Tumor Angiogenesis: from Bench to Bedside

1

Judah Folkman

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1.1 Introduction

An association between cancer and blood vessels has been observed for more than a century. These reports dealt mainly with angioarchitecture of tumors, vascular patterns unique to tumors, effects of irradiation on tumor vasculature, alteration in tumor blood flow, increased vascularity of the peripheral shell of tumors, and the delivery of intravital dyes and anti-cancer drugs to the tumor bed (Thiersch 1865; Goldmann 1907; Thiessen 1936). In a few reports, experimental tumors were transplanted into transparent chambers in the mouse or rabbit. In some reports, the tumor and the host vascular bed were separated by a micropore filter to determine whether a diffusible substance was released from tumors that could stimulate blood vessel growth (Ide et al. 1939; Algire 1943; Algire and Legallais 1947; Toolan 1951; Greene 1952; Day et al. 1959; Zweifach 1961; Gullino and Grantham 1962; Goldacre and Sylvén 1962; Warner 1964; Greenblatt and Shubik 1968; Greenblatt et al 1969; Rubin and Casarett 1966; Tannock 1970). For a definitive historical review from 1865 to 1970, see Peterson (1979). Furthermore, surgeons often reported excessive bleeding from tumors, and "serpentiginous veins" on the surface of tumors. Numerous explanations for these findings were offered in the literature. The vascularity of tumors was attributed to vasodilation, inflammation, dying tumor cells, increased tumor metabolism, overproduction of lactic acid or uric acid, or hypoxia from "tumors outgrowing

their blood supply." There were no molecular mediators of angiogenesis in these reports. One of the most common assumptions prior to 1970 was that excessive vascularity of tumors was a *side effect* of growing tumors or dying tumor cells.

In this chapter I have set out to review: (1) the original concepts; (2) the development of bioassays; (3) the discovery of the first angiogenesis regulatory molecules; and (4) the genetic basis upon which modern research in tumor angiogenesis has been founded. I briefly outline how these studies in tumor angiogenesis evolved to a larger field of angiogenesis research and to clinical validation. Research in tumor angiogenesis itself is now a wide-ranging field described in more than 30 books and monographs. Angiogenesis inhibitors alone are the subject of more than 39,000 reports in the scientific literature. The very informative chapters in this book describe recent progress and new directions in tumor angiogenesis research.

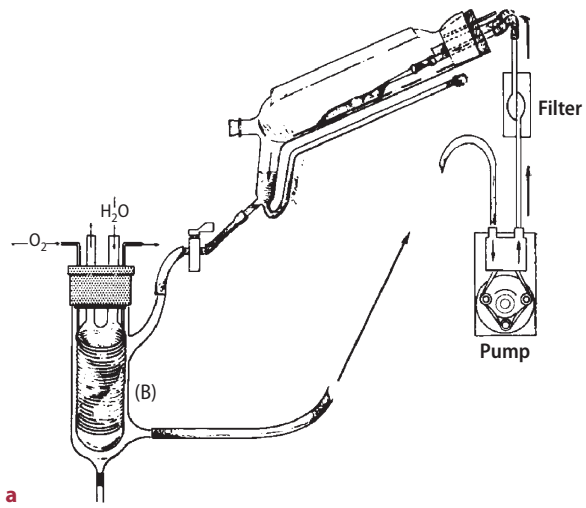
In 1962, I perfused hemoglobin solutions into the carotid artery of rabbit and canine thyroid glands isolated in glass chambers during a study to develop blood transfusion substitutes for the US Navy (Fig. 1.1). When murine melanomas were implanted into the glands, tiny tumors grew up to ~1 mm^3, but did not undergo further expansion (Folkman et al. 1962, 1963, 1966; Folkman and Gimbrone 1971). When these microscopic tumors were transplanted to syngeneic mice, the tumors grew to more than 1,000 times their original volume in the perfused thyroid gland. Large tumors in mice were highly neovascularized, in contrast to tumors in the isolated organs, which were viable, but not vascularized. This difference suggested that in the absence of neovascularization, tumors would stop growing at a volume in the range of ~1 mm^3, most likely due to the limits of oxygen diffusion.

The hemoglobin solution was acellular; it did not contain red cells, leukocytes, or platelets. When we subsequently perfused isolated thyroid glands with platelet-rich medium, endothelial vascular integrity was preserved (Gimbrone et al. 1969). This result implied that absence of platelets was a possible mechanism for lack of neovessels in the earlier experiments of thyroid glands perfused with hemoglobin solution. Today it is known that platelets contain endothelial mitogens and survival factors such as bFGF and VEGF (Folkman et al. 2001).

1.1.1 Hypothesis That Tumor Growth is Angiogenesis-dependent

In 1971 I first published a hypothesis that "tumor growth is angiogenesis dependent" (Folkman 1971). This paper also: (1) predicted that most tumors would be unable to grow beyond a microscopic size of 1–2 mm^3 without recruiting new blood vessels; (2) introduced the concept that tumors would be found to secrete diffusible angiogenic molecules; (3) described a model of tumor dormancy due to blocked angiogenesis; (4) proposed the term antiangiogenesis to mean the prevention of new capillary sprouts from being recruited into an early tumor implant; (5) predicted the future discovery of angiogenesis inhibitors; and (6) advanced the idea that an antibody to a tumor angiogenic factor (TAF), could be an anti-cancer drug. The hypothesis itself was formulated not only from the 1962 experiments of restricted tumor growth in the absence of neovascularization in isolated organs, but also from experiments completed in my laboratory during 1971, and a year later. We had demonstrated tumor dormancy at a microscopic size due to blocked angiogenesis of tumors in the aqueous humor of the anterior chamber of the rabbit eye (Gimbrone et al. 1972). We also had demonstrated DNA synthesis by autoradiography, induced in endothelial cells of a tumor bed in vivo (Cavallo et al. 1972). The concept that tumor growth is angiogenesis-dependent was extended and supported in subsequent invited reviews (Folkman 1974a, 1974b, 1975, 1978; Folkman et al. 1974; Brem et al. 1975; Folkman and Gimbrone 1975; Folkman and Klagsbrun 1975; Folkman and Cotran 1976).

It has been stated that the 1971 *New England Journal of Medicine* paper initiated the field of angiogenesis research (Folkman 1971). However, this field



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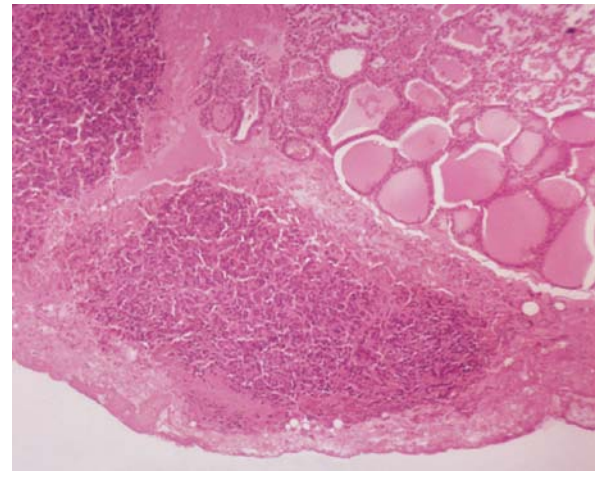

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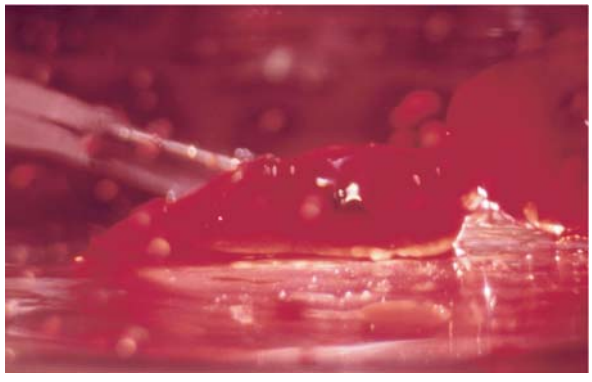

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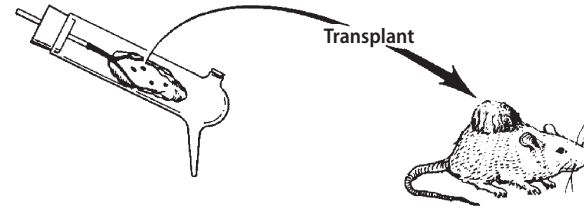

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Fig. 1.1a–d. Perfusion of isolated canine thyroid gland through the carotid artery with hemoglobin solution. **a** The perfusion circuit includes a silicone rubber oxygenator and a roller pump with silicone rubber tubing. **b** Transilluminated canine thyroid gland in the perfusion chamber, containing a transplanted murine melanoma that grew to ~1 mm^3 and stopped expanding. **c** Histologic section of thyroid gland showing viable tumor embedded among viable thyroid follicles. **d** When the tiny, non-expanding tumor was transplanted to a syngeneic mouse, it grew to more than 1000 times its initial volume in the perfused thyroid gland. The large tumor in the mouse was highly neovascularized, in contrast to its precursor tumor which was not vascularized. The hemoglobin solution was acellular, i.e., it did not contain red cells, leukocytes, or platelets. Reprinted from Folkman (2007) with permission of the publisher. Also, see Folkman et al. (1962, 1963)

was slow to develop. Throughout the 1970s, very few scientists believed that tumors needed new blood vessels, and there were hardly any papers from other investigators (Fig. 1.2) (see also Folkman 2007). The conventional wisdom was that tumor vascularity was non-specific inflammation. Skeptics challenged the hypothesis that tumor growth depended on angiogenesis. Reviewers complained that the conclusions of the experiments reached beyond the data. At the time the hypothesis was published, there were no bioassays for angiogenesis, no endothelial cells in long-term culture, and no angiogenesis regulatory molecules. During the 1970s, we set out to remedy these deficiencies so that reagents and methods would eventually become available to isolate and purify proangiogenic and antiangiogenic factors. These advances would be needed to find supporting evidence for the beleaguered hypothesis.

