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The Role of the Vascular Phase in Solid Tumor Growth: A Historical Review

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Abstract

Angiogenesis is a biological process by which new capillaries are formed from pre-existing vessels. It occurs in both physiological conditions such as embryo development, cyclically in the female genital system and during wound repair, and pathological conditions, such as arthritis, diabetic retinopathy and tumors. In solid tumor growth, a specific critical turning point is the transition from the avascular to the vascular phase. Having developed an intrinsic vascular network, the neoplastic mass is able to grow indefinitely (unlike all the other forms, tumor angiogenesis is not limited in time) both *in situ* and at distant sites (metastasis) in so far as an intrinsic vascular network enables its cells to enter the vascular bed and colonize other organs. Tumor angiogenesis depends mainly on the release by neoplastic cells of growth factors specific for endothelial cells and able to stimulate growth of the host's blood vessels. This review describes its history as traced by the main contributions to the international medical literature and their contents. The specific new paradigm discussed here has been gaining general approval and considerable confirmation, thanks to its possible applications, as recently highlighted by the introduction of anti-angiogenic substances in adjuvant tumor management.

Keywords: angiogenesis, history of medicine, solid tumor.

Introduction

Angiogenesis is a biological process by which new capillaries are formed. It is essential in many physiological (embryo development, ovulation and wound repair) and pathological conditions, such as arthritis, diabetic retinopathy, and tumors.

Solid tumors are endowed with angiogenic capability and their growth, invasion and metastasis are angiogenesis-dependent. Neoplastic cell populations can only form a clinically observable tumor if the host produces a vascular network sufficient to sustain their growth. Furthermore, new blood vessels provide them with a gateway through which they enter the circulation and metastasize to distant sites. Tumor angiogenesis is essentially mediated by angiogenic molecules elaborated by tumor cells.

This paper offers a historical account of the relevant literature. It also emphasizes the crucial and paradigmatic role of angiogenesis as a biological process and the significance of the anti-angiogenic approach to the treatment of solid tumors.

Early Evidence of the Vascular Phase and its Importance in Tumor Growth

Virchow, the founder of pathological anatomy, drew attention to the huge number of blood vessels in a tumor mass as long ago as 1865. Tumor vascularization was first studied systematically by Goldman [1], who described the vasoproliferative response of the organ in which a tumor develops as follows: "The normal blood vessels of the organs in which the tumor is developing are disturbed by chaotic growth, there is a dilatation and spiralling of the affected vessels, marked capillary budding and new vessel formation, particularly at the advancing border."

When Clark *et al.* [2] and Clark and Clark [3] perfected the implantation of transparent chambers in a rabbit's ear, the morphological characteristics of blood vessels could be studied *in vivo*, including the use of contrast media.

Early Evidence of Tumor Cells Releasing Specific Growth Factor for Blood Vessels

In 1939, Ide *et al.* [4] were the first to suggest that tumors release specific factors capable of stimulating the growth of blood vessels.

In 1945, Algire and Chalkley [5] used a transparent chamber implanted in a cat's skin to study the vasoproliferative reaction secondary to a wound or implantation of normal or neoplastic tissues. They showed that the vasoproliferative response induced by tumor tissues was more substantial and earlier than that induced by normal tissues or following a wound. They concluded that the growth of a tumor is closely connected to the development of an intrinsic vascular network.

In 1956, Merwin and Algire [6] found that the vasoproliferative response of normal or neoplastic tissues transplanted into muscle was not significantly different with

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respect to the time of onset of new blood vessels, though it was stronger when the implantation was performed in a resection area. In addition, while normal tissues induced a vasoproliferative response confined to the host, tumor tissues induced the formation of neovessels that pierced the implant. Lastly, the intensity of the response seemed to be influenced by the distance between the implant and the host's vessels: normal tissue was unable to induce a response if placed more than 50 μm away, whereas tumor tissue had a longer activity range.

Greenblatt and Shubik [7] implanted Millipore chambers (pore size 0.45 μm) into a hamster's cheek pouch and placed some tumor fragments around them. In a few days, the growing tumor mass engulfed the whole chamber, whose pores were permeable to the tumor interstitial fluid, but not to the tumor cells. New blood vessels, however, were formed in any case very likely through the release of a diffusible factor that could pass through the pores.

Ehrmann and Knoth [8] confirmed these data with tumor fragments laid on Millipore filters planted on the chick embryo chorioallantoic membrane (CAM).

Isolation of the First Angiogenic Tumor Factor

An angiogenic factor was first isolated by Folkman *et al.* [9] in 1971. The homogenate of a Walker 256 carcinoma—a breast tumor of Sprague-Dawley rats—was fractionated by gel filtration on Sephadex G-100. The fraction that exhibited the strongest angiogenic activity had a molecular weight of about 10,000 Da and consisted of 25% RNA, 10% proteins, and 58% carbohydrates, plus a possible lipid residue. It was inactivated by digestion with pancreatic ribonuclease, or by heating at 56°C for 1 hour, and was not modified when kept at 4°C for 3 months, nor when treated with trypsin for more than 3 days. This active fraction was subsequently called "tumor angiogenesis factor" (TAF) [9]. Both the cytoplasmic and the nuclear fractions of tumor cells stimulated angiogenesis. In the nuclear fraction, this was found to be associated with non-histonic proteins [10]. TAF has since been non-destructively extracted from several tumor cell lines, and several low-molecular weight angiogenic factors have been isolated, again from the Walker 256 carcinoma. These factors induced a vasoproliferative response *in vivo* when tested on rabbit cornea or chick CAM, and *in vitro* on cultured endothelial cells [11–13].

Purification of Other Angiogenic Factors

The 1980s saw the discovery of the first molecules that mediate angiogenesis. Heparin-affinity chromatography was employed to purify basic fibroblast growth factor (FGF-2) [14,15] and vascular endothelial growth factor (VEGF) [16,17].

In 1984, Shing *et al.* [15], at the Children's Hospital in Boston, discovered that a tumor-derived factor bound with such high affinity to heparin that it could be purified 200,000-fold by a single passage over a heparin-affinity column. This purified protein had a molecular weight of 14,800 and stimulated the proliferation of capillary endothelial cells. These workers later used the chick CAM to show that it stimulated new vessel growth [18]. FGF-2 was highly purified from bovine pituitary [19], and bovine brain [20]. Its amino acid sequence was determined by Esch *et al.* [21]. It has since been isolated from a variety of tissues and cell lines.

In 1983, Senger *et al.* [23] described the partial purification of a tumor product that promotes increased vascular permeability (vascular permeability factor, VPF) in guinea pig skin with a potency some 50,000 times than histamine.

In 1989, Ferrara and Henzel [16] and Plouet *et al.* [17] independently reported the purification (to homogeneity) and sequencing of an endothelial cell-specific mitogen, which they, respectively, called VEGF and vasculotropin. The subsequent molecular cloning of VEGF and VPF [23–25] unexpectedly revealed that both activities are embodied in the same molecule. VEGF has been isolated from the conditioned media of a number of cell lines including bovine pituitary follicular cells [16,26], guinea pig tumor [27], NB41 neuroblastoma [28] and rat glioma cells [25]. It elicits a potent angiogenic response when tested in the chick CAM [23] or rat cornea [27].

VEGF/VPF is expressed by numerous tumor cell lines both *in vitro* and *in vivo*, and receptors for VEGF/VPF occur only on peritumoral capillaries and not on distant endothelial cells [29]. Like VPF/VEGF, FGF-2 is expressed by many tumor lines. Synergistic angiogenesis of VPF/VEGF and FGF-2 has been shown both *in vitro* and *in vivo* [30–32] .

The Avascular and Vascular Phases of Solid Tumor Growth

Solid tumor growth consists of an avascular and a subsequent vascular phase. Assuming that it is dependent on angiogenesis and that this depends on the release of angiogenic factors, acquisition of angiogenic capability can be seen as an expression of progression from neoplastic transformation to tumor growth and metastasis.

Practically all solid tumors, including tumors of the colon, lung, breast, cervix, bladder, prostate and pancreas, evolve through these two phases. Brem *et al.* [33,34] and Maiorana and Gullino [35] observed that experimental breast cancer in rat and mouse gave rise to marked breast angiogenic activity that was lacking in adult gland. Moreover, just like the hyperplastic and dysplastic breast lesions more frequently subject to neoplastic change, preneoplastic lesions also induce a strong vasoproliferative response long before any morphological sign of neoplastic transformation can be observed.

