

Editorial Focus

Angiogenesis and the progress of vascular and tumor biology: A tribute to Judah Folkman

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"Discovery is seeing what everybody else has seen, and thinking what nobody else has thought." Szent-Gyorgi

On February 24, 2008, Judah Folkman would have celebrated his 75th birthday. However, as announced in the February issue of *Thrombosis and Haemostasis*, he suddenly passed away on January 14, 2008, while crossing the US continent pursuing his mission: to communicate the results and the clinical benefits gained from 40 years of endless, dedicated research. Among the over hundred awards and prizes he received world wide, he was invited to deliver on March 29, 2006, the prestigious "John Paul II Lecture" at the Centre for High Technology Research and Education in Biomedical Sciences of the Catholic University at Campobasso (Italy). On that occasion, the scientific community of the Catholic University was honored to have the opportunity to discuss with him his seminal work on angiogenesis and reflect on the history of the conceptual evolution behind the results obtained by Judah Folkman and his coworkers over the past 40 years.

During the last decades, angiogenesis, a microvascular process of formation of new blood vessels out of pre-existing capillaries, changed our concepts of vascular biology and some of our views on the mechanisms of tumor growth and tumor treatment. But the idea that tumor growth can be accompanied by increased vascular proliferation was introduced over a hundred years ago (see [1]). During the first half of the XXth century, several investigators examined different tumors and their vasculature; however, this did not generate very much interest. The general assumption of those studies was that cancer cells might produce growth factors promoting vascular development which precedes rapid tumor growth (1). But the available methods for further investigations on this subject were still poor and in-vitro conditions for growing cancer cells were considered highly artificial as compared with those *in vivo*.

Judah Folkman, at that time a young surgeon at Harvard Medical School in Boston, was already interested in the field of

tumor genesis and began by developing a technique for the in-vitro growth of tumors in solid form, in a system where they could grow and metastasize as if in the living host. In the 1960s he and his colleagues presented a method of cancer cell implantation in isolated perfused organs, where the "cohesiveness, shedding rate, invasiveness and behaviour of tumor cells in circulation" could be investigated (2). They predicted that it could not only provide a method for estimating metastatic potential of different tumors, but also offer a possibility to test chemotherapeutic agents and their therapeutic potential.

Subsequently, Folkman and a group of fellow workers reported the isolation of a soluble factor, from human and animal tumors, able to stimulate new vessel formation in a rat dorsal air-sac model (3). The hypothesis that solid tumors are angiogenesis-dependent, and, acting through a tumor angiogenesis factor, can attract endothelial cells to form new vessels, was proposed. But this was opposed to the existing dogma that tumors grow on pre-existing vessels and invade them, and that the redness, morphologically detectable in association with tumors, is due to non-specific inflammation, not to tumor vascular tissue.

A few months later, in a pivotal paper in the *New England Journal of Medicine (NEJM)*, Folkman proposed a revolutionary idea for treating cancers by developing angiogenesis inhibitors (4). Then, step by step, during the following 37 years until his death, he performed experiments to support this hypothesis and drew the clinical conclusions.

Within the decade immediately following the 1971 *NEJM* paper, the field of angiogenesis grew exponentially (165 papers as opposed to 6 papers between 1950 and 1971) and Judah Folkman was the author of 32 (20%) of those papers.

From a Pubmed search, the following logarithmic growth of publications on angiogenesis records 996 papers from 1981 to 1990, 8,364 from 1991 to 2000 and 24,573 from 2001 until the week of Judah Folkman's death. Figure 1 represents in a very schematic way some of the crucial steps in the development of Folkman's concepts and their translation into practice and clinical application.

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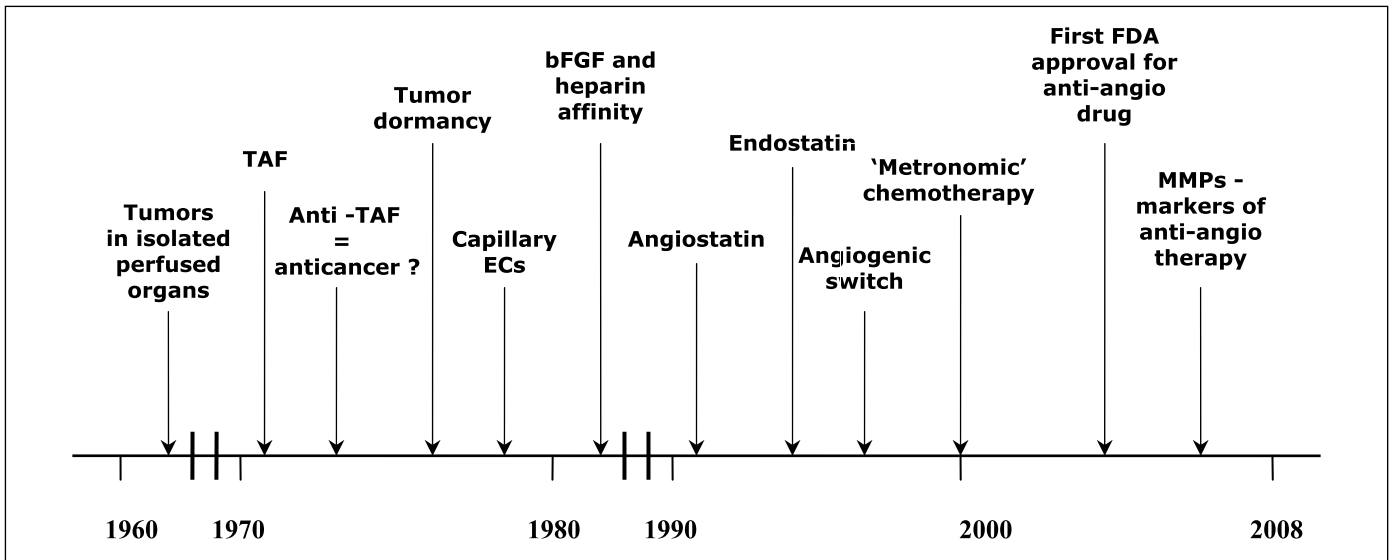