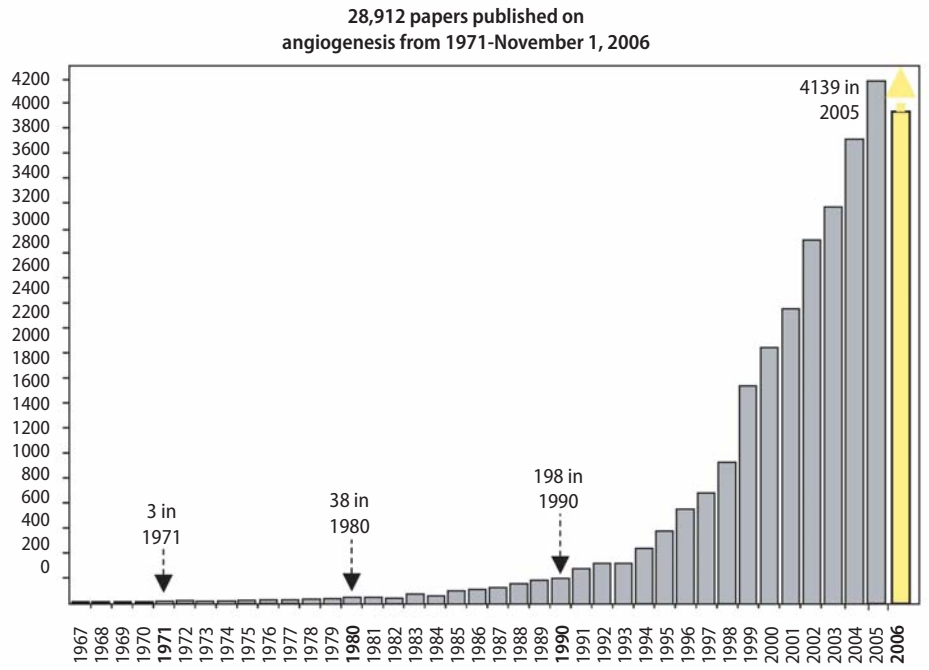


Fig. 1.2. Publications with "angiogenesis" in the title from 1967 to November 2006. There are almost 29,000 papers since the 1971 publication in the *New England Journal of Medicine*, but very few in the first 10 years thereafter

1.1.2 Development of Bioassays for Angiogenesis Research

1.1.2.1 Corneal Neovascularization

In the early 1970s, a challenging problem was how to maintain an in vivo tumor separate from its vascular bed in order to prove that tumors secreted diffusible "angiogenic" molecules. Methods for growing tumor implants on micropore filters in transparent chambers in animals had become more sophisticated (Greenblatt and Shubik 1968; Ehrmann and Knoth 1968), but it was difficult to rule out the possibility that pseudopodia of tumor cells had made contact with the underlying vascular bed. Young scientists who are beginning angiogenesis research today are incredulous at how hard it was in the 1970s to convince scientific colleagues that a diffusible angiogenic substance existed. Michael Gimbrone, a post-doctoral fellow, and I implanted tumors (of approximately 0.5 mm^3) into the stromal layers of the rabbit cornea at distances of up to 2 mm from the limbal edge (Fig. 1.3). New capillary blood vessels grew from the limbus, invaded the stroma of the avascular corneas, and reached the edge of the tumor over a period of approximately 8–10 days. When tumors were implanted beyond 3 mm from the limbus (or in the center of the rabbit cornea, which is approximately 12 mm in diameter), no neovascularization was observed (Gimbrone et al. 1974a, 1974b).

Vascularized tumors turned from white to red, grew exponentially in three dimensions, and became exophytic and protruded from the cornea within 2–3 weeks. Non-vascularized tumors in the center of the cornea expanded slowly in two dimensions, as thin, flat, translucent, intracorneal lesions until one edge extended to within ~2 mm of the limbus and recruited new blood vessels (Folkman 1978). This method demonstrated that a diffusible "angiogenic factor" existed, and that such a putative angiogenic molecule could possibly be isolated from tumors. However, when tumor extracts were

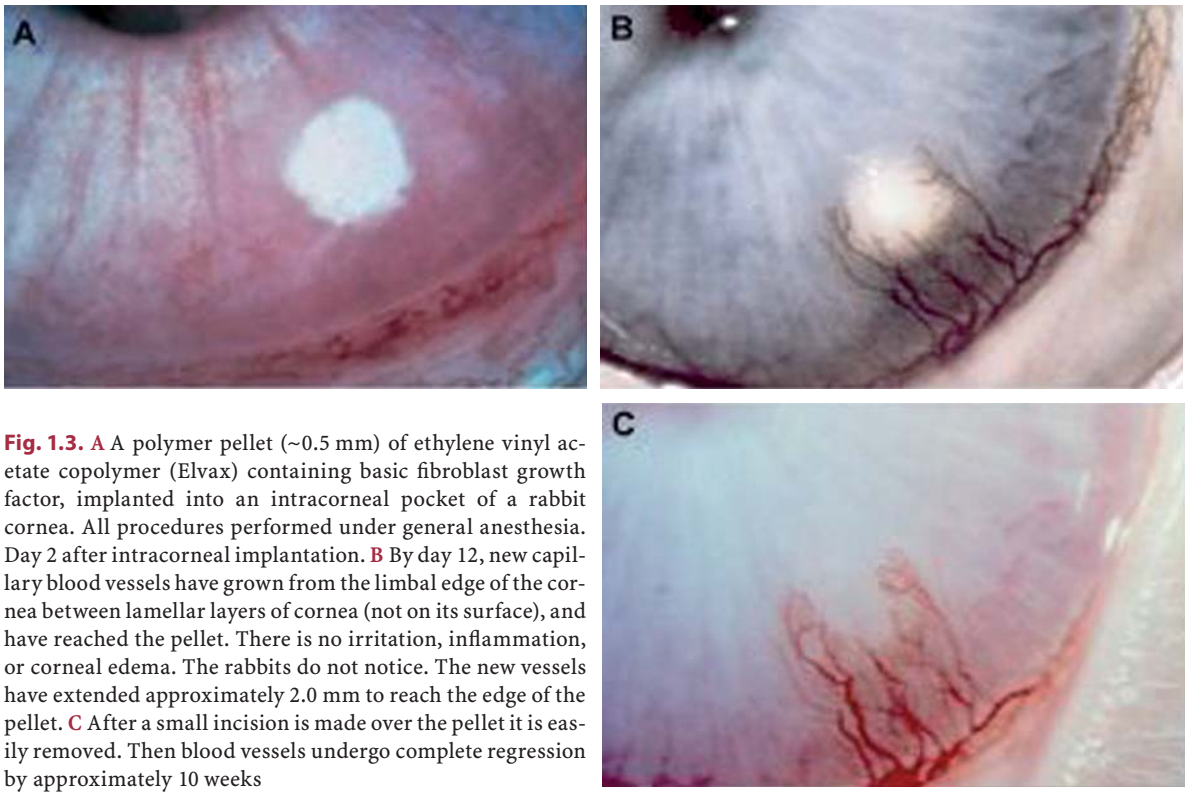


Fig. 1.3. A A polymer pellet (~0.5 mm) of ethylene vinyl acetate copolymer (Elvax) containing basic fibroblast growth factor, implanted into an intracorneal pocket of a rabbit cornea. All procedures performed under general anesthesia. Day 2 after intracorneal implantation. B By day 12, new capillary blood vessels have grown from the limbal edge of the cornea between lamellar layers of cornea (not on its surface), and have reached the pellet. There is no irritation, inflammation, or corneal edema. The rabbits do not notice. The new vessels have extended approximately 2.0 mm to reach the edge of the pellet. C After a small incision is made over the pellet it is easily removed. Then blood vessels undergo complete regression by approximately 10 weeks

implanted into the cornea to mimic a tumor implant, the extracts rapidly diffused away into the cornea. A focal steady-state concentration gradient of angiogenic activity, similar to a tumor implant, could not be established. Silicone rubber capsules that we had previously found to steadily release small molecules (< 500 Da) by diffusion through the polymer itself (Folkman and Long 1964), could not release proteins.

