung metastases [52].

## 8. Concluding remarks

The conventional angiogenic model predicts that tumours progress from a low microvessel density to more aggressive stages, which are related to increases in angiogenic activity. Angiogenesis inhibitors may generally be most effective in preventing the growth of the neovasculature or in causing regression of the neovasculature, but have little or no effect on the mature microvasculature.

The ability of the tumour to support continued growth through non angiogenesis-dependent pathways, such as vascular co-option, vasculogenic mimicry, recruitment of bone marrow-derived stem cells and lymphoangiogenesis, represents an important mechanism of tumour resistance to conventional anti-angiogenic treatments and requires adjustment of these therapies to counter such new pathways.

Moreover, further studies on the mechanisms underlying the initiation of tumour cell dissemination and of the interactions of tumour cells with blood and lymphatic endothelial cells should advance our current understanding of tumour progression and its invasive potential.

## Acknowledgements

This work was supported by grants from the Fondazione Italiana per la Lotta al Neuroblastoma, Genoa, and Associazione Italiana per la Ricerca sul Cancro, Milan, Italy.

## 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. Folkman J. Angiogenesis. In Jaffe EA, ed. *Biology of endothelial cell*. Amsterdam, Martinus Nijhoff Publishing, 1984, 412–428.
3. Folkman J. Tumor angiogenesis. In Mendelshon J, Howley PM, Israel MA, Liotta LA, eds. *The molecular basis of cancer*. Philadelphia, WB Saunders, 1995, 206–232.
4. Hanahan D, Folkman J. Patterns and emerging mechanisms of the angiogenic switch during tumorigenesis. *Cell* 1996, **86**, 353–364.
5. Hanahan D. Heritable formation of pancreatic beta-cell tumors in transgenic mice expressing recombinant insulin/simian virus 40 oncogene. *Nature* 1985, **315**, 115–122.
6. Folkman J, Watson KM, Ingber DE, Hanahan D. Induction of angiogenesis during the transition from hyperplasia to neoplasia. *Nature* 1989, **339**, 58–61.
7. Dvorak HF. Vascular permeability factor/vascular endothelial growth factor: a critical cytokine in tumor angiogenesis and a potential target for diagnosis and therapy. *J Clin Oncol* 2002, **20**, 4368–4380.
8. Crowther M, Brown NJ, Bishop ET, Lewis CE. Microenvironmental influence on macrophage regulation of angiogenesis in wounds and malignant tumors. *J Leukoc Biol* 2001, **70**, 478–490.