The avascular phase appears to correspond to the histopathological picture presented by a small colony of neoplastic cells (500,000 to 1 ml cells/1–2 mm in diameter) that reaches a steady state before it proliferates and becomes rapidly invasive. Here, metabolites and catabolites are transferred by simple diffusion through the surrounding tissue. The cells at the periphery of the tumor continue to reproduce, whereas those in the deeper portion die away.

The Importance of Cell Population Geometry in Tumor Growth

When tumor growth becomes three-dimensional, the above steady state is established. If it is two-dimensional, as at the bottom of a Petri dish, cells will proliferate to as many as 1 billion before a steady state is attained. The specific geometry of tumor growth is thus a most important variable. In a spherical tumor (the "spheroid"), the cell volume grows according to the cube of the radius, whereas its surface area only increases in proportion to the square of the radius. Conversely, in a two-dimensionally growing tumor, volume and surface area both increase in parallel, so that diffusion is not a limiting factor for cell proliferation. This has been illustrated in *in vitro* experiments in which different kinds of tumors were cultivated in agar. Spherical colonies 0.1 mm in diameter were formed in about 6 to 7 days and continued to grow until they reached a steady state, in which they remained vital for 3 to 5 months, because the cells at the periphery continued to divide, whereas those in the deeper portion died [36].

To obtain an experimental model of avascular three-dimensional growth *in vivo*, small tumor fragments were suspended in the aqueous humor of the rabbit anterior chamber at varying distances from the iris vessels. Their behavior was compared with that of tumors directly implanted into the iris or the cornea. When the tumor was suspended at a considerable distance from the iris, it did not undergo vascularization, nor did it grow beyond 1 mm, though it stayed vital because it drew nourishment from diffusible substances in the aqueous humor. The tumor became vascularized when it was suspended less than 6 mm from the iris vessels. When it was implanted directly into the iris, vascularization developed and it grew rapidly to reach a 16,000-fold volume in 2 weeks [37].

First Evidence of Existence of the Two Phases

The earliest evidence of the existence of the two phases was obtained by Folkman *et al.* [38] in 1963, who perfused the lobe of a thyroid gland with plasma and inoculated a suspension of melanoma B16 tumor cells through the perfusion fluid. These cells grew into small, clearly visible black nodules. The nodules did not exceed 1 mm in diameter and did not connect with the host's vascular network. Their outer third generally remained vital, while the interior portion underwent necrosis. Reimplanted nodules, on the other hand, equipped themselves with a vascular network and grew very rapidly. The conclusion was thus drawn that the absence of vascularization limits the growth of solid tumors.

Further research by Cavallo [39,40] resulted in an experimental system in which the tumor, or its extracts, could be separated from the vascular bed. This system was based on subcutaneous insufflation to lift the skin of a rat and form a poorly vascularized region below it. When Millipore filters containing Walker 256 cancer cells or their cytoplasmic or nuclear extracts were implanted in this region, a vasoproliferative reaction appeared under the filter 48 hours later—this reaction was demonstrated histologically, ultrastructurally and by autoradiography.

In another series of experiments, Gimbrone *et al.* [41] implanted 1 mm fragments from Brown-Pearce and V2 carcinomas into the avascular stroma of a rabbit cornea 1 to 6 mm away from the limbic vessels, and observed the tumor growth daily with a stereomicroscope. After 1 week, new blood vessels had invaded the cornea starting from the edge closer to the site of implantation and developed in that direction at 0.2 mm and then about 1 mm/day. Once the vessels had reached the tumor, it grew very rapidly to permeate the entire globe within 4 weeks.

How Tumor Cells Switch to the Angiogenic Phenotype

Spontaneously arising tumor cells are not usually angiogenic at first [42]. The phenotypic switch to angiogenesis is usually accomplished by a subset that induces new capillaries which then converge toward the tumor. These new vessels perfuse the tumor with blood, which transports nutrients and oxygen to the tumor and catabolites away from it, and their endothelial cells produce a spectrum of growth factors with a paracrine stimulatory effect on the tumor cells [43].

The mechanism of this switch was inaccessible to analysis until a report in 1985 by Hanahan [44], who developed transgenic mice in which the large "T" oncogene is hybridized to the insulin promoter. The pancreatic β cells become hyperplastic and progressed to tumors via a reproducible and predictable multistep process. One step occurs at 6 to 7 weeks, when angiogenesis is switched on in approximately 10% of preneoplastic islets. Vascularized tumors arise from these islets and are fatal by 12 weeks. This onset pattern closely resembles that of angiogenesis in human tumors. Folkman *et al.* [42] subsequently showed that the islets are chemotactic to capillary endothelial cells *in vitro* when they become angiogenic.

The switch depends on increased production of one or more of the positive regulators of angiogenesis. These can be exported from tumor cells [45], mobilized from extracellular matrix [46], or released from host cells (e.g., macrophages) recruited to the tumor [47]. The switch clearly involved more than simple upregulation of angiogenic activity and was thus thought to be the result of a net balance of positive and negative regulators.

In 1989, Rastinejad *et al.* [48] reported that the switch during the tumorigenesis of transformed hamster cells was associated with downregulation of an inhibitor of angiogenesis, thrombospondin 1 (TSP1) [49,50]. They showed that BHK 21/cl 13 cells, an immortal but non-tumorigenic line of hamster fibroblasts, could be converted to malignancy and anchorage independence by loss of a functioning tumor suppressor gene. These cells were highly tumorigenic in nude mice and neonatal hamsters, and potently angiogenic *in vivo*. Normal BHK cells and suppressed hybrids generated by fusing transformed BHK cells with either non-transformed BHK or normal human fibroblasts were unable to induce neovascularization when cells or their concentrated conditioned media were introduced into rat corneas, whereas

transformed BHK cells and transformed segregants from the suppressed hybrids were angiogenic under the same conditions. Mixing experiments showed that normal cells elaborated an inhibitor of neovascularization whose production was blocked coincidentally with suppressor loss. When endothelial cell chemotaxis was used as an *in vitro* corollary of angiogenesis in the rat cornea assay, the inhibitor was purified and shown to be TSP1 [51]. This was the first illustration of a new function for a tumor suppressor gene, namely regulation of the production of a naturally occurring inhibitor of angiogenesis.

In another set of experiments, Dameron *et al.* [50] established a direct link between the *p53* tumor suppressor gene, tumor angiogenesis and TSP1. To examine the effect of *p53* on angiogenesis, they used cultured fibroblasts from patients with the Li-Fraumeni syndrome who have inherited one wild-type allele and one mutant allele of the *p53* gene. When the wild-type allele was lost, these cells acquired potent angiogenic activity coincidental with loss of TSP1 production. Transfection assay revealed that *p53* stimulated the endogenous TSP1 gene and positively regulated the TSP1 promoter sequences.

The first direct evidence that tumors are angiogenesis-dependent also comes from this period [52–54].

Neovascularization also Facilitates Metastasis

It has been shown experimentally that the onset of neovascularization coincides with increased shedding of tumor cells into the circulation and metastasis [55]. Entry into the circulation is enhanced by a growing density of immature, highly permeable blood vessels that have little basement membrane and fewer intercellular junctional complexes than normal mature vessels [56]. As many as 2×10^6/day mammary carcinoma cells can be shed from a 1-cm primary tumor [57].

The number of metastases is generally proportional to the number of tumor cells shed. Consequently, decreased angiogenesis by a given metastatic tumor should result in fewer metastatic colonies [58].

The list of angiogenesis inhibitors that also inhibit tumor metastasis includes the steroids [59], thalidomide [60], the fumagillin analogue TNP-470 (AGM-1470) [61–63], TSP [64], angiostatin [65], endostatin [66], and platelet factor IV [67].

Dormancy of Micrometastases may be Governed by Angiogenesis

Endogenous inhibition of angiogenesis may be maintained in dormant state lung metastases [65,68,69]. Folkman et al. found that metastases were suppressed when a primary tumor was implanted and allowed to grow in nude mice, whereas they underwent neovascularization and became clinically evident when primary neoplasm was removed. In the absence of angiogenesis, micrometastases rarely exceeded 0.2 mm diameter and contained many proliferating tumor cells balanced by many apoptotic cells. When they were allowed to become angiogenic, they grew rapidly. Dormancy may be generalizable to a variety of tumors in which blocked angiogenesis results in balanced tumor cell proliferation and apoptosis [64,68].

Tumor Angiogenesis as a Prognostic Indicator

Angiogenesis is a prognostic indicator for a wide variety of tumors. If the vessel density is low, the prognosis is good. Tumor angiogenesis was initially measured by using a histological index based on endothelial cell morphology and vascular density [69]. The most widely used method today is the microvessel density technique first proposed by Weidner *et al.* [70] and summarized by Gasparini and Harris [71]. Endothelial cells from a tissue biopsy are stained with an immunoperoxidase method using an anti–factor VIII-related antigen/von Willebrand's factor antibody. Vessels are counted in the region of the highest density as determined by the pathologist.