Robert Langer, a post-doctoral fellow, solved the problem. He dissolved the polymer polyhydroxy ethylmethacrylate (polyhema), into alcohol and added lyophilized protein. When the solvent was evaporated, the protein remained trapped in a rubbery polymeric pellet. When the pellet was implanted into the cornea, water diffused into the pellet. This caused the formation of microchannels around the protein. Protein diffused out from these channels at zero-order kinetics for weeks to months (Langer and Folkman 1976; Brown et al. 1983). Another polymer, ethylene vinyl acetate copolymer (Elvax) dissolved in ethylene chloride was also used. These polymers did not irritate the cornea. Robert Auerbach reported that the mouse cornea could also be implanted with tumors or polymer pellets (Muthukkaruppan and Auerbach 1979). This advance permitted genetic experiments, and mice are now routinely employed for corneal neovascularization bioassays.

The corneal neovascularization bioassay and the method of implanted sustained-release corneal implants have played an important role in elucidating the process of tumor angiogenesis (Fig. 1.4b).

In 1978, we reported that removal of an angiogenic stimulus from the cornea resulted in regression of neovasculature by a series of sequential morphologic events (Ausprunk et al. 1978). This finding demonstrated that newly induced neovasculature does not become "established," as was the conventional wisdom at the time. Regression of corneal neovascularization also predicted that future angiogenesis

inhibitors could possibly cause new blood vessels to regress. These results provided a compelling rationale for the future attempts to discover angiogenesis inhibitors, and for the efforts to develop them.

These results also helped in the debate with certain colleagues who argued that the search for an angiogenesis inhibitor was a "fruitless exercise." Lymphangiogenesis was first dissociated from angiogenesis in the cornea; the two processes were induced by different concentrations of bFGF, and inhibited by different mechanisms (Chang et al. 2004).

Recently, corneal avascularity has been shown to be due to soluble VEGF receptor-1 (sflt-1), which binds VEGF and is highly concentrated in the cornea (Ambati et al. 2006). In the cornea, sflt-1 is expressed predominantly by corneal epithelium. The corneas of *corn1* mice are deficient in slft-1 and are spontaneously neovascularized (Smith et al. 1996). These mice could possibly be used to test antiangiogenic activity of systemically administered molecules, or to develop a standardized unit of antiangiogenic activity to compare different inhibitors.

1.1.2.2 Vascular Endothelial Cells In Vitro

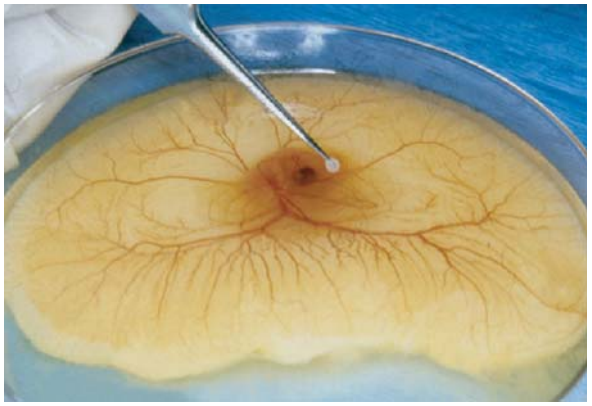
Before the 1970s, it was thought that vascular endothelial cells could not survive in vitro, to say nothing of their long-term passage. In 1973, Gimbrone in my laboratory (Gimbrone et al. 1973, 1974a, 1974b) and Eric Jaffe's laboratory at Cornell (Jaffe et al. 1973), were independently the first to report long-term passage of vascular endothelial cells (from human umbilical veins) in vitro. The first long-term passage of cloned capillary endothelial cells came later and was reported in 1979 (Folkman et al. 1979), followed by the demonstration of angiogenesis in vitro (Folkman and Haudenschild 1980) (Fig. 1.4d). Endothelial cultures also facilitated discovery of endothelial mitogens and suppressors of endothelial cell proliferation that could then be tested in vivo for pro- or antiangiogenic activity. However, endothelial cell cultures were still not useful for guiding purification of endothelial mitogens until we found that vascular endothelial cells become refractory to virtually any mitogen once the cells had reached confluence, in contrast to confluent fibroblasts, which still responded to mitogens (Haudenschild et al. 1976).

It became clear that vascular endothelial cells were among the most stringently regulated cells at high cell density. This report deserves emphasis because those who are unaware of it today risk being misled by their experiments as researchers were 30 years ago. Until the mid-1970s, it was conventional practice to guide the purification of growth factors with fibroblast cell cultures. Fibroblasts (3T3 cells) were grown to confluence. When a putative growth factor was added, one or two additional rounds of DNA synthesis ensued. However, when *endothelial* cells were used to guide purification of endothelial mitogens, confluent endothelial cells did *not* undergo additional DNA synthesis, and investigators assumed that their tumor extracts were inactive. Therefore, it was necessary to incubate endothelial cells with a putative mitogen when the cells were sparse, not when they were confluent – a critical detail – and just the opposite of employing 3T3 fibroblasts to purify a mitogen for fibroblasts.

Changes in cell shape during confluence in vitro were later found to be a central mechanism of suppression of DNA synthesis in endothelial cells (Folkman and Moscona 1978). Shape control of DNA synthesis (Folkman and Moscona 1978) appears to have eluded discovery until the advent of successful in vitro growth of vascular endothelial cells. This mechanism was further elucidated by Donald Ingber, who showed how changes in cell shape can signal through integrins to regulate gene expression and DNA synthesis. He went on to develop an entirely new field of investigation of cell biology based on the mechanisms by which mechanical forces modify DNA synthesis and gene expression (Ingber et al. 1987; Ingber and Folkman 1989a, 1989b, 1989c; Huang and Ingber 2005). The experiments of Mina Bissell on cell shape and differentiation of function also yielded information on the role of cell shape in cell growth (Bissell et al. 1977).

When angiogenesis in vitro was demonstrated (Folkman and Haudenschild 1980) it became possible to elucidate the morphologic and molecular events of lumen formation in microvessels (Kuo et al. 2001).

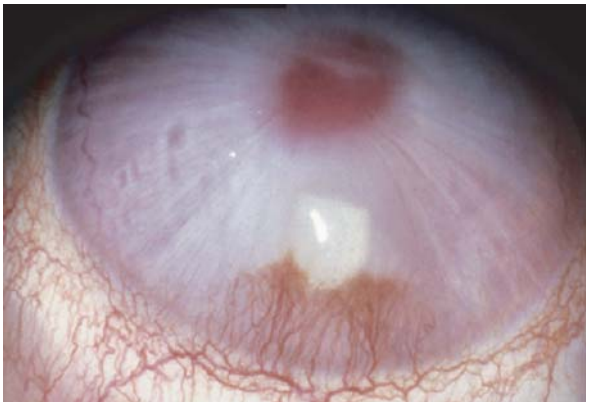
Shell-less **chick embryo**:
Choriallantoic membrane



a

1974 Devel Biology, 41:391

1) **Cornea micropocket** and
2) Sustained release polymers



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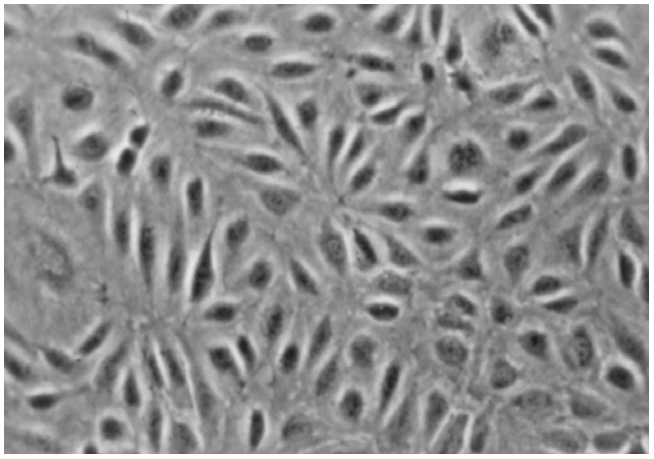
1974 JNCI, 52:514, **1976** Nature, 236:797

Cloned capillary **endothelial cells**



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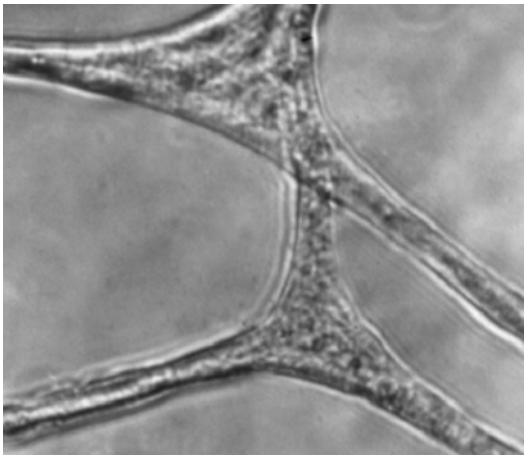
1973 Series Haematol 6:453, **1979** Proc Natl Acad Sci 76:5217

Angiogenesis in vito



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1980 Nature, 288:551

Fig. 1.4a–d. Bioassays for angiogenesis developed during the 1970s. **a** The chick embryo chorioallantoic membrane. **b** Corneal neovascularization stimulated by an implanted polymer releasing an angiogenic protein. **c** Capillary endothelial cells in vitro. **d** Angiogenesis in vitro

The development of the shell-less chick embryo that could be routinely cultured in large numbers from day 3 to at least day 18 permitted the growing chorioallantoic membrane to be employed as a vascularized substrate to test antiangiogenic and angiogenic molecules, starting from day 5 (Auerbach et al. 1974; Ausprunk et al. 1974) (Fig. 1.4a). It was demonstrated that normal and neoplastic tissues had quantitatively different mechanisms of vascularization after being grafted to the chorioallantoic membrane (Ausprunk et al. 1975). Therefore, 7 years before angiogenic molecules were discovered, these experiments showed that the angiogenic activity of tumor tissue was significantly greater than that of equivalent normal tissue. Today, this difference is understood in part by the ratio of expression or generation of pro- and antiangiogenic proteins by a tumor.