9. Maragoudakis ME. Angiogenesis in health and disease. *Gen Pharmacol* 2002, **35**, 225–226.
10. Eberhard A, Kahlert S, Goede V, Hemmerlein B, Plate KH, Augustin HG. Heterogeneity of angiogenesis and blood vessel maturation in human tumors: implications for antiangiogenic tumor therapies. *Cancer Res* 2002, **60**, 1388–1393.
11. Turner HE, Nagy Z, Gatter KC, Esiri MM, Harris AL, Wass JA. Angiogenesis in pituitary adenomas and in the normal pituitary gland. *J Clin Endocrinol Metab* 2000, **85**, 1159–1162.
12. Sterinberg F, Rohrborn HJ, Otto T, Scheufler KM, Streffer C. NIR reflection measurements of hemoglobin and cytochrome da3 in healthy tissue and tumors. Correlation to oxygen consuption: preclinical and clinical data. *Adv Exp Med Biol* 1997, **428**, 69–77.
13. Graeber TG, Osmanian C, Jacks T, *et al*. Hypoxia-mediated selection of cells with diminished apoptotic potential in solid tumours. *Nature* 1996, **379**, 88–91.
14. Pezzella F, Pastorino U, Tagliabue E, *et al*. Non-sall-lung carcinoma tumor growth without morphological evidence of neoangiogenesis. *Am J Pathol* 1997, **151**, 1417–1423.
15. Holash J, Maisonpierre PC, Compton D, *et al*. Vessel cooption, regression and growth in tumors mediated by angiopoietins and VEGF. *Science* 1999, **284**, 1994–1998.
16. Yancopoulos GD, Davis S, Gale NW, Rudge JS, Wiegand SJ, Holash J. Vascular specific growth factors and blood vessels formation. *Nature* 2000, **407**, 242–248.
17. Maniotis AJ, Folberg R, Hess A, *et al*. Vascular channel formation by human melanoma cells in vivo and in vitro: vasculogenic mimicry. *Am J Pathol* 1999, **155**, 739–752.
18. Pastorino U, Andreola S, Tagliabue E, *et al*. Imunohistochemical markers in stage I lung cancer: relevance to prognosis. *J Clin Oncol* 1997, **15**, 2858–2865.
19. Wesseling P, Van Der Laak JA, De Leeuw H, Ruiter DJ, Burger PC. Quantitative immunohistological analysis of the microvasculature in untreated human glioblastoma multiforme. Computer-assisted image analysis of whole tumor sections. *J Neurosurg* 1994, **81**, 902–9009.
20. Pezzella F. Evidence for novel non-angiogenic pathway in breast-cancer metastasis. Breast Cancer Progression Working Parthy. *Lancet* 2000, **355**, 1787–1788.
21. Guidi AJ, Berry DA, Broadwater G, *et al*. Association of angiogenesis in lymph node metastases with outcome of breast cancer. *J Natl Cancer Inst* 2000, **92**, 486–492.
22. Naresh NK, Nerurkar AY, Borges AM. Angiogenesis is a redundant for tumour growth in lymph node metastases. *Histopathology* 2001, **38**, 466–470.
23. Zagzag D, Hooper A, Friedlander DR, *et al*. In situ expression of angiopoietins in astrocytomas identifies angiopoietin-2 as an early marker of tumor angiogenesis. *Exp Neurol* 1999, **159**, 391–400.
24. Holash J, Wiegand SJ, Yancopoulos GF. New model of tumor angiogenesis: dynamic balance between vessel regression and growth mediated by angiopoietins and VEGF. *Oncogene* 1999, **18**, 5356–5362.
25. Vajkoczy P, Farhadi M, Gaumann A, *et al*. Microtumor growth initiates angiogenic sprouting with simulatenous expression of VEGF, VEGF receptor-2, and angiopoietin-2. *J Clin Invest* 2002, **109**, 777–785.
26. Seftor REB, Seftor EA, Koshikawa N, *et al*. Cooperative interactions of laminin 5 gamma-2 chain, matrix metalloproteinase-2, and membrane type-1-matrix/metalloproteinase are required for mimicry of embryonic vasculogenesis by aggressive melanoma. *Cancer Res* 2001, **61**, 6322–6327.
27. McDonald DM, Munn L, Jain RK. Vasculogenic mimicry: how convincing, how novel, and how significant. *Am J Pathol* 2000, **156**, 383–388.
28. Jensen OA. The Knapp-Ronne type of malignant melanoma of the choroid: a haemangioma-like melanoma with a typical clinical picture. So-called "preretinal malignant choroid melanoma". *Acta Ophthalmol* 1976, **54**, 41–54.
29. Duke-Elder S, Perkins ES. Cysts and tumors of the uveal tract. In Duke-Elder S, ed. *System of ophthalmology*. St. Louis, CV, Mosby Company, 1996, 754–937.
30. Francois J, Neetens A. Physico-anatomical studies of spontaneous and experimental intraocular new growths: vascular supply. *Bibl Anat* 1967, **9**, 403–411.