Quantification with one of these techniques provides prognostic information for a number of cancer types. The first report appeared in 1988, when Srivastava *et al.* [72] found that the degree of histologic staining for vessels in melanoma patients was associated with a probability of metastasis. Roughly a hundred investigations of this kind have been published. In most of them, a significant correlation was found between a high microvascular count and metastatic disease with a poor prognosis. Weidner et al. [70] showed a direct correlation between vascular density and metastasis in human breast cancer. This finding has since been extended to carcinoma of the prostate [73,74], lung [75,76], stomach [77], cervix [78] and ovary [79], squamous cell carcinoma of the head and neck [80], multiple myeloma [81–83] and lymphoma [84–86].

The angiogenic capacity of a tumor can also be assessed by means of a biochemical assay that detects angiogenic factors in a patient's serum, urine, or cerebrospinal fluid. Children with brain tumors have increased levels of FGF-2 in their cerebrospinal fluid [87], men with prostate cancer have elevated serum FGF-2 levels [88], and increased levels of FGF-2 have been found in the urine of patients with a variety of cancers [89].

Hypoxic Regulation of Tumor Angiogenesis

There is a complex interrelationship between tumor hypoxia and tumor angiogenesis. Hypoxia in tumors develops as chronic hypoxia, resulting from long diffusion distances between tumor vessels, and/or acute hypoxia, resulting from a transient collapse of tumor vessels. Many tumors contain hypoxic microenvironment, a condition that is associated with poor prognosis and resistance to treatment. Production of several angiogenic cytokines, such as FGF-2, VEGF, TGF-β, TNF-α and IL-8, is regulated by hypoxia. VEGF-mRNA expression is rapidly and reversibly induced by exposure of cultured endothelial cells to low pO_2 [90]. Many tumor cell lines were reported to show hypoxic induced expression of VEGF [91–95]. In a rat glioma model, VEGF

gene expression is activated in a distinct tumor cell subpopulation by two distinct hypoxia-driven mechanisms [96].

Hypoxia-inducible factor (HIF)-1 helps restore oxygen homeostasis by inducing glycolysis, erythropoiesis and angiogenesis [97]. Tumor vascularization is largely controlled by HIF-1, in part, as a result of upregulation of VEGF [98].

The Role of Inflammatory Cells in Tumor Angiogenesis

The peritumoral inflammatory infiltrate surrounding the newly formed blood vessels consists of fibroblasts, macrophages, mast cells and other leukocytes that may contribute to the induction of angiogenic response by secreting angiogenic cytokines and proteolytic enzymes, which, in turn, mobilize angiogenic factors stored in the extracellular matrix [99].

In tumors, macrophages are recruited and activated via several factors secreted by tumor cells, such as chemokines [100], FGF-2 and VEGF [101]. Activated macrophages secrete several angiogenic factors, such as FGF-2, VEGF, TNF-α, IL-8, tissue factor, hepatocyte growth factor/scatter factor and insulin-like growth factor-1 [102,103].

Experimentally induced tumors display mast cell accumulation close to the tumor cells before the onset of angiogenesis [104], and those induced in mast-cell-deficient mice display both reduced angiogenesis and ability to metastasize [105]. Mast cells are strikingly associated with angiogenesis in hemangioma, carcinomas, B-cell non-Hodgkin lymphomas and multiple myeloma [83,85,106,107]. Mast cells are recruited and activated by several factors secreted by tumor cells: the c-kit receptor, or stem cell factor [106], FGF-2, VEGF and PD-ECGF [108]. In turn, mast cells synthesize a variety of angiogenic cytokines, such as FGF-2, VEGF, TNF-α, IL-8 [107,109–111], implicated in tumor-associated angiogenesis.

Lymphocytes synthesize and secrete FGF-2 and VEGF [112,113]. T lymphocytes infiltrating human cancers express VEGF [113].

Angiopoietins and the Role of Mural Cells (Pericytes and Muscle Cells) in Vascular Remodeling and Tumor Angiogenesis

Recently, a novel family of angiogenic factors, designated as angiopoietins (Ang), has been identified by Davis *et al.* [114] and Maisonpierre *et al.* [115]. Ang-1 was discovered originally as a ligand for Tie-2, a member, with the originally cloned isoform Tie-1, of the tyrosine kinase with immunoglobulin and epidermal growth factor homology receptor (Tie) family [114,116–118]. Ang-2 also binds Tie-2 [115]. Although both Ang-1 and Ang-2 bind Tie-2, no ligand for Tie-1 has been identified. Ang plays a role in vascular stabilization [119]. Ang-1 is associated with developing vessels and its absence leads to defect in vessel remodeling. Ang-2, which antagonizes the action of Ang-1, plays a role in the destabilization of existing vessels (it is found in tissues like the ovary, uterus and placenta that undergo transient or periodic growth and vascularization, followed by regression). In the absence of Ang-1, angiogenic factors like VEGF may produce immature vessels that are hemorrhagic and display poor contact with underlying matrix material. Tie-2 [120] or Ang-1 [121] knock-out mice show immature vascularization pattern as well as lack of periendothelial mesenchymal cells, such as pericytes and immature smooth muscle cells (SMCs), leading to death around 11.0 to 12.5 days of gestation. Targeted disruption of the Tie-1 gene indicates that it is required for the maintenance of vascular integrity [120]. Transgenic mice overexpressing Ang-2 also died during embryogenesis with similar vascular defects as mice lacking Ang-1 or Tie-2 [115].

Ang-2 seems to be the earliest marker of blood vessels that has perturbed by invading tumor cells [122]. Ang-2 is overexpressed in tumor microvasculature of human glioblastoma and hepatocellular carcinoma [123,124].

Periendothelial cells, e.g., pericytes around capillaries and SMCs around larger vessels, provide structural strength and participate in the maturation of the vasculature by controlling several endothelial cell functions. Pericytes restrict the proliferation of endothelial cells in coculture,

Table 1. Major factors acting as agonists and antagonists of the vascular phase.

Agonist	Year of discovery	Authors	Reference number
Tumor angiogenic factor (TAF)	1971	Folkman *et al.*	[9]
Vascular permeability factor (VPF)*	1983	Senger *et al.*	[23]
Basic fibroblast growth factor (FGF-2)	1984	Maciag *et al.*	[14]
Vascular endothelial growth factor (VEGF)*	1989	Shing *et al.*	[15]
		Ferrara and Henzel	[16]
		Plouet *et al.*	[17]
Antagonist	**Year of discovery**	**Authors**	**Reference number**
Angiostatic steroids	1985	Crum *et al.*	[59]
Thrombospondin 1	1989	Rastinejad *et al.*	[48]
TNP-470 (AGM1470)	1990	Ingber *et al.*	[61]
Antibodies to FGF-2	1991	Hori *et al.*	[52]
Antibodies to VEGF	1993	Kim *et al.*	[53]
Angiostatin	1994	O'Reilly *et al.*	[65]
Endostatin	1997	O'Reilly *et al.*	[66]

*The molecular cloning of VEGF and VPF has revealed that both activities are embodied in the same molecule.

where a single pericyte could contact and inhibit the growth up to the endothelial cells [125]. This inhibition, requiring contacts between endothelial cells and pericytes, has been attributed to activation of TGF-β [126]. Platelet-derived growth factor-BB (PDGF-BB) is involved in endothelial cell to pericyte signaling, stimulating pericyte migration and proliferation [127]. A current hypothesis suggests that loosening of endothelial cell/pericyte contact may be necessary for the initiation of angiogenesis in mature adult vessels [128]. In a hypoxic condition (see above), VEGF can also act as a pericyte mitogen [129]. Moreover, hypoxia promotes the *in vitro* growth of pericytes through the autocrine action of VEGF induced in this cell type.

Pericytes may differentiate into SMCs. A study of mesenteric capillary growth in rats [130] suggests that fibroblasts transform into pericytes which, in turn, become SMC. Targeted disruption of the PDGF-BB gene resulted in a defective development of the SMCs [131].

Pericytes or SMC may contribute to tumor neovascularization, as demonstrated in the microvasculature of glioblastoma multiforme, where many α-SMC actin-positive cells, reacting also with an antibody against activated pericytes, are detectable [132]. Examination of TGF-β positive cells in Kaposi's sarcoma reveals the precursors in both SMC and pericytes as well as in the spindle-shaped Kaposi's sarcoma cells [133].