Ribatti et al. improved the chick chorioallantoic membrane bioassay (Ribatti et al. 1997a), and also studied the modification of angiogenesis on the chorioallantoic membrane, by heparin (Ribatti et al. 1995), bFGF (Ribatti et al. 1997b), and interferon alpha (Ribatti et al. 2002). Nguyen and Folkman (Nguyen et al. 1994), converted the chick chorioallantoic membrane to a quantitative angiogenesis bioassay by implanting the test protein in a white opaque gel sandwiched between two squares of nylon mesh. New microvessel sprouts grew *vertically* through the mesh. When separate sprouts protruded vertically through the mesh and anastomosed to form a capillary loop enveloping a nylon thread, this was compelling proof that the microvessels were new (and not just dilated vessels). They could be accurately quantified by the ratio of squares of mesh containing a microvessel to empty squares of mesh.

The chick embryo chorioallantoic membrane also made it possible to dissociate the early pre-angiogenic phase of tumor growth from the angiogenic growth phase (Knighton et al. 1977). This evidence further supported the concept that tumor growth was restricted in the absence of angiogenesis.

For recent reviews of in vivo models of angiogenesis see Murray (2001) and Norrby (2006).

1.1.3 Discovery of Angiogenic Molecules

It first became possible to isolate angiogenic molecules when the chick embryo chorioallantoic membrane was employed together with bioassays based on endothelial cell migration and proliferation in vitro, and with corneal neovascularization in vivo. At best, these were semiquantitative bioassays, but served the purpose of isolating and purifying angiogenic proteins.

In 1984, Yuen Shing and Michael Klagsbrun in my laboratory employed heparin-affinity chromatography to isolate and purify to homogeneity the first angiogenic endothelial cell mitogen from a tumor (Shing et al. 1984, 1985; Folkman and Klagsbrun 1987). When this protein was subsequently purified from bovine pituitary and sequenced by Esch et al. (1985), it proved to be basic fibroblast growth factor (bFGF). FGF had previously been isolated from the pituitary by Gospodarowicz and shown to be mitogenic for 3T3 fibroblasts and for vascular endothelial cells (Gospodarowicz 1974). It was also a survival factor for these cells (Gospodarowicz 1974; Gospodarowicz et al. 1976), but had not been completely purified.

By 1989, Rosalind Rosenthal in my laboratory had isolated and purified to homogeneity a second angiogenic protein from a different tumor that did not express bFGF. We had not yet sequenced this new protein, when we received a call from Napoleone Ferrara of Genentech, who had purified a novel angiogenic protein from pituitary cells. He had heard about the new angiogenic protein in our lab, and suggested that the two labs compare their proteins because Ferrara had already sequenced his protein. The two proteins were identical, and were named vascular endothelial growth factor (VEGF) by Ferrara. Ferrara's report was published in mid-1989 (Ferrara and Henzel 1989), and our paper reporting the first VEGF from a tumor was published in 1990, with Ferrara as a co-author (Rosenthal et al. 1990). In 1983, Senger and Dvorak had purified a vascular permeability factor (VPF) from tumor cells that promoted accumulation of ascites (Senger et al. 1983). By 1990, it was clear that VPF was also the same as VEGF. Thus, VEGF had an auspicious start, having been purified from three different sources, but first sequenced in Ferrara's laboratory.

Many other proangiogenic molecules have since been discovered and are discussed by other authors in this book. Recently, Klagsbrun discovered that neuropilin-1 is another receptor for VEGF and stimulates angiogenesis (Miao et al. 2000).

1.1.3.1 Storage of an Angiogenic Protein in Extracellular Matrix

In 1987, Israel Vlodavsky, Klagsbrun and Folkman reported that bFGF was stored in extracellular matrix, where it was bound to heparan sulfate proteoglycans (Vlodavsky et al. 1987; Folkman et al. 1988). This finding opened a new avenue of research.

Angiogenesis regulatory proteins were sequestered in extracellular matrix and basement membrane, where they were protected from degradation by their heparin affinity. This storage mechanism prevented endothelial cells in the vasculature from overexposure to these biologically active molecules. Because angiogenic molecules could be released at a site of extracellular membrane disruption, this provided a mechanism by which angiogenic activity was localized to a wound site. Angiogenesis regulatory proteins were also releasable by heparitinase and additionally by heparin-like molecules (Bashkin et al. 1989). Of interest is that Descemet's membrane in the cornea contains a high concentration of bFGF (Folkman et al. 1988).

1.1.4 Discovery of Angiogenesis Inhibitors

Throughout the 1970s, evidence that tumors were angiogenesis-dependent was based almost solely on blocking angiogenesis by mechanical separation of tumors from their nearest vascular bed. More compelling evidence would depend on pharmacologic blockade of angiogenesis. By 1980, the availability of bioassays and of angiogenic proteins facilitated the search for molecules with *anti*angiogenic activity.

Bruce Zetter in the Folkman lab reported that very low concentrations of interferon alpha specifically suppressed migration of endothelial cells in vitro (Brouty-Boye and Zetter 1980). Dvorak and Gresser (1989) and also Sidky and Borden (1987) then reported that interferon alpha inhibited angiogenesis in experimental animals. In 1988, I received a call from Carl White, a pulmonary specialist at Denver Jewish Hospital who was caring for a teenager who had progressive hemangiomatosis of both lungs and hemoptysis. The patient had failed all therapy for this disease, which up to that time was fatal.

I suggested a trial of frequent low-dose interferon alpha, based on our experimental elucidation of its antiendothelial properties and its antiangiogenic activity in animals (Dvorak and Gresser 1989), and because of its FDA approval. With very low doses of interferon alpha every second day, the patient made a complete recovery after several months and was treated for an additional 5 years (by subcutaneous self-injection), while he completed his education (White et al. 1989; Folkman 1989). He has a normal chest film and is in good health today, 18 years later. This is the first recorded case of antiangiogenic therapy. It was subsequently found in other patients that these low doses are antiangiogenic, but are neither cytotoxic nor immunosuppressive.

Stephanie Taylor and I showed that two antagonists of heparin, protamine and platelet factor 4, blocked angiogenesis in the chick embryo and produced dose-dependent avascular zones in the growing chorioallantoic membrane (Taylor and Folkman 1982).

Until the early 1980s, corticosteroids were classified as either glucocorticoid or mineralocorticoid. With Rosa Crum and Sandor Szabo, we discovered a third class of steroids, named "angiostatic steroids" (Crum et al. 1985). The most potent of these was tetrahydrocortisol, a pure angiogenesis inhibitor that had no glucocorticoid or mineralocorticoid activity. (Tetrahydrocortisol is currently in phase III clinical trials for the treatment of macular degeneration). In studies of the mechanism of action of angiostatic steroids, we found that they were potentiated by an arylsulfatase inhibitor (synthesized by Professor E.J. Corey), that inhibited desulfation of endogenous heparin (Chen et al. 1988). In 1994, after Fotsis et al. reported that the steroid 2-methoxyestradiol inhibited angiogenesis (Fotsis et al. 1994), Robert D'Amato in my lab reported that this angiostatic steroid inhibited tubulin polymerization by interacting at the colchicine site (D'Amato et al. 1994).

Eleven angiogenesis inhibitors were discovered in the Folkman laboratory (Table 1.1). Five of them were previously unknown molecules (i.e., TNP-470 [a synthetic analogue of fumagillin], angiostatin, endostatin, caplostatin, and cleaved antithrombin III). Of the known molecules in which antiangiogenic activity was a new function (i.e., platelet factor 4, interferon alpha, etc.), eight are *endogenous* angiogenesis inhibitors in the blood or in extracellular matrix. Many other laboratories joined this research effort (Auerbach and Auerbach 1994), and at the time of writing there are 28 known endogenous angiogenesis inhibitors (Folkman 2004; Nyberg et al. 2005).

1.1.4.1 Angiostatin and Endostatin: Their Role in Suppression of Metastasis by a Primary Tumor

Angiostatin (O'Reilly et al. 1994) and endostatin (O'Reilly et al. 1997; Boehm et al. 1997), are the first endogenous angiogenesis inhibitors found to be internal fragments of larger proteins which themselves do not regulate angiogenesis. Surgeons had long observed that the removal of certain primary tumors was followed by rapid growth of metastases at remote sites. This phenomenon was assumed to be due to release of tumor cells into the circulation during surgical removal of the primary tumor. This explanation, however, was inconsistent with the explosive growth of metastasis after removal of a primary tumor in humans and animals. An alternative hypothesis, that a primary tumor could suppress its remote metastasis, seemed counterintuitive, until the discovery that certain tumors could enzymatically cleave angiostatin (O'Reilly et al. 1994) from plasminogen, or endostatin from collagen XVIII (O'Reilly et al. 1997; Boehm et al. 1997). Expression of proangiogenic proteins, such as VEGF, within the primary tumor exceeded the generation of antiangiogenic proteins such as angiostatin or endostatin, resulting in vascularization and growth of the primary tumor (Roy et al. 2004; Cao et al. 1998). However, the angiogenesis inhibitors accumulate in the circulation because of their longer half-life.