Figure 1: Schematic outline of the sequence of discoveries by Folkman’s group in the field of angiogenesis. TAF, tumor angiogenesis factor; ECs, endothelial cells; MMPs, matrix metalloproteinases; bFGF, basic fibroblast growth factor.

Tumor vasculature as a therapeutic target: A story spanning over 35 years

A pivotal observation at the beginning of this sequence of results was that there is a possibility to induce “dormancy” (i.e. a condition associated with halt or blockade of growth) in solid tumors by preventing neo-vascularization in rabbit models of anterior chamber cancer implants (5). Under these conditions, tumors, unable to elicit new vasculature, would survive in a very small size, reached only by simple diffusion. In this “avascular” phase of growth, tumors devoid of their malignancy, could remain in the host until death without showing any symptoms (5). From that moment the extensive search for factors inhibiting or inducing angiogenesis began; it was a very ‘logical’ sequence of cellular and animal experiments which led to the present clinical applications.

In 1975, using the basic knowledge that capillaries are not present in cartilage, Folkman and his group showed that in the presence of neonatal scapular cartilage capillary proliferation induced by tumors is inhibited (6). This led to the conclusion that the human body in physiologic conditions is able to produce an-

giogenesis inhibitors which in animal models may inhibit also pathologic vascularization.

Since angiogenesis is a process typically taking place in the microvasculature, the newly developed umbilical cord endothelial cells model (7, 8) was found to be not perfectly suited. Therefore an original method was developed to obtain long-term culture of capillary endothelial cells (9). This opened new possibilities to investigate basic processes in proliferation and motility of capillary endothelial cells, which already were thought to play a pivotal role not only in tumor growth and metastasis, but also in diseases like diabetic retinopathy, psoriasis or arthritis. Many scientists from that moment on devoted their efforts to look for angiogenic factors.

In the years that followed, an idea, again from Folkman’s group, made the researchers’ efforts easier. In 1984 they purified basic fibroblast growth factor (bFGF) – one of the proangiogenic factors – after discovering its high affinity to heparin (10). The latter was already known to have a huge structural similarity to heparan sulfate, a major glycosaminoglycan on the surface of endothelial cells – basal cells for the angiogenic process. Heparin binding of growth factors also led to identification and purification of the vascular endothelial growth factor (VEGF) (1). Since the discovery of bFGF and then VEGF, several other proangiogenic factors have been purified and described (Table 1). However VEGF, as the first vascular endothelium-specific growth factor (11), was shown to have a crucial role in tumor angiogenesis: this resulted in preparation of the first inhibitors against this growth factor, to achieve inhibition of tumor vasculogenesis (1).

The search for inhibitors of angiogenesis naturally existing in the human body led to the discovery and purification of two prototype factors in the 1990s, namely angiostatin and endostatin (12, 13). During animal experimentation endostatin was especially found to have a number of advantages: lack of side effects, active on different types of tumors and not inducing drug resistance (14). Endostatin entered clinical trials in a small

Table 1: Main pro-angiogenic factors, subdivided according to their specificity for vascular endothelial cells (11). VEGF, vascular endothelial growth factor; FGF, fibroblast growth factor; bFGF, basic fibroblast growth factor (FGF2); aFGF, acidic fibroblast growth factor (FGF1); TGF, transforming growth factor; PDGF, platelet-derived growth factor; HGF, hepatocyte growth factor.

Vascular endothelium-specific growth factors	Vascular endothelium-nonspecific growth factors
VEGF family (VEGF-A, VEGF-B, VEGF-C)	FGF family (bFGF, aFGF, etc.)
Angiopoietin family (Ang1, Ang2)	TGF family (TGF- α , TGF- β)
Ephrins family (Ephr-B1, Ephr-B2)	PDGF
	HGF

number of patients in the U.S. as a single agent, but the trials were discontinued in phase II in August 2005, in part because the small company that manufactured endostatin was unable to continue production. However, in China, Endostar in a phase III, placebo-controlled trial, in combination with chemotherapy resulted in statistically significant and clinically meaningful improvement in response to treatment and median time to progression in patients with advanced non-small cell lung cancer (15). In 2005 Endostar was approved by the Chinese State Food and Drug Administration (FDA) for treatment of non-small-cell lung cancer.