Inhibition of Angiogenesis

The existence of specific angiogenesis inhibitors was first postulated by Folkman [134] in an editorial. The term ''anti-angiogenesis'' was introduced to describe treatment designed to prevent the induction of new blood vessels and perhaps reduce the number of those already present.

Anti-angiogenic activity was first described in cartilage. Eisenstein *et al.* [135] observed the formation of avascular

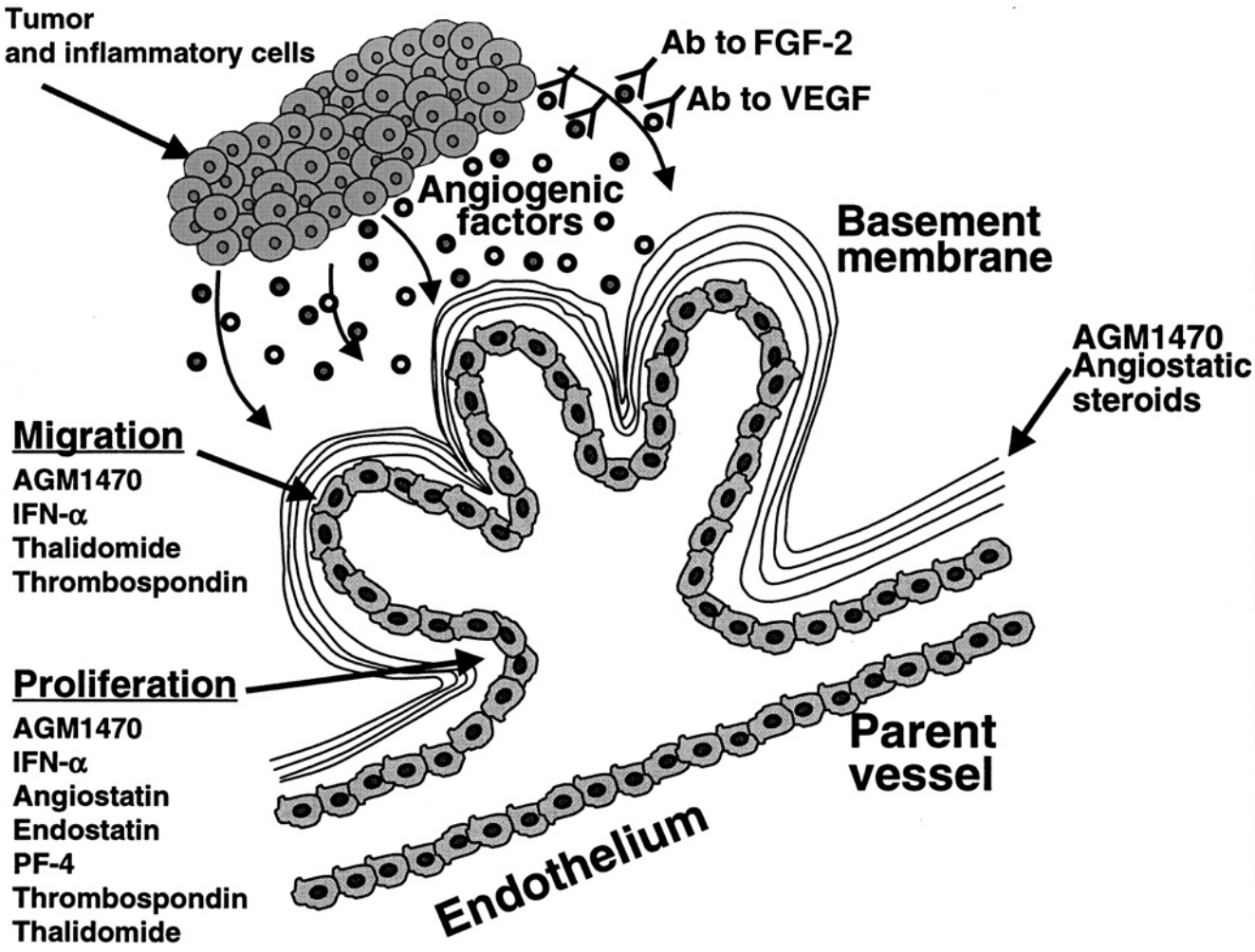

Figure 1. *Relationships between angiogenic cascade and angiogenic inhibitory agents in the tumor vascular phase. Tumor angiogenesis is a multistep process involving secretion of angiogenic growth factors by the tumor and inflammatory cells, invasion, migration and proliferation of endothelial cells (EC) through the basement membrane and growth of the new-formed vessels into the tumor stroma. Inhibition on angiogenesis is given by several molecules acting on distinct targets: AGM1470 inhibits EC proliferation and migration and destroys the basement membrane; angiostatic steroids destroy the basement membrane; angiostatin and endostatin inhibit EC proliferation; antibodies (Ab) against FGF-2 and VEGF inhibit angiogenic cytokines; platelet factor-4 (PF-4) inhibits EC proliferation and capillary formation; IFN-α, thalidomide and thrombospondin inhibit EC migration and proliferation.*

areas around cartilage fragments placed on the surface of the chick CAM.

Angiogenesis inhibitors act by: i) inhibiting the production and/or expression of angiogenic factors. Short-term administration of antibodies against specific angiogenic peptides, such as FGF-2 [52] or VEGF [53], has inhibited tumor growth in animals; ii) preventing the proliferation and migration of endothelial cells in response to angiogenic factors, e.g., the fungal derivative TNP-470 (AGM-1470) [61]; iii) combination of therapies directed against both endothelial and tumor cells.

Anti-angiogenesis was proposed as a cancer therapy over 20 years ago. Clinical trials of potential inhibitors, however, are very recent. Several phases I–II studies on TNP-470 in North America have so far shown some antitumoral effects with no significant systemic toxic side-effects [136]. Interferon-alpha has effectively accelerated the regression of hemangiomas in infants and children [137].

Marimastat, a tissue metalloproteinase inhibitor, is an orally active drug which inhibits tumor cell invasiveness and metastasis as well as angiogenesis. A preliminary multicentric study of its effects in different types of solid or advanced solid tumors suggested that it decreases the levels of some serum markers of malignancy [138].

Brooks *et al.* [139] and Friedlander *et al.* [140] have shown that the integrins $\alpha_v\beta_3$ and $\alpha_v\beta_5$ are essential for angiogenesis and that the former and the latter are involved in the specific angiogenic pathways stimulated by FGF-2 and VEGF, respectively. Humanised monoclonal antibodies against VEGF and integrin $\alpha_v\beta_3$ are being investigated in phase I trials in North America [141].

O'Reilly *et al.* [65] have isolated two new angiogenesis inhibitors, namely angiostatin and endostatin. Angiostatin, a specific inhibitor of endothelial cell proliferation, is an internal fragment of plasminogen containing at least three of its kringles. It was isolated from a subclone of Lewis lung carcinoma in which the primary tumor inhibited the growth of its metastases. It was generated by the primary tumor and potently inhibited angiogenesis. Systemic therapy with angiostatin, in fact, led to the maintenance of metastases in a microscopic dormant state defined by a balance of apoptosis and proliferation of the tumor cells [65,68]. It has since been shown that this treatment also inhibits the growth of three types of murine primary tumors, even if it is not begun until they correspond to 2% of body weight [142].

Endostatin is a terminal fragment of collagen XVIII. It specifically inhibits endothelial proliferation and is a potent inhibitor of angiogenesis and tumor growth [66]. Primary tumors regressed to dormant microscopic lesions and immunohistochemistry revealed blocked angiogenesis accompanied by high tumor cell proliferation balanced by apoptosis. Further experiments have shown that drug resistance does not develop in three tumor types treated with endostatin and that repeated courses are followed by prolonged tumor dormancy without further therapy.

These findings form the background to a new approach to the treatment of human tumors since the biological difference between human and murine endothelial cells is only marginal. A further advance may be achieved by combining anti-angiogenic treatment and chemotherapy so as to reduce the doses of chemotherapeutic drugs and hence their adverse side-effects.

Conclusions

This historical review has illustrated the importance of the vascular phase in the growth of solid tumors and has described the major factors acting as agonists or antagonists of this process (Table 1). It also shows that this scientific paradigm, to use the term imparted to this concept by Kuhn, the philosopher of science, has been repeatedly confirmed. The strength of this paradigm has now been increased by the mounting of clinical and not just experimental evidence of the prognostic value of the vascular component in both tumor growth and metastasis [70,143]. The complex relationships between angiogenic cascade and anti-angiogenic agents in the tumor vascular phase (Figure 1, Table 1), as well as further identification and characterization of angiogenesis inhibitors, have indicated that anti-angiogenesis can be seriously considered as a strategy for the adjuvant therapy of solid tumors.