Table 1.1. Molecules with antiangiogenic activity published from the Folkman laboratory between 1980 and 2005

Year	Molecule(s)	Reference
1980	Interferon α/β, new activity	Brouty-Boye and Zetter 1980
1982	Platelet factor 4, protamine	Taylor and Folkman 1982
1985	Angiostatic steroids	Crum et al. (Folkman) 1985
1990	TNP-470, a fumagillin analogue	Ingber et al. (Folkman) 1990
1994	Angiostatin	O'Reilly et al. (Folkman) 1994
1994	Thalidomide	D'Amato et al. (Folkman) 1994
1994	2-Methoxyestradiol (*see footnote*)	D'Amato et al. (Folkman) 1994
1997	Endostatin	O'Reilly et al. (Folkman) 1997
1999	Cleaved antithrombin III	O'Reilly et al. (Folkman) 1999
2002	3-Amino thalidomide	Lentzsch et al. (D'Amato) 2002
2003	DBP-maf (*see footnote*)	Kisker et al. 2003
2005	Caplostatin	Satchi-Fainaro et al. (Folkman) 2005

Ten of these molecules were discovered either as new molecules (e.g., angiostatin and endostatin), or as new functions of known molecules (e.g., interferon alpha and the angiostatic steroid tetrahydrocortisol). *2-Methoxyestradiol* was first found to be an angiogenesis inhibitor by Fotsis et al. (1994). Subsequently, D'Amato et al. (1994) reported its mechanism as an inhibitor of tubulin polymerization by acting at the colchicine site. *DBP-maf* was first discovered by Yamamoto and Kumashiro (2003) and found to be an angiogenesis inhibitor by Kisker et al. (2003). Eight of these are endogenous angiogenesis inhibitors (interferon alpha, platelet factor 4, angiostatic steroids (including 2-methoxyestradiol and tetrahydrocortisol), angiostatin, endostatin, cleaved antithrombin III, and DBP-maf (vitamin D binding protein-macrophage activating factor). (From Folkman J, *Nature Reviews Drug Discovery* 2007)

Removal of the primary tumor leads to a decrease in circulating inhibitor, over a period of about a week, as revealed by incubations of proliferating endothelial cells with serum samples taken each day after removal of the primary tumor (O'Reilly et al. 1994; Cao et al. 1998). Microscopic metastases become angiogenic and begin rapid growth within 4–5 days after removal of the primary tumor (Holmgren et al. 1995). Systemic replacement of the angiogenesis inhibitor lost after removal of the primary tumor, or after its regression by ionizing irradiation, prevents growth of remote metastases (Camphausen et al. 2001). Kalluri et al. have discovered other endogenous angiogenesis inhibitors that are internal fragments of collagens (Kamphaus et al. 2000; Hamano and Kalluri 2005).

1.1.5 The Switch to the Angiogenic Phenotype

Douglas Hanahan and I found that spontaneous murine tumors arising in his transgenic mice first appeared in a non-angiogenic state at a microscopic size of less than 1–2 mm^3. A small percentage (~10%) then switched to the angiogenic phenotype after a predictable time period (for example 6–7 weeks after birth for transgenic islet cell carcinomas) (Hanahan and Folkman 1996). The angiogenic tumors recruited new blood vessels and underwent rapid expansion of tumor mass. Subsequent studies revealed that the angiogenic switch resulted from a change in the net balance of positive and negative regulators of angiogenesis, i.e., increased expression of VEGF and other proangiogenic proteins accompanied by decreased expression of thrombospondin-1 and other antiangiogenic proteins.

1.1.5.1 The Angiogenic Switch in Human Tumors in Immunodeficient Mice

A similar switch to the angiogenic phenotype has been demonstrated in human cancers transplanted to SCID immunodeficient mice (Achilles et al. 2001; Udagawa et al. 2002; Almog et al. 2006; Naumov et al. 2006a, 2006b). Non-angiogenic and angiogenic tumor cells were cloned from human cancers obtained from discarded tumor specimens in the operating room, or from the American Tissue Culture Collection. These cells form either non-angiogenic or angiogenic tumors when injected subcutaneously, or into orthotopic sites such as the mammary fat pad in mice. Non-angiogenic tumors remain dormant at a microscopic size of less than 1 mm^3. They do not recruit blood vessels. The angiogenic tumors become highly neovascularized and grow rapidly. Non-angiogenic tumor cells proliferate at approximately the same rate as angiogenic tumor cells. In contrast, the apoptotic rate of tumor cells in the non-angiogenic tumors is significantly higher than that of tumor cells in angiogenic tumors. The non-angiogenic tumors remain at a microscopic size for a predictable period of time (from months to over a year), before switching to the angiogenic phenotype. The switch itself can be accurately quantified by bioluminescence if the tumor cells are infected with luciferase. For example, 95% of non-angiogenic human liposarcomas reproducibly switched to the angiogenic phenotype at a median of ~133 days ± 2 weeks (Almog et al. 2006). In contrast, approximately 60% of human breast cancers became angiogenic at a median of 80 days. A human osteogenic tumor did not switch to the angiogenic phenotype for more than a year, and then only 5% of tumors become angiogenic (Udagawa et al. 2002).

The initiating events that drive the angiogenic switch are unknown. However, a predictable, reproducible animal model now exists. Preliminary data have begun to fill in pieces of the puzzle. The angiogenic switch can be significantly accelerated by transfecting non-angiogenic human tumor cells with Ras (Udagawa et al. 2002). This induces a 38% increase in VEGF expression, and a 50% suppression of thrombospondin-1 expression within approximately 1 week in a human osteogenic sarcoma that in the absence of Ras transfection would not become angiogenic spontaneously for more than a year (Udagawa et al. 2002). Analyses of gene expressions before and after the angiogenic switch are under way for five different human cancers. At the time of writing, the mechanism of the angiogenic switch

in human cancer is unknown. If it were understood how to prevent or reverse the angiogenic switch, this could become a novel conceptual advance in controlling cancer.

1.1.6 U-shaped Dose–Efficacy Curve of Angiogenesis Inhibitors

Singh et al., in Isaiah Fidler's laboratory, first reported that low-dose interferon alpha inhibits angiogenesis by suppressing expression of bFGF from human cancer cells (Singh et al. 1995). Fidler and his associates further showed that dose–efficacy of the antiangiogenic activity of interferon alpha could be expressed as a biphasic curve that was U-shaped (Slaton et al. 1999). Higher doses are less effective than low doses (Fig. 1.5a).

Since these reports, other angiogenesis inhibitors have also shown a biphasic, U-shaped dose–response curve in experimental tumor models. These include rosiglitazone (Panigrahy et al. 2002), endostatin protein therapy (Celik et al. 2005) (Fig. 1.5b), and endostatin gene therapy (Tjin Tham Sjin et al. 2006). In fact, when endothelial cells are incubated in vitro with endostatin at increasing concentrations, gene expressions also reveal a U-shape (Abdollahi et al. 2004). Furthermore, anti-cancer cytotoxic chemotherapy, administered at frequent *low* doses, is more effective in tumor-bearing mice than high doses of chemotherapy administered less frequently. Low-dose frequent chemotherapy, also called 'antiangiogenic therapy' or 'metronomic therapy,' is more effective against mouse tumors that have become resistant to high-dose chemotherapy (Browder et al. 2000; Klement et al. 2000; Hanahan et al. 2000).

Conventional cytotoxic chemotherapy is generally administered on a linear dose–efficacy curve and is guided by the concept of "maximum tolerated dose." In contrast, if the experimental finding of a biphasic dose–response curve for angiogenesis inhibitors is validated in the clinic in the future, "maximum tolerated dose" (MTD) may become less useful for administering antiangiogenic therapy, and specific biomarkers of antiangiogenic activity may become more useful.

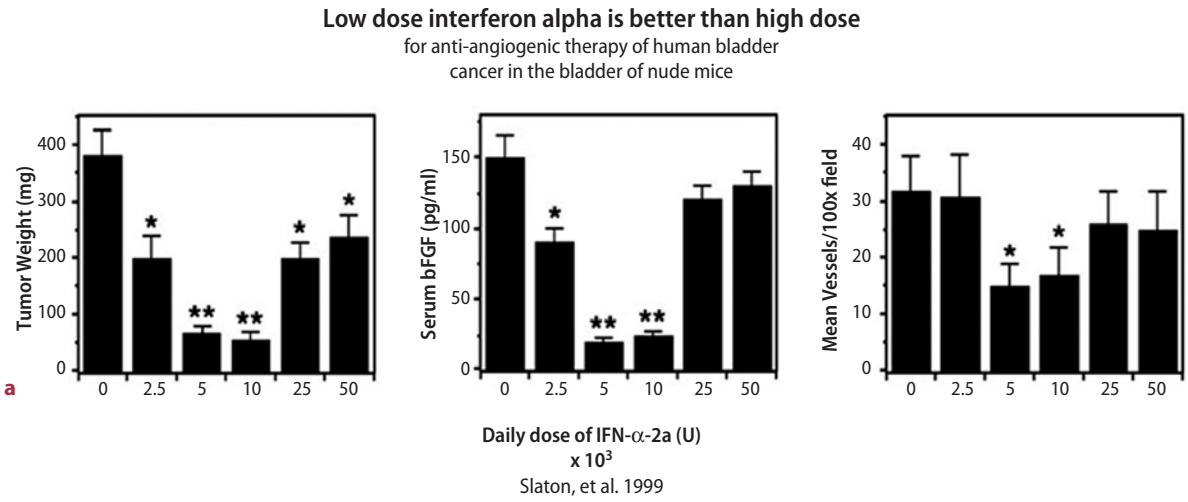
The mechanism of the biphasic response of vascular endothelium to different angiogenesis inhibitors is unclear, but its physiological function may be, in part, to protect vascular endothelium from surges in plasma concentrations of endothelial regulatory molecules.