31. Warren BA. The vascular morphology of tumors. In Patherson HI, ed. *Tumor blood circulation: angiogenesis, vascular morphology and blood flow of experimental and human tumors*. Boca Raton, CRC Press Inc, 1979, 1–48.
32. Prause JU, Jensen OA. Scanning electron microscopy of frozen-cracked, dry-cracked and enzyme-digested tissue of human malignant choriodal melanomas. *Albrecht Von Gralfes Arch Klin Exp Ophthalmol* 1980, **212**, 217–225.
33. Hammersen F, Endrich B, Messmer K, The fine structure of tumor blood vessels I. Partecipation of non-endothelial cells in tumor angiogenesis. *Int J Microcirc Clin Exp* 1985, **4**, 31–43.
34. Baron JA, Monzon F, Galaria N, Murphy GF. Angiomatoid melanoma: a novel pattern of differentiation in invasive periocular desmoplastic malignant melanoma. *Hum Pathol* 2000, **31**, 1520–1522.
35. Sood AK, Seftor EA, Fletcher MS, *et al*. Molecular determinants of ovarian cancer plasticity. *Am J Pathol* 2001, **158**, 1279–1288.
36. Shirakawa K, Shibuya M, Heike Y, *et al*. Tumor-infiltrating endothelial cells and endothelial precursor cells in inflammatory breast cancer. *Int J Cancer* 2002, **99**, 344–351.
37. Chang YS, di Tomaso E, Mc Donald DM, Jones R, Jain RK, Munn LL. Mosaic blood vessels in tumors: frequency of cancer cells in contact with flowing blood. *Proc Natl Acad Sci USA* 2000, **97**, 14608–14613.
38. Asahara T, Takahashi T, Masuda H, *et al*. VEGF contributes to postnatal neovascularization by mobilizing bone-marrow derived endothelial progenitor cells. *EMBO J* 1999, **18**, 3964–3972.
39. Hattori K, Dias S, Heissig B, *et al*. Vascular endothelial growth factor and angiopoietin-1 stimulate postnatal hematopoiesis by recruitment of vasculogenic and hematopoietic stem cells. *J Exp Med* 2000, **193**, 1005–1014.
40. Takahashi T, Kalka C, Masuda H, *et al*. Ischemia- and cytokine-induced mobilization of bone marrow-derived endothelial progenitor cells for neovascularization. *Nature Med* 1999, **5**, 434–438.
41. Lyden D, Hattori K, Dias S, *et al*. Impaired recruitment of bone-marrow-derived endothelial and hematopoietic precursor cells blocks tumor angiogenesis and growth. *Nature Med* 2001, **7**, 1194–1201.
42. Reyes M, Dudek A, Jahagirdar B, Koodie L, Marker PH, Verfaillie CM. Origin of endothelial progenitors in human postnatal bone marrow. *J Clin Invest* 2002, **109**, 337–346.
43. Clarijs R, Ruiter DJ, De Wall RM. Lymphangiogenesis in malignant tumours: does it occur? *J Pathol* 2001, **193**, 143–146.
44. Pepper MS. Lymphangiogenesis and tumor metastasis: myth or reality? *Clin Cancer Res* 2001, **7**, 462–468.
45. Makinen T, Veikkola T, Mustjoki S, *et al*. Isolated lymphatic endothelial cells transduce growth, survival and migratory signals via the VEGFC/D receptor VEGFR-3. *EMBO J* 2001, **20**, 4762–4773.
46. Stacker SA, Baldwin ME, Achen MG. The role of tumor lymphangiogenesis in metastatic spread. *FASEB J* 2002, **16**, 922–934.
47. Shirakawa K, Kobayashi H, Heike Y, *et al*. Hemodynamics in vasculogenic mimicry and angiogenesis of inflammatory breast cancer xenograft. *Cancer Res* 2002, **62**, 560–566.
48. Scappaticci FA. Mechanisms and future directions for angiogenesis-based cancer therapies. *J Clin Oncol* 2002, **20**, 3906–3927.
49. Kerbel RS. Inhibition of tumor angiogenesis as a strategy to circumvent acquired resistance to anti-cancer therapeutic agents. *BioEssay* 1991, **13**, 31–36.

50. Klement G, Baruchel S, Rak J, *et al*. Continuous low-dose therapy with vinblastine and VEGF receptor-2 antibody induces sustained tumor regression without overt toxicity. *J Clin Invest* 2000, **105**, R15–R24.
51. Davidoff AM, NG CYC, Brown, P, *et al*. Bone marrow-derived cells contribute to tumor neovasculature and, when modified to express an angiogenesis inhibitor, can restrict tumor growth in mice. Clin Cancer Res 2001, 7, 2870-2879.
52. He Y, Kozaki K, Karpanen T, *et al*. Suppression of tumor lymphangiogenesis and lymph node metastasis by blocking vascular endothelial growth factor receptor 3 signaling. *J Natl Cancer Inst* 2002, **94**, 819–825.