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References

[1] Goldman E (1907). The growth of malignant disease in man and the lower animals with special reference to the vascular system. *Lancet* **ii**, 1236–1240.

[2] Clark ER, Hitschler WJ, Kirby-Smith HT, Rex RO, and Smith JH (1931). General observations on the ingrowth of new blood vessels into standardized chambers in the rabbit's ear, and the subsequent changes in the newly grown vessels over a period of months. *Anat Rec* **50**, 129–167.

[3] Clark ER, and Clark EI (1939). Microscopic observations on the growth of blood capillaries in the living mammals. *Am J Anat* **64**, 251–301.

[4] Ide AG, Baker NH, and Warren SL (1939). Vascularization of the Brown-Pearce rabbit epithelioma transplant as seen in the transparent ear chambers. *AJR* **32**, 891–899.

[5] Algire GH, and Chalkley HW (1945). Vascular reactions of normal and malignant tissue *in vivo*. *J Natl Cancer Inst* **6**, 73–85.

[6] Merwin RM, and Algire GH (1956). The role of graft and host vessels in vascularization of grafts of normal and neoplastic tissues. *J Natl Cancer Inst* **17**, 23–33.

[7] Greenblatt M, and Shubik P (1968). Tumor angiogenesis: transfilter diffusion studied in the hamster by the transparent chamber technique. *J Natl Cancer Inst* **41**, 111–124.

[8] Ehrmann RL, and Knoth M (1968). Choriocarcinoma: transfilter stimulation of vasoproliferation in the hamster cheek pouch studied by light and electron microscopy. *J Natl Cancer Inst* **41**, 1239–1241.

[9] Folkman J, Merler E, Abernathy C, and Williams G (1971). Isolation of a tumor fraction responsible for angiogenesis. *J Exp Med* **133**, 275–288.

[10] Tuan D, Smith S, Folkman J, and Merler E (1973). Isolation of the nonhistone proteins of rat Walker 256 carcinoma. Their association with tumor angiogenesis. *Biochemistry* **12**, 3159–3165.

[11] McAuslan BR, and Hoffman H (1979). Endothelium stimulating factor from Walker carcinoma cells. Relation to tumor angiogenic factor. *Exp Cell Res* **119**, 181–190.
[12] Weiss JB, Brown RA, Kumar S, and Phillips P (1979). An angiogenic factor isolated from tumours: a potent low-molecular weight compound. *Br J Cancer* **40**, 493–496.
[13] Fenselau A, Kaiser D, and Wallis K (1981). Nucleoside requirements for the *in vivo* growth of bovine aortic endothelial cells. *J Cell Physiol* **108**, 375–384.
[14] Maciag T, Mehlman T, Friesel R, and Schreiber AM (1984). Heparin binds endothelial cell growth factor, the principal cell mitogen in bovine brain. *Science* **225**, 932–935.
[15] Shing Y, Folkman J, Sullivan R, Butterfield C, Murray J, and Klagsbrun M (1984). Heparin-affinity: purification of a tumor-derived capillary endothelial cell growth factor. *Science* **223**, 1296–1298.
[16] Ferrara N, and Henzel WJ (1989). Pituitary follicular cells secrete a novel heparin-binding growth factor specific for vascular endothelial cells. *Biochem Biophys Res Commun* **161**, 851–855.
[17] Plouet J, Schilling J, and Gospodarowicz D (1989). Isolation and characterization of a newly identified endothelial cell mitogen produced by AtT20 cells. *EMBO J* **8**, 3801–3807.
[18] Shing Y, Folkman J, Haudenschild C, Lund D, Crum R, and Klagsbrun M (1985). Angiogenesis is stimulated by a tumor-derived endothelial cell growth factor. *J Cell Biochem* **29**, 275–287.
[19] Bohlem P, Baird A, Esch F, Ling N, and Gospodarowicz D (1984). Isolation and partial molecular characterization of pituitary fibroblast growth factor. *Proc Natl Acad Sci USA* **81**, 5364–5368.
[20] Gospodarowicz D, Cheng J, Lui GM, Baird A, and Bohlen P (1984). Isolation of brain fibroblast growth factor by heparin–sepharose affinity chromatography: identity with pituitary fibroblast growth factor. *Proc Natl Acad Sci USA* **81**, 6963–6967.
[21] Esch F, Baird A, Ling N, Veno N, Hill F, Denoroy L, Klepper R, Gospodarowicz D, Bohlen P, and Guillemin R (1985). Primary structure of bovine pituitary basic fibroblast growth factor (FGF) and comparison with the amino-terminal sequence of bovine brain acidic FGF. *Proc Natl Acad Sci USA* **82**, 6507–6511.
[22] Leung DW, Cachianes G, Kuang WJ, Goeddel DV, and Ferrara N (1989). Vascular endothelial growth factor is a secreted angiogenic mitogen. *Science* **246**, 1306–1309.
[23] Senger DR, Galli SJ, Dvorak AM, Perruzzi CA, Harvey VS, and Dvorak HF (1983). Tumor cells secrete a vascular permeability factor that promotes accumulation of ascites fluids. *Science* **219**, 983–985.
[24] Kech PJ, Hauser SD, Krivi G, Sanzo K, Warren T, Feder J, and Connolly DT (1989). Vascular permeability factor, an endothelial cell mitogen related to platelet derived growth factor. *Science* **246**, 1309–1312.
[25] Conn G, Bayne ML, Soderman DD, Kwok PW, and Sullivan KA (1990). Amino acid and cDNA sequences of a vascular endothelial cell mitogen that is homologous to platelet-derived growth factor. *Proc Natl Acad Sci USA* **82**, 2628–2632.
[26] Gospodarowicz D, Abraham JA, and Schilling J (1989). Isolation and characterization of a vascular endothelial cell mitogen produced by pituitary-derived folliculostellate cells. *Proc Natl Acad Sci USA* **86**, 7311–7315.
[27] Connolly DT, Heuvelman DM, Nelson R, Olander JV, Eppley B, Delfino JJ, Seigel NR, Leimgruber RM, and Feder J (1989). Tumor vascular permeability factor stimulates endothelial cell growth and angiogenesis. *J Clin Invest* **84**, 1470–1478.
[28] Levy AP, Tamargo R, Brem H, and Nathans D (1989). An endothelial cell growth factor from the mouse neuroblastoma cell line NB41. *Growth Factors* **2**, 9–19.
[29] Senger D, Brown L, Claffey K, and Dvorak A (1995). Vascular permeability factor, tumor angiogenesis and stroma generation. *Invasion Metastasis* **14**, 385–394.
[30] Pepper MS, Ferrara N, Orci L, and Montesano R (1992). Potent synergism between vascular endothelial growth factor and basic fibroblast growth factor in the induction of angiogenesis *in vitro*. *Biochem Biophys Res Commun* **189**, 824–831.
[31] Goto F, Goto K, Weindel K, and Folkman J (1993). Synergistic effects of vascular endothelial growth factor and basic fibroblast growth factor on bovine capillary endothelial cells within collagen gels. *Lab Invest* **69**, 508–571.
[32] Asahara T, Bauters C, Zheng L, Takeshita S, Bunting S, Ferrara N, Symes JF, and Isner JM (1995). Synergistic effect of vascular endothelial growth factor and basic fibroblast growth factor on angiogenesis *in vivo*. *Circulation* **92**, S365–S371.
[33] Brem SS, Gullino PM, and Medina P (1977). Angiogenesis: a marker for neoplastic transformation of mammary papillary hyperplasia. *Science* **195**, 880–882.
[34] Brem SS, Jensen HM, and Gullino PM (1978). Angiogenesis as a marker of preneoplastic lesions of the human breast. *Cancer* **41**, 239–244.
[35] Maiorana A, and Gullino PM (1978). Acquisition of angiogenic capacity and neoplastic transformation in the rat mammary gland. *Cancer Res* **38**, 4409–4414.
[36] Folkman J, and Hochberg M (1973). Self-regulation of growth in three-dimension. *J Exp Med* **138**, 745–753.
[37] Gimbrone MA Jr, Leapman S, Cotran RS, and Folkman J (1972). Tumor dormancy *in vivo* by prevention of neovascularization. *J Exp Med* **136**, 261–276.
[38] Folkman J, Lang DM, and Becker FF (1963). Growth and metastasis of tumor in organ culture. *Cancer* **16**, 453–467.
[39] Cavallo T, Sade R, Folkman J, and Cotran RS (1972). Tumor angiogenesis: rapid induction of endothelial mitosis demonstrated by autoradiography. *J Cell Biol* **54**, 408–420.
[40] Cavallo T, Sade R, Folkman J, and Cotran RS (1973). Ultrastructural autoradiographic studies of the early vasoproliferative response in tumor angiogenesis. *Am J Pathol* **70**, 345–362.
[41] Gimbrone MA Jr, Cotran RS, Leapman SB, and Folkman J (1974). Tumor growth and neovascularization: an experimental model using the rabbit cornea. *J Natl Cancer Inst* **52**, 413–427.
[42] Folkman J, Watson K, Ingber D, and Hanahan D (1989). Induction of angiogenesis during the transition from hyperplasia to neoplasia. *Nature* **339**, 58–61.
[43] Nicosia RF, Tchao R, and Leighton J (1986). Interactions between newly formed endothelial channels and carcinoma cells in plasma clot culture. *Clin Exp Metastasis* **4**, 91–104.
[44] Hanahan D (1985). Heritable formation of pancreatic beta-cell tumors in transgenic mice expressing recombinant insulin/simian virus 40 oncogene. *Nature* **315**, 115–122.
[45] Kandel J, Bossy-Wetzel E, Radvanyl F, Klagsbrun M, Folkman J, and Hanahan D (1991). Neovascularization is associated with a switch to the export of bFGF. *Cell* **66**, 1095–1104.
[46] Vlodavsky I, Korner G, Ishai-Michaeli R, Bashkin P, Bar-Shavit R, and Fuks Z (1990). Extracellular matrix-resident growth factors and enzymes: possible involvement in tumor metastasis and angiogenesis. *Cancer Metastasis Rev* **9**, 203–226.
[47] Leibovich SJ, Polverini PJ, Shepard HM, Wiseman DM, Shively V, and Nuseir N (1987). Macrophage-induced angiogenesis is mediated by tumor necrosis factor-α. *Nature* **329**, 630–632.
[48] Rastinejad F, Polverini PJ, and Bouck NP (1989). Regulation of the activity of a new inhibitor of angiogenesis by a cancer suppressor gene. *Cell* **56**, 345–355.
[49] Good DJ, Polverini PJ, Rastinejad F, Le Beau MM, Lemons RS, Frazier WA, and Bouck NP (1990). A tumor suppressor-dependent inhibitor of angiogenesis is immunologically and functionally indistinguishable from a fragment of thrombospondin. *Proc Natl Acad Sci USA* **87**, 6624–6628.
[50] Dameron KM, Volpert OV, Tainsky MA, and Bouck N (1994). Control of angiogenesis in fibroblasts by *p53* regulation of thrombospondin-1. *Science* **265**, 1582–1584.
[51] Bornstein P (1992). Thrombospondins: structure and regulation of expression. *FASEB J* **6**, 3290–3293.
[52] Hori A, Sasada D, Matsutani E, Naito K, Samura Y, Fijta T, and Kozay Y (1991). Suppression of solid tumor growth by immunoneutralizing monoclonal antibody against human basic fibroblast growth factor. *Cancer Res* **51**, 6180–6184.
[53] Kim KJ, Li B, Winer J, Armanini M, Gillett N, Phillips HS, and Ferrara N (1993). Inhibition of vascular endothelial growth factor-induced growth *in vivo*. *Nature* **362**, 841–844.
[54] Millauer B, Shawyer LK, Plate KH, Risau W, and Ullrich A (1994). Glioblastoma growth inhibited *in vivo* by a dominant-negative Flk-1 mutant. *Nature* **367**, 576–579.
[55] Liotta LA, Kleinerman J, and Saidel GM (1974). Quantitative relationships of intravascular tumor cells, tumor vessels, and pulmonary metastases following tumor implantation. *Cancer Res* **34**, 997–1004.
[56] Dvorak HF, Brown LF, Detmar M, and Dvorak AM (1995). Vascular permeability factor/vascular endothelial growth factor, microvascular hyperpermeability, and angiogenesis. *Am J Pathol* **146**, 1029–1039.
[57] Butler TP, and Gullino PM (1975). Quantitation of cell shedding into efferent blood of mammary adenocarcinoma. *Cancer Res* **35**, 512–516.
[58] Zetter BR (1998). Angiogenesis and tumor metastasis. *Ann Rev Med* **49**, 407–424.