1.1.7 The Platelet Angiogenesis Proteome

Platelets may also function as a circulating storage depot that protects vascular endothelium from surges in plasma concentrations of angiogenesis regulatory molecules. Platelets have been reported to be associated with both positive and negative regulators of angiogenesis (Folkman et al. 2001). Klement et al. reported that platelets can continuously scavenge angiogenesis regulatory molecules from plasma and sequester them within the alpha granules of platelets (Klement et al. 2004, 2006). Angiogenesis regulatory proteins sequestered by platelets are not completely released into serum during coagulation. Therefore, serum levels of angiogenesis regulatory proteins may not reflect the total content of these proteins in the blood. For example, Avastin (bevacizumab) administered to patients is taken up by platelets, where it binds VEGF and neutralizes its angiogenic activity (Verheul et al. 2006).

This new platelet biology also explains why pharmacodynamics of an angiogenic protein such as VEGF based only on serum may not always correlate with the burden of cancer in a patient. Furthermore, platelets exclude many other proteins, such as albumin, even though some of these proteins are more concentrated in plasma. Italiano et al, recently made the surprising discovery that proangiogenic proteins are contained together in one set of alpha granules and antiangiogenic proteins are contained in a different set of alpha granules (Italiano et al. 2006). Furthermore, the angiogenesis regulatory molecules that are segregated into two types of alpha granules in platelets may be released separately.

In preliminary studies, Klement et al. showed that platelets can scavenge angiogenesis regulatory proteins that are released into plasma from microscopic human tumors in SCID mice (Klement et al. 2004, 2006).



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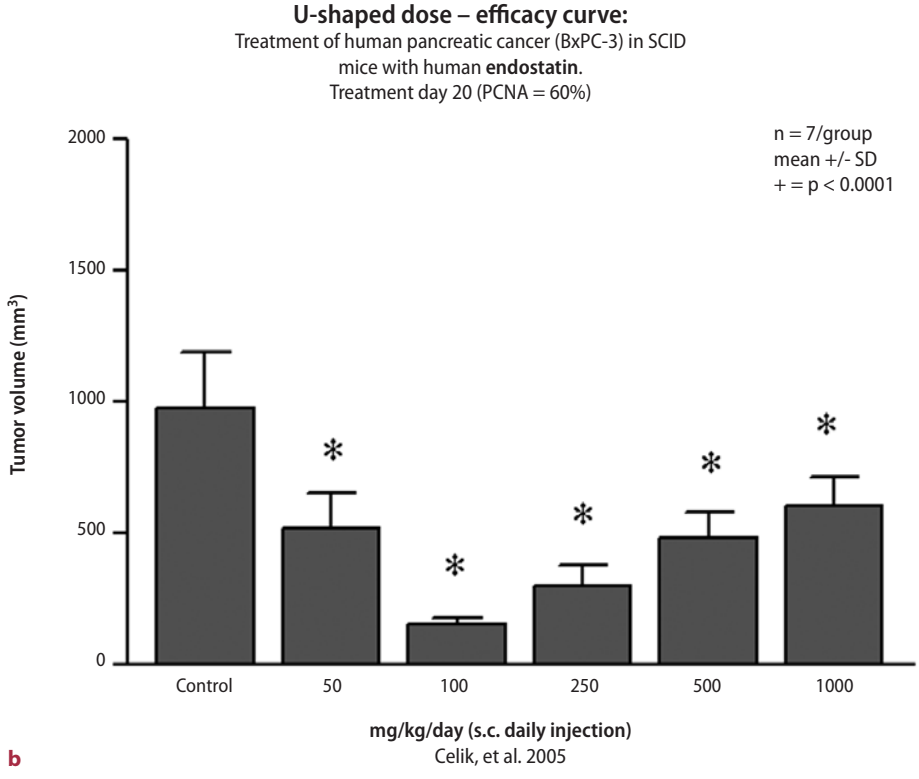

b

Fig. 1.5. a The interferon alpha suppression of bFGF expression by a human bladder cancer is revealed as a biphasic, U-shaped dose–efficacy curve. As a result, inhibition of tumor growth (tumor volume) and of microvessel density in the tumor are also U-shaped (adapted from Slaton et al. 1999). b A similar biphasic, U-shaped curve of efficacy is revealed when endostatin is administered systemically to SCID immunodeficient mice bearing human pancreatic cancer (from Celik et al. 2005)

1.1.8 Genetic Regulation of Angiogenesis

Soon after proangiogenic and antiangiogenic proteins were discovered, genes that regulate these proteins began to be elucidated. The genetic regulation of angiogenesis is itself a burgeoning field that can only briefly be discussed here. However, certain fundamental early reports deserve mention. Arbiser et al. immortalized vascular endothelial cells by transfection with the SV-40 large T oncogene (Arbiser et al. 1997). These cells formed microscopic, dormant, non-angiogenic tumors in vivo, but were not lethal to their host mice. After a second transfection, with the H-ras oncogene, the tumors became angiogenic, grew rapidly as angiosarcomas, and metastases killed the mice. This experiment, 10 years ago, revealed that the switch to the angiogenic phenotype, for at least one tumor type, was controlled by sequential transfection of two different oncogenes.

Rak et al. (2000) reported the sets of proangiogenic proteins (i.e., VEGF) up-regulated by oncogenes, and the antiangiogenic proteins down-regulated by oncogenes. D'Amato and colleagues reported a hierarchy of different mouse strains that have increasing angiogenic responsiveness to the same dose of a given angiogenic protein (Rohan et al. 2000). Interestingly, increasing angiogenic responsiveness correlated directly and precisely with increasing output of bone marrow-derived endothelial precursor cells (Udagawa et al. 2006; Shaked at al. 2005). Udagawa et al. showed that Ras transfection of human non-angiogenic tumor cells switched them to the angiogenic phenotype (Udagawa et al. 2002). Microscopic dormant tumors of which only 5% became angiogenic after ~1 year all became angiogenic at approximately 1 week after Ras transfection. The angiogenic switch was preceded by a 38% increased expression of VEGF and a 50% decreased expression of thrombospondin-1.

Folkman's laboratory in collaboration with Dean Felsher's laboratory reported that tumorigenesis by activated oncogenes is angiogenesis-dependent (Giuriato et al. 2006). The previous conventional wisdom was that inactivation of an oncogene responsible for tumorigenesis would lead to regression of the tumor (Folkman and Ryeom 2005; Chin et al. 1999; Jain et al. 2002). This phenomenon, called "oncogene addiction," implied that oncogene-driven tumor cell proliferation was necessary and sufficient to induce expansion of tumor mass, and that inactivation of the oncogene was necessary and sufficient to regress the tumor. However, Giuriato et al. showed that Myc inactivation alone did not lead to sustained tumor regression unless thrombospondin-1 expression was elevated, or p53 activity was normal. In both cases, elevated thrombospondin-1 expression is necessary to suppress angiogenesis, which then leads to tumor regression (Giuriato et al. 2006). In fact, Dameron et al. first showed that the tumor suppressor gene wild-type p53 inhibited tumorigenesis not only by inhibiting tumor cell proliferation, but also by maintaining up-regulated expression of thrombospondin-1 that inhibited angiogenesis (Dameron et al. 1994). Recently, Teodoro et al. reported an additional mechanism by which wild-type p53 could inhibit angiogenesis, and they demonstrated that p53 mobilized endostatin and tumstatin from their respective collagens XVIII and IV, through up-regulation of alpha(II) collagen prolyl-4-hydroxylase (Teodoro et al. 2006).

Endostatin controls perhaps the widest spectrum of genes that regulate angiogenesis. A gene array analysis of 90% of the human genome revealed that human endostatin down-regulates a broad spectrum of signaling pathways in human microvascular endothelium associated with proangiogenic activity including bFGF, bFGF receptors, HGF, EGFR, HIF-1 alpha, Id1, Id4, TNF-alpha receptor and others (Abdollahi et al. 2004; Folkman 2006). Endostatin simultaneously up-regulated many antiangiogenic genes including those encoding thrombospondin-1, maspin, APC (adenomatous polyposis coli) and others. In fact, endostatin can molecularly reset en masse the set of gene expressions underlying the angiogenic balance in tissues. This broad spectrum of antiangiogenic activity appears to be responsible, in part, for the lack of drug resistance observed with endostatin in experimental animals (Boehm et al. 1997).