But the first pure anti-angiogenic drug accepted by the U.S. FDA for treatment of metastatic colorectal cancer was bevacizumab, a monoclonal antibody against VEGF. The approval was based on a phase III clinical trial of bevacizumab added to standard chemotherapy, demonstrating the increased median overall survival rate by 30% in the treatment group, compared to the placebo group, and increased median progression free survival from 6.2 months in the placebo group to 10.6 in the bevacizumab group (16). Over 30 years after Folkman had first proposed a theory of antiangiogenic treatment of cancers, the idea finally became clinical practice.

In the mean time, looking for the mechanisms leading to angiogenesis, Folkman had proposed the concept of the so called “angiogenic switch” which could be defined as the conversion from a dormant to an activated state: as long as there is a balance in the human body between the activators and the inhibitors of angiogenesis, cancer cells present in different tissues remain in a dormant state, not growing and not presenting symptoms (17, 18). When this balance is disturbed and proangiogenic factors are in excess, rapid vascularization and growth of tumors may occur (18, 19). Which factors initiate the angiogenic switch still remains an unsolved issue.

As it often happens in research on fundamental biological systems, looking for new angiogenesis inhibitors with exclusively anti-angiogenic actions, the concept was developed that drugs, already used for other indications, might also be found to possess anti-angiogenic function (20). After years of investigation, several agents used as antibiotics, non-steroidal anti-inflammatory drugs, bone-targeted bisphosphonates and many others were shown to inhibit angiogenesis when administered in long-term therapy in modified doses (Table 2) (20, 21). Similar inhibitory influences on endothelial cell functions were linked to cytotoxic agents (22). Based on this idea, a novel approach to the use of chemotherapeutics in tumor treatment, termed “anti-angiogenic chemotherapy”, was introduced (23). Chemotherapeutic agents, when administered at lower doses, but more frequently at variance with conventional chemotherapy, acted as anti-angiogenics, without generating side-effects typical of high-dose intermittent chemotherapy (23, 24). By influencing genetically stable endothelial cells of the tumor bed, derived from host endothelium, on the one hand, antiangiogenic chemotherapeutics and antiangiogenic agents reduce drug resistance, typical for conventional chemotherapy scheduling (22, 25). But, on the other hand, drugs administered alone or in combination with conventional chemotherapy and acting on endothelium may increase the risk of thromboembolic events during therapy (26–28). While the beneficial effects of anticoagulant treatment alone are not always obvious in cancer patients, inhibition of pro-

Table 2: Selected endogenous and exogenous angiogenesis inhibitors (reviewed in [20]). Endogenous angiogenesis inhibitors are the substances produced by different tissues of the human body, and present in direct (interferon, thrombospondin) or cryptic (endostatin, angiostatin, tumstatin) form. Some of them influence only angiogenesis (endostatin), and some may also influence other processes (interferon). From many of them drugs were derived, which are already in use, or still in clinical trials.

Endogenous angiogenesis inhibitors	Angiogenesis inhibitors developed as drugs	
	Drugs with pure antiangiogenic activity	Drugs already in use for other indications
<ul style="list-style-type: none"> – interferon alfa – platelet factor 4 – angiostatin – endostatin – thrombospondin – tumstatin 	<ul style="list-style-type: none"> – endostatin – anti-VEGF antibody – VEGF-trap – angiostatin – thrombospondin 	<ul style="list-style-type: none"> – celecoxib – rosiglytazone – zolendronic acid – thalidomide – interferon alfa – doxycycline

coagulant activity may become a real challenge for the clinicians when combinant anti-angiogenic therapy becomes common use in oncology.

The application of angiogenesis inhibitors in clinical conditions also raises the problem of how to monitor the therapy and what could be the best marker for therapy initiation, even when the tumor is still dormant. Recently, a method was introduced for therapy monitoring, by quantitative analysis of the urinary levels of matrix metalloproteinases (MMPs) that allow migration of endothelial cells contributing to the development of new blood vessels by destroying extracellular matrix components (29). In patients with lymphangio-leiomyomatosis, treatment with the MMP-inhibitor doxycyclin was found to be effective (30).

Past, present and future

More than 35 years after Folkman put forward his hypothesis, it is hard to think of solid tumors disregarding angiogenesis or anti-angiogenic therapy. Moreover, these studies introduced a new concept of tumor treatment, whereby cancer cell targeting is important, but influencing host endothelial cells may be equally efficient in some instances to inhibit the disease progression and to prolong survival, although such treatments are not devoid of side-effects such as increased clotting activation and thrombogenesis.

To summarize the scientific and clinical achievements obtained in this field, we could quote Folkman’s words, which also show the future direction of his and other investigators’ work. “It is possible that one day cancer might be treated as a chronic manageable disease, such as diabetes or heart disease” (31).

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