[59] Crum R, Szabo S, and Folkman J (1985). A new class of steroids inhibits angiogenesis in the presence of heparin or a heparin fragment. *Science* **230**, 1375–1378.
[60] D'Amato RJ, Loughnan MS, Flynn E, and Folkman J (1994). Thalidomide is an inhibitor of angiogenesis. *Proc Natl Acad Sci USA* **91**, 4082–4085.
[61] Ingber DE, Fujita T, Kishimoto S, Sudo K, Kanamanu K, Brem H, and Folkman J (1990). Angioinhibins: synthetic analogues of fumagillin which inhibit angiogenesis and suppress tumor growth. *Nature* **348**, 555–557.
[62] Konno H, Tanaka T, Matsuda I, Kanai T, Maruo Y, Nishino N, Nakamura S, and Baba S (1995). Comparison of the inhibitory effect of the angiogenesis inhibitor, TNP-470, and mitomycin C on the growth and liver metastasis of human colon cancer. *Int J Cancer* **61**, 268–271.
[63] Mori S, Ueda T, Kuratsu S, Hosono N, Izawa K, and Uchida A (1995). Suppression of pulmonary metastasis by angiogenesis inhibitor TNP-470 in murine osteosarcoma. *Int J Cancer* **61**, 148–152.
[64] Weinstat-Saslow DL, Zabrenetzky VS, VanHoutte K, Frazier WA, Roberts DD, and Steegs PS (1994). Transfection of thrombospondin 1 complementary DNA into a human breast carcinoma cell line reduces primary tumor growth, metastatic potential, and angiogenesis. *Cancer Res* **54**, 6504–6511.
[65] O'Reilly MS, Holmgren L, Shing Y, Chen C, Rosenthal RA, Moses M, Lane WS, Cao Y, Sage EH, and Folkman J (1994). Angiostatin: a novel angiogenesis inhibitor that mediates the suppression of metastases by a Lewis lung carcinoma. *Cell* **79**, 315–328.
[66] O'Reilly MS, Boehm T, Shing Y, Fukai N, Vasios G, Lane WS, Flynn E, Birkhead JR, Olsen BR, and Folkman J (1997). Endostatin: an endogenous inhibitor of angiogenesis and tumor growth. *Cell* **88**, 277–285.
[67] Kolber DL, Knisely TL, and Maione TE (1995). Inhibition of development of murine melanoma lung metastases by systemic administration of recombinant platelet factor 4. *J Natl Cancer Inst* **87**, 304–309.
[68] Holmgren L, O'Reilly, and Folkman J (1995). Dormancy of micrometastases: balanced proliferation and apoptosis in the presence of angiogenesis suppression. *Nat Med* **1**, 149–153.
[69] Brem S, Cotran R, and Folkman J (1972). Tumor angiogenesis: a quantitative method for histologic grading. *J Natl Cancer Inst* **48**, 347–356.
[70] Weidner N, Sample JP, Welch WR, and Folkman J (1991). Tumor angiogenesis and metastasis. Correlation in invasive breast carcinoma. *New Engl J Med* **324**, 1–8.
[71] Gasparini G, and Harris A (1995). Clinical importance of the determination of tumor angiogenesis in breast carcinoma: much more than a new prognostic tool: review. *J Clin Oncol* **13**, 765–782.
[72] Srivastava A, Laider P, Davies R, Horgan K, and Hughes L (1988). The prognostic significance of tumor vascularity in intermediate thickness (0.76–4 mm thick) skin melanoma: a quantitative histologic study. *Am J Pathol* **133**, 419–423.
[73] Weidner N, Carroll PR, Flax J, Blumenfeld W, and Folkman J (1993). Tumor angiogenesis correlates with metastasis in invasive prostate carcinoma. *Am J Pathol* **143**, 1–9.
[74] Brawer MK (1996). Quantitative microvessel density, a staging and prognostic marker for human prostatic carcinoma. *Cancer* **78**, 345–349.
[75] Yamazaki K, Abe S, Tekekawa H, Sukoh N, Watanabe N, Ogura S, Nakajima I, Isobe H, Inoue K, and Kawakami Y (1994). Tumor angiogenesis in human lung adenocarcinoma. *Cancer* **74**, 2245–2250.
[76] Angeletti CA, Lucchi M, Fontanini G, Mussi A, Chella A, Ribechini A, Vignati S, and Bevilacqua G (1996). Prognostic significance of tumoral angiogenesis in completely resected late stage lung carcinoma (stage IIIA-N2): impact of adjuvant therapies in a subset of patients at high risk of recurrence. *Cancer* **78**, 409–415.
[77] Maeda K, Chung YS, Takasuka S, and Ogawa Y (1995). Tumor angiogenesis is a predictor of recurrence in gastric carcinoma. *J Clin Oncol* **13**, 477–481.
[78] Wiggins DL, Granai CO, Steinhoff MM, and Calabresi P (1995). Tumor angiogenesis as a prognostic factor in cervical carcinoma. *Gynecol Oncol* **56**, 353–356.
[79] Hollingsworth HC, Kohn EC, Steinberg SM, Rothengerg ML, and Merino MJ (1995). Tumor angiogenesis in advanced stage ovarian carcinoma *Am J Pathol* **147**, 33–41.
[80] Gasparini G, Weidner N, Maluta S, Pozza F, Boracchi P, Mezzetti M, Testolin A, and Bevilacqua P (1993). Intratumoral microvessel density and p53 protein: correlation with metastasis in head and neck squamous-cell carcinoma. *Int J Cancer* **55**, 739–744.
[81] Vacca A, Ribatti D, Roncali L, Ranieri G, Serio G, Silvestris F, and Dammacco F (1994). Bone marrow angiogenesis and progression in multiple myeloma. *Br J Hematol* **87**, 503–508.
[82] Vacca A, Ribatti D, Presta M, Minischetti M, Iurlaro M, Ria R, Albini A, Bussolino F, and Dammacco F (1999a). Bone marrow neovascularization, plasma cell angiogenic potential and matrix metalloproteinase-2 secretion parallel progression of human multiple myeloma. *Blood* **93**, 3064–3073.
[83] Ribatti D, Vacca A, Nico B, Quondamatteo F, Ria R, Minischetti M, Marzullo A, Herken R, Roncali L, and Dammacco F (1999). Bone marrow angiogenesis and mast cell density increase simultaneously with progression of human multiple myeloma. *Br J Cancer* **79**, 451–455.
[84] Ribatti D, Vacca A, Nico B, Fanelli M, Roncali L, and Dammacco F (1996). Angiogenesis spectrum in the stroma of B-cell non-Hodgkin's lymphomas. An immunohistochemical and ultrastructural study. *Eur J Hematol* **56**, 45–53.
[85] Ribatti D, Nico B, Vacca A, Marzullo A, Calvi N, Roncali L, and Dammacco F (1998). Do mast cells help angiogenesis in B-cell non-Hodgkin's lymphomas? *Br J Cancer* **77**, 1900–1906.
[86] Vacca A, Ribatti D, Ruco L, Giacchetta F, Nico B, Quondamatteo F, Ria R, Iurlaro M, and Dammacco F (1999b). Angiogenesis extent and macrophage density increase simultaneously with pathological progression in B-cell non-Hodgkin's lymphomas. *Br J Cancer* **79**, 965–970.
[87] Li VW, Folkerth R, Watanabe H, Yu C, Rupnick M, Barnes P, Scott RM, Black PM, Sallan SE, and Folkman J (1994). Microvessel count and cerebrospinal fluid basic fibroblast growth factor in children with brain tumours. *Lancet* **344**, 82–86.
[88] Meyer G, Yu E, Siegal J, Petteway J, Blumenstein B, and Brawer M (1995). Serum basic fibroblast growth factor in men with and without prostate cancer. *Cancer* **76**, 2304–2311.
[89] Nguyen M, Watanabe H, Budson A, Richie J, Hayes D, and Folkman J (1994). Elevated levels of an angiogenic peptide, basic fibroblast growth factor, in the urine of patients with a wide spectrum of cancers. *J Natl Cancer Inst* **86**, 356–361.
[90] Levy AP, Levy NS, Wegner S, and Goldberg MA (1995). Transcriptional regulation of the rat vascular endothelial growth factor gene by hypoxia. *J Biol Chem* **270**, 13333–13340.
[91] Plate KH, Breier G, Widch HA, and Risau W (1992). Vascular endothelial growth factor is a potent tumor angiogenesis factor in human gliomas *in vivo*. *Nature* **359**, 845–848.
[92] Shweiki D, Itin A, Soffer D, and Keshet E (1992). Vascular endothelial growth factor induced by hypoxia may mediate hypoxia-initiated angiogenesis. *Nature* **359**, 843–845.
[93] Hlatky L, Tsionou C, Hahnfeldt P, and Coleman CN (1994). Mammary fibroblasts may influence breast tumor angiogenesis via hypoxia-induced vascular endothelial growth factor upregulation and protein expression. *Cancer Res* **54**, 6083–6086.
[94] Potgens AJ, Lubsen NH, van Altena MC, Schoenmakers JG, Ruiter DJ, and De Waal RM (1995). Vascular permeability factor expression influences tumor angiogenesis in human melanoma lines xenografted to nude mice. *Am J Pathol* **146**, 197–209.
[95] Claffey KP, Brown LF, Del Aguila LF, Tognazzi K, Yeo KT, Manseau EJ, and Dvorak HF (1996). Expression of vascular permeability factor/vascular endothelial growth factor by melanoma cells increases tumor growth, angiogenesis, and experimental metastasis. *Cancer Res* **56**, 172–181.
[96] Damert A, Machein M, Breier G, Fujita MQ, Hanahan D, Risau W, and Plate KH (1997). Upregulation of vascular endothelial growth factor expression in a rat glioma is conferred by two distinct hypoxia-driven mechanisms. *Cancer Res* **57**, 3860–3864.
[97] Semenza GL (1996). Transcriptional regulation by hypoxia-inducible factor-1. *Trends Cardiovasc Med* **6**, 151–157.
[98] Carmeliet P, Dor Y, Herbert JM, Fukumura D, Brusselmans K, Dewerchin M, Neeman M, Bono F, Abramovitch R, Maxwell P, Koch CJ, Ratcliffe P, Moons L, Jain RK, Collen D, and Keshet E (1998). Role of HIF-1 in hypoxia-mediated apoptosis, cell proliferation and tumor angiogenesis. *Nature* **394**, 485–490.
[99] Folkman J, and Berm H (1992). Angiogenesis and inflammation. In: Gallin J, Goldstein M, and Synderman R (Eds.), *Inflammation: Basic Principles and Clinical Applications*. Raven Press, New York, pp. 821–839.
[100] Rollins BJ (1997). Chemokines. *Blood* **90**, 909–928.
[101] Watanabe K, Mc Cormick KL, Volker K, Ortaldo JR, Wiggington JR,