These results may explain in part why individuals with Down syndrome are the most protected humans against cancer, having only one tenth the general incidence of all cancers except for testicular cancer and a megakaryocytic leukemia (Yang et al. 2002). Individuals with Down syndrome have a 1.6-fold circulating level of endostatin due to an extra copy of the gene for collagen XVIII on the trisomic chromosome 21 (Zorick et al. 2001).

Perhaps the most heuristic recent discovery of a genetic mechanism that regulates angiogenesis is the Notch ligand, delta-like 4, that negatively regulates formation of tip cells during angiogenesis (Ridgway et al. 2006; Noguera-Troise et al. 2006; Hellstrom et al. 2007; Lobov et al. 2007; Suchting et al. 2007). Delta-like 4 is a transmembrane ligand for Notch receptors that is expressed in arterial blood vessels and sprouting endothelium. VEGF stimulation of capillary sprouts induces delta-like 4 as a negative feed-back on endothelial tip cells (Ausprunk and Folkman 1977), which lead capillary sprout extension during angiogenesis.

1.2 Clinical Translation of Antiangiogenic Therapy

1.2.1 Low-Dose Interferon Alpha

The first use of antiangiogenic therapy in a human was in 1989 (see Sect. 1.1.4). A 12-year-old boy with life-threatening bilateral pulmonary hemangioendotheliomas was treated with low-dose daily interferon alpha for 7 months with complete regression of the pulmonary lesions and resolution of symptoms, after which the treatment was discontinued (White et al. 1989; Folkman 1989). The disease recurred within a month and the same treatment was resumed for the next 6 years. There was rapid resolution of the disease, and the patient has remained perfectly healthy and active during the past 12 years off therapy. High-grade giant cell tumors and angioblastomas, which failed all conventional therapy, have been treated with low-dose daily interferon and the results reported (Kaban et al. 1999, 2002; Marler et al. 2002; Folkman 2002). Two of 2 patients with refractory angioblastomas and 27 of 27 patients with high-grade giant-cell tumors remain off therapy and free of tumor for up to 8 years. The use of urinary bFGF as a biomarker (Nguyen et al. 1993) has been helpful in determining when to discontinue therapy (Marler et al. 2002).

1.2.2 Antiangiogenic Therapy of Cancer

Avastin (bevacizumab) is the first angiogenesis inhibitor that was tested in multi-center, placebo-controlled, double-blind clinical trials against cancer. It is also the first angiogenesis inhibitor that demonstrated significant prolongation of survival and significant prolongation of time to recurrence in patients with advanced colon cancer. At this writing eight drugs with antiangiogenic activity and varying degrees of other activities have received FDA approval for cancer therapy in the United States. Some of them have also been approved in more than 30 other countries, including the European Union and China (Table 1.2). Endostar is a modified form of endostatin approved in China for lung cancer. Twenty-two other drugs with antiangiogenic activity are in phase III clinical trials (Table 1.2). The majority of them target VEGF or one of its receptors. Therefore, currently most of the FDA-approved drugs as well as those in phase III clinical trials target a single proangiogenic protein. Approximately 30 drugs with antiangiogenic activity are in phase II clinical trials for the treatment of cancer (Table 1.3). Many of these are beginning to target more than one proangiogenic protein.

1.2.3 Mono-antiangiogenic Therapy Versus Broad-Spectrum Antiangiogenic Therapy

While approximately 60% of human cancers express VEGF, Avastin (bevacizumab) or other drugs that inhibit expression of VEGF or its receptors can be very effective against such tumors, especially if used in

combination with chemotherapy (Fig. 1.6). However, most human cancers, for example breast cancer, can also express up to five or six proangiogenic proteins (Relf et al. 1997). Therefore, eventually other angiogenic proteins (i.e., bFGF, PDGF, HGF or others) may be expressed by a tumor in which only VEGF is inhibited and give the clinical appearance of acquired "drug resistance." The "resistance" may in fact be a form of drug evasion (Casanovas 2005). Therefore, in the future, angiogenesis inhibitors may be administered in combination with other angiogenesis inhibitors, or in combination with antiangiogenic chemotherapy (metronomic, low-dose chemotherapy). Broad-spectrum angiogenesis inhibitors such as endostatin may be less susceptible to the development of "drug resistance" (Fig. 1.7). However, until these broad-spectrum angiogenesis inhibitors receive FDA approval, it is not clear whether they will encounter significantly less drug resistance. Jain has shown in both human and animals that certain angiogenesis inhibitors can "normalize" tumor blood vessels. This can lead to decreased vascular leakage, decreased intratumoral tissue pressure, temporarily increased blood flow (Jain 1988), and increased delivery of chemotherapy (Jain 1994; Carmeliet and Jain 2000). Also, other therapies delivered by the vasculature may be potentiated by antiangiogenic therapy (Jain 2001; Jain 2005; Batchelor et al. 2007).

Table 1.2. New drugs with antiangiogenic activity approved by the US Food and Drug Administration for clinical use in the USA, and by the appropriate regulatory agencies in more than 30 other countries. Velcade was approved as a proteasome inhibitor and subsequently was reported to be a potent angiogenesis inhibitor. (From Folkman J, *Nature Reviews Drug Discovery* 2007)

Date approved	Drug	Place	Disease
May 2003	Velcade (bortezomib)	USA (FDA)	Multiple myeloma
December 2003	Thalidomide	Australia	Multiple myeloma
February 2004	Avastin (bevacizumab)	USA (FDA)	Colorectal cancer
November 2004	Tarceva (erlotinib)	USA (FDA)	Lung cancer
December 2004	Avastin	Switzerland	Colorectal cancer
December 2004	Macugen	USA (FDA)	Macular degeneration
January 2005	Avastin	European Union (25 countries)	Colorectal cancer
September 2005	Endostatin (Endostar)	China (SFDA)	Lung cancer
November 2005	Tarceva	USA (FDA)	Pancreatic cancer
December 2005	Nexavar (sorafenib)	USA (FDA)	Kidney cancer
December 2005	Revlimid	USA (FDA)	Myelodysplastic syndrome
January 2006	Sutent (sunitinib)	USA (FDA)	Gastric (GIST); kidney cancer
June 2006	Lucentis	USA (FDA)	Macular degeneration
June 2006	Revlimid	USA (FDA)	Multiple myeloma
August 2006	Lucentis	Switzerland	Macular degeneration
September 2006	Lucentis	India	Macular degeneration
October 2006	Avastin	USA (FDA)	Lung cancer
November 2006	Lucentis	EU (provisional approval)	Macular degeneration

Table 1.3. Drugs with antiangiogenic activity and varying degrees of other activities in phase III trials for the treatment of cancer. (From Folkman J, *Nature Reviews Drug Discovery* 2007)

Agent	Target
AG3340 (Prinomastat) (Agouron Phamaceuticals)	MMP inhibitor
Avastin (Genentech)	VEGF
AZD2171 (AstraZeneca)	VEGFR-1, -2, -3, PDGFR
BMS-275291 (Bristol Myers Squibb)	MMP inhibitor
CCI-779 (Wyeth)	VEGFR, MTOR inhibitor
Ceflatonin (homoharringtonine) (ChemGenex)	Downregulates BEG in leukemic cells
Celebrex (celecoxib) (Pfizer)	Increases endostatin
GW786034 (pazopanib) (GlaxoSmithKline)	VEGFR
LY317615 (Enzastaurin) (Eli Lilly)	VEGF
Neovastat (Benefin/AE941) (Aetema Zentaris)	VEGFR-2, MMP inhibitor
Nexavar (sorafenib/BAY439006) (Bayer/Onyx)	VEGFR-2, PDGFR-beta
PTK787 (vatalanib) (Novartis)	VEGFR-1, -2, PDGFR
RAD001 (everolimus) (Novartis)	VEGFR, MTOR
Revlimid (lenalidomide/CC5013) (Celgene)	VEGF, precursor endothelial cells
Suramin (NCI)	IGF-1, EGFR, PDGFR, TGF-b; inhibits VEGF & bFGF
Sutent (SU11248) (Pfizer)	VEGFR-1, -2, -3, PDGFR
Tarceva (OSI774/erlotinib) (Genentech/OSI)	HER1, EGFR
Tetrathiomolybdate (TM) (Univ. of Michigan)	VEGF, Copper chelator
Thalidomide (Celgene Corporation)	VEGF, precursor endothelial cells
VEGF Trap (Regeneron Pharm.)	VEGF
Velcade (PS341/bortezomib) (Millennium Pharm.)	VEGF
ZD6474 (Zactima/vandetanib) (AstraZeneca)	VEGFR-2, EGFR

1.2.4 Treatment of Neovascular Age-related Macular Degeneration

Research in tumor angiogenesis became the basis in part for the current antiangiogenic therapy of age-related macular degeneration by two FDA-approved drugs, Macugen (pegaptanib) and Lucentis (ranibizumab), and off-label use of Avastin. In a series of publications beginning in 1993, Folkman and his collaborators Anthony Adamis, Patricia D'Amore, and Joan Miller demonstrated that VEGF was the major mediator of ocular neovascularization in the non-human primate and also reported correlative evidence for humans. In 1993 they reported that VEGF was synthesized and secreted by human retinal cells (Adamis et al. 1993). In 1994, they showed that iris neovascularization correlated spatially and temporally with intraocular VEGF levels (Miller et al. 1994). Moreover, retinal VEGF expression was shown to be upregulated in ischemic retina. Also in 1994, they reported that the vitreous of human eyes with