Brunda MJ, Wiltrout RH, and Fogler WE (1997). Regulation of host-mediated anti-tumor mechanism of cytokines. Direct and indirect effects on leukocyte recruitment and angiogenesis. *Am J Pathol* **150**, 1869–1880.

[102] Sunderkotter C, Steinbrink K, Goebeler M, Bhardwaj R, and Sorg C (1994). Macrophages and angiogenesis. *J Leukocyte Biol* **55**, 410–422.

[103] Leek RD, Lewis CE, and Harris AL (1997). The role of macrophages in tumour angiogenesis. In: Bicknell R, Lewis CE and Ferrara N (Eds.), *Tumour Angiogenesis*. Oxford University Press, Oxford, pp. 81–89.

[104] Kessler DA, Langer RS, Pless NA, and Folkman J (1976). Mast cell and tumor angiogenesis. *Int J Cancer* **18**, 703–709.

[105] Starkey JR, Crowle PK, and Taubenberger S (1988). Mst cell-deficient W/Wv mice exhibit a decreased rate of tumor angiogenesis. *Int J Cancer* **42**, 48–52.

[106] Norrby K, and Whooley D (1993). Role of mast cells in mitogenesis and angiogenesis in normal tissues and tumor tissues. *Adv Biosci* **89**, 71–136.

[107] Qu Z, Leibler JM, Powers MR, Galey T, Ahmadi P, Huang XN, Ansel JC, Butterfield JH, Planck SR, and Rosembaum JT (1995). Mast cell are a major source of basic fibroblast growth factor in chronic inflammation and cutaneous hemangiomas. *Am J Pathol* **147**, 564–573.

[108] Gruber BL, Marchese MJ, and Kew R (1995). Angiogenic factors stimulate mast cell migration. *Blood* **86**, 2488–2493.

[109] Grutzkau A, Kruger-Krasagakes S, Kogel H, Moller A, Lippert U, and Henz BM (1997). Detection of intracellular interleukin-8 in human mast cells: flow cytometry as a guide for immunoelectron microscopy. *J Histochem Cytochem* **45**, 935–945.

[110] Grutzkau A, Kruger-Krasagakes S, Baumesteir H, Schwarz C, Kogel H, Welker P, Lippert U, Henz BM, and Moller A (1998). Synthesis, storage and release of vascular endothelial growth factor/vascular permeability factor (VEGF/VPF) by human mast cells: implications for the biological significance of VEGF 206. *Mol Biol Cell* **9**, 875–884.

[111] Moller A, Henz BM, Grutzkau A, Lippert U, Aragane Y, Schwarz T, and Kruger-Krasagakes S (1998). Comparative cytokine gene expression: regulation and release by human mast cells. *Immunology* **93**, 289–295.

[112] Blotnick S, Peoples GE, Freeman MR, Eberlein TJ, and Klagsbrun M (1995). T lymphocytes synthesize and export heparin-binding epidermal growth factor-like growth factor and basic fibroblast growth factor, mitogens for vascular cells and fibroblasts: differential production and release by CD4+ and CD8+ cells. *Proc Natl Acad Sci USA* **91**, 2890–2894.

[113] Freeman MR, Schneck FX, Gagnon ML, Corless C, Soker S, Nikdenjad K, Peoples GE, and Klagsbrun M (1995). Peripheral blood T lymphocytes and lymphocytes infiltrating human cancer express vascular endothelial growth factor: a potential role for T cells in angiogenesis. *Cancer Res* **55**, 4140–4145.

[114] Davis S, Aldrich TH, Jones PF, Acheson A, Compton DL, Jain V, Ryan TE, Bruno J, Radziejwski C, Maisonpierre PC, and Yancopoulos GD (1996). Isolation of angiopoietin-1, a ligand for the TIE2 receptor, by secretion-trap expression cloning. *Cell* **87**, 1161–1169.

[115] Maisonpierre PC, Suri C, Jones PF, Bartunkova S, Wiegand S, Radziejwski C, Compton D, Mc Clain J, Aldrich TH, Papadopoulos N, Daly TJ, Davis S, Sato TN, and Yancopoulos GD (1997). Angiopoietin-2, a natural antagonist for Tie-2 that disrupts *in vivo* angiogenesis. *Science* **277**, 55–60.

[116] Dumont DJ, Yamaguchi TP, Conlon RA, Rossant J, and Breitman RL (1992). *tek*, a novel tyrosine kinase gene located on mouse chromosome 4, is expressed in endothelial cells, and their presumptive precursors. *Oncogene* **7**, 1471–1480.

[117] Partanen J, Armstrong E, Makela TP, Korhonen J, Sandberg M, Renkonen R, Knuutila S, Huebner K, and Alitalo K (1992). A novel endothelial cell surface receptor tyrosine kinase with extracellular epidermal growth factor homology domains. *Mol Cell Biol* **12**, 1698–1707.

[118] Sato TN, Quin Y, Kozak CA, and Audus KL (1993). Tie-1 and Tie-2 define another class of putative receptor tyrosine kinase genes expressed in early embryonic vascular system. *Proc Natl Acad Sci USA* **90**, 9355–9358.

[119] Beck L Jr, and D'Amore PA (1997). Vascular development: cellular and molecular regulation. *FASEB J* **11**, 365–373.

[120] Sato TN, Tazawa Y, Deutsch U, Wolburg-Bucholz K, Fujiwara Y, Gendron-Maguire M, Gridley T, Wolburg H, Risau W, and Quin Y (1995). Distinct roles of the receptor tyrosine kinase Tie-1 and Tie-2 in blood vessel formation. *Nature* **376**, 70–74.

[121] Suri C, Jones PF, Patan S, Bartunkova S, Maisonpierre PC, Davis S, Sato TN, and Yancopoulos GD (1996). Requisite role of angiopoietin-1, a ligand for the TIE2 receptor, during embryonic angiogenesis. *Cell* **87**, 1171–1180.

[122] Holash J, Maisonpierre PC, Compton D, Bolano P, Alexander CR, Zagzag D, Yancopoulos GD, and Wiegand SJ (1999). Vessel cooption, regression and growth in tumors mediated by angiopoietins and VEGF. *Science* **284**, 1994–1998.

[123] Stratmann A, Risau W, and Plate KH (1998). Cell-type specific expression of angiopoietin-1 and angiopoietin-2 suggests a role in glioblastoma angiogenesis. *Am J Pathol* **153**, 1459–1466.

[124] Tanaka S, Mori M, Sakamoto Y, Makuuchi M, Sugimachi K, and Wands JR (1999). Biologic significance of angiopoietin-2 expression in human hepatocellular carcinoma. *J Clin Invest* **103**, 341–345.

[125] Orlidge A, and D'Amore PA (1987). Inhibition of capillary endothelial cell growth by pericytes and smooth muscle cells. *J Cell Biol* **105**, 1455–1462.

[126] Antonelli-Orlidge A, Saunders KB, Smith SR, and D'Amore PA (1989). An activated form of transforming growth factor-β is produced by cocultures of endothelial cells and pericytes. *Proc Natl Acad Sci USA* **86**, 4544–4548.

[127] Lindahl P, Johansson BR, Leveen P, and Betsholtz C (1997). Pericyte loss microaneurysm formation in PDGF-B deficient mice. *Science* **277**, 242–245.

[128] Hanahan D (1997). Signaling vascular morphogenesis and maintenance. *Science* **277**, 48–50.

[129] Yamagishi S, Yonekura H, Yamamoto Y, Fujimori H, Sakurai S, Tanaka N, and Yamamoto H (1999). Vascular endothelial growth factor acts as a pericyte mitogen under hypoxic conditions. *Lab Invest* **79**, 501–509.

[130] Rhodin JAG, and Fujita H (1989). Capillary growth in mesentery of normal young rats. Intravital video and electron microscope analyses. *J Submicrosc Cytol Pathol* **21**, 1–34.

[131] Leeven P, Perny M, Gebre-Medhin S, Swolin B, Larsson E, and Betsholtz C (1994). Mice deficient for PDGF-B show renal, cardiovascular and hematological abnormalities. *Genes Dev* **8**, 1875–1887.

[132] Wesseling P, Schlingemann RO, Reiveld FJ, Link M, Burger PC, and Ruiter DJ (1995). Early and extensive contribution of pericytes/vascular smooth muscle cells to microvascular proliferation in glioblastoma multiforme: an immunolight and immunoelectron microscopic study. *J Neuropathol Exp Neurol* **54**, 304–310.

[133] Williams AO, Ward JM, Jackson MA, and Flanders KC (1995). Immunohistochemical localization of transforming growth factor-beta 1 in Kaposi's sarcoma. *Hum Pathol* **26**, 469–473.

[134] Folkman J (1971). Tumor angiogenesis: therapeutic implications. *New Engl J Med* **285**, 1182–1186.

[135] Eisenstein R, Sorgente R, Soble LW, Miller A, and Kuettner KE (1973). The resistance of certain tissues to invasion: penetrability of explanted tissues by vascularized mesenchyme. *Am J Pathol* **73**, 765–774.

[136] Castronovo V, and Belotti D. (1996). TNP-470 (AGM-1470): mechanisms of action and early clinical development. *Eur J Cancer* **32A**, 2520–2527.

[137] Schweigerer L, and Fotsis T (1991). Angiogenesis and angiogenesis inhibitors in paediatric diseases. *Eur J Pediatr* **151**, 472–476.

[138] Talbot DC, and Brown PD (1996). Experimental and clinical studies on the use of matrix metalloproteinase inhibitors for the treatment of cancer. *Eur J Cancer* **32A**, 2523–2533.

[139] Brooks PC, Clark RAF, and Cheresh DA (1994). Requirement of vascular integrin $\alpha_v\beta_3$ for angiogenesis. *Science* **264**, 569–571.

[140] Friedlander M, Brooks PC, Shaffer RW, Kincaid CM, Varner JA, and Cheresh DA (1995). Definitions of two angiogenic pathways by distinct integrins. *Science* **270**, 1500–1502.

[141] Gasparini G (1997). Anti-angiogenic drugs as a novel anticancer therapeutic strategy. Which are the more promising agents? What are the clinical developments and indications? *Crit Rev Oncol Hematol* **26**, 142–162.

[142] O'Reilly MS, Holmgren L, Chen C, and Folkman J (1996). Angiostatin induces and sustains dormancy of human primary tumors in mice. *Nat Med* **2**, 689–692.

[143] Macchiarini P, Fontanini G, Hardin MJ, Squartini F, and Angeletti CA (1992). Relation of neovascularization to metastasis of non-small cell-lung cancer. *Lancet* **340**, 145–146.