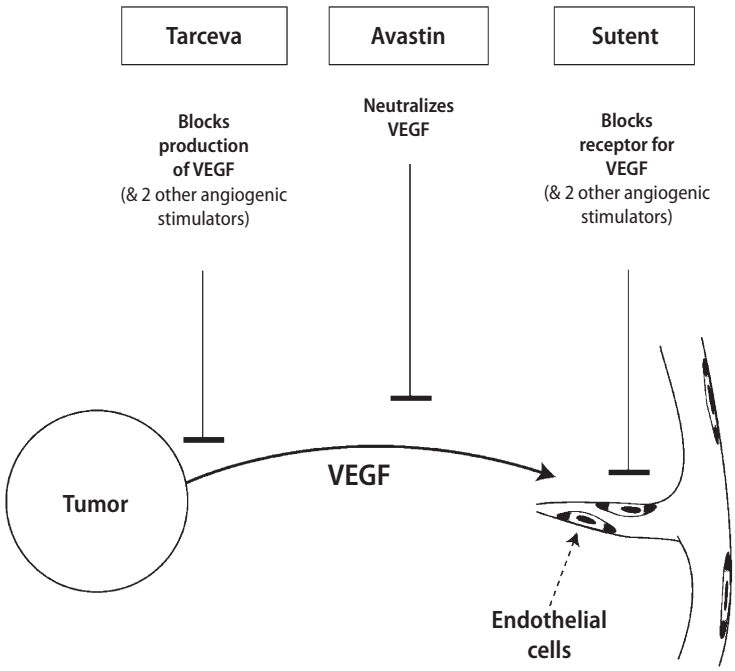

Fig. 1.6. The three general mechanisms of angiogenesis inhibitors that block VEGF: *left*, inhibition of tumor cell expression of VEGF; *center*, inhibition of the ligand; *right*, inhibition of the endothelial receptor(s) for VEGF. (From Folkman J, *Nature Reviews Drug Discovery* 2007)

proliferative diabetic retinopathy contained significantly increased VEGF levels (Adamis et al. 1994). Aiello et al., also reported high VEGF in diabetic vitreous. These were among the first papers in the scientific literature to characterize the role of VEGF in eye disease. In 1995, Folkman's laboratory, in collaboration with Napoleone Ferrara (of Genentech), reported that VEGF was the major endothelial mitogen made by hypoxic retinal cells (Shima et al. 1995). The Folkman laboratory then collaborated with Adamis, D'Amore, Miller, and Ferrara to prove that intravitreal injection of a neutralizing antibody to VEGF (the precursor to Avastin, produced by Ferrara at Genentech), inhibited retinal ischemia-associated neovascularization in a non-human primate (Adamis et al. 1996).

These findings led to the development of Macugen (pegaptanib), an anti-VEGF aptamer, by Eyetech and Lucentis, an anti-VEGF Fab fragment (ranibizumab), by Genentech. Macugen was approved by the FDA in 2004 for the treatment of age-related macular degeneration. Lucentis was approved in 2006. The very significant improvements in eyesight of patients with age-related macular degeneration have been described by Stone (2006).

1.3 Future Directions

As angiogenesis inhibitors come to be used in combinations, or together with other anti-cancer modalities, it is possible that cancer may eventually be treated as a "chronic manageable disease" (Folkman quoted in Ezzell 1998).

If biomarkers in blood or urine can be developed to achieve the accuracy of detecting recurrent cancer as early as serum calcitonin can detect recurrent medullary thyroid cancer, then it may eventually be possible to guide cancer therapy by biomarkers. For example, could angiogenesis-based biomarkers in blood (e.g., in platelets), or in urine (Roy et al. 2004), be used to detect recurrent cancer years before symptoms have appeared, or even before tu-

Table 1.4. Drugs with antiangiogenic activity and varying degrees of other activities in phase II trials for the treatment of cancer. (From Folkman J, *Nature Reviews Drug Discovery* 2007)

Agent	Target
A6 (Angstrom Pharmaceuticals)	Binds to uPA cell surface receptor
ABT-510 (Abbott Laboratories)	Thrombospondin-1 receptor CD36
Actimid (CC4047) (Celgene Corp.)	Downregulates TNF-α
AG-013736 (Pfizer)	VEGF, PDGFR
AMG706 (Amgen)	VEGF, PDGFR, KITR, RetR
AP23573 (Ariad Pharmaceuticals)	VEGF, MTOR inhibitor
AS1404 (Antisoma)	Vascular disrupting: releases TNF-α and vWF
Atiprimod (Callisto Pharm.)	VEGF, bFEGF, IL6
ATN-161 (Attenuon)	Alpha 5 beta 1 antagonist
BIBF1120 (Boehringer Ingelheim)	VEGF, PDGF, FGF receptor kinases
BMS-582664 (Bristol Myers Squibb)	VEGFR-2
CDP-791 (ImClone)	VEGFR-2, KDR
Combretastatin (Oxigene)	VE-Cadherin
E7820 (Elsai)	Inhibits integrin alpha 2 subunit on endothelium
EMD 121974 (cilengitide) (EMD)	Alpha v beta 3 and 5 antagonist
Genistein (McKesson Health Solutions)	Suppresses VEGF, neuropilin, and MMP-9
INGN 241 (Introgen Therapeutics)	VEGF, MDA-7
Interleukin-12 (NCI)	Upreregulates IP10
MEDI 522 (Abergrin) (Medimmune)	Antibody alpha V beta 3
MLN518 (tandutinib) (Millennium)	FLT3, PDGFR, cKit, CSF-1R
Panzem (2ME2) (EntreMed)	Inhibits tubulin polymerization
PI-88 (Progen Industries/Medigen)	bFGF, stimulates release of TSP1
PKC412 (Novartis)	VEGFR-2
PXD101 (CuraGen Corporation)	HDAC inhibitor
SUO14813 (Pfizer)	VEGFR-3, PDGFR-a, PDGFR-b, RET, FLT3
Tempostatin (Collard Biopharm.)	Extracellular matrix proteins
XL647 (Exelisis)	VEGFR, EGFR, HER2
XL784 (Exelisis)	ADAM-10, MMPs
XL880 (Exelisis)	VEGFR-2, C-met, RTK
XL999 (Exelisis)	VEGFR, PDGFR, FGFR, Flt-3, Scr

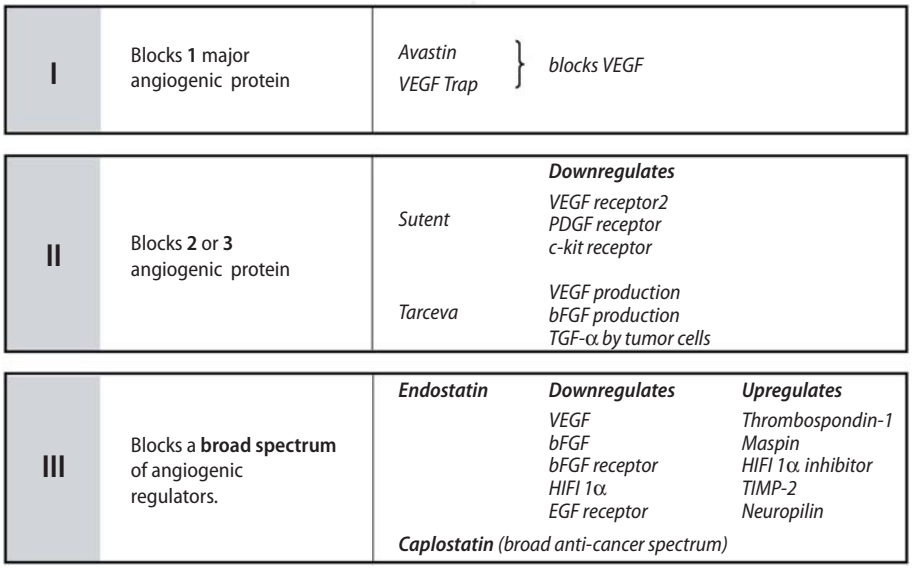

Fig. 1.7. Three types of angiogenesis inhibitors: *I* those that block a single proangiogenic protein; *II* those that can block two or three proangiogenic proteins; *III* those that are broad-spectrum angiogenesis inhibitors. (From Folkman J, *Nature Reviews Drug Discovery* 2007).

mors could be anatomically located? If this is possible, the management of cancer may eventually be liberated from dependency on determining anatomical location. This would be analogous to the history of treating infections. Before 1930, when very few drugs were available, many infections became abscesses. These had to be anatomically located for surgeons to drain them. Today, physicians treat infections guided mainly by blood biomarkers, such as the white blood cell count. For example, most patients with colon cancer are operated upon. At least 50–60% of colon cancers are cured by surgery. The rest will recur at approximately 5 years. If biomarkers could accurately detect the recurrence when it is still at a microscopic size, it could be possible to "treat the biomarker" with relatively non-toxic angiogenesis inhibitors, without having to know the anatomical location of the recurrent tumors.

It is also possible that the long-term management of age-related macular degeneration and of diabetic retinopathy by antiangiogenic therapy could be greatly improved if guided by angiogenesis-based biomarkers.

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