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Angiogenesis

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Preface

We dedicate this volume to the memory of Judah Folkman, an inspirational leader and a mentor to many, whose pioneering research in angiogenesis has led to a dramatic change in the way cancer is treated and to extraordinary improvements in the treatment of many eye diseases.

Blood vessels are formed by two primary mechanisms: angiogenesis, which is defined as the sprouting of new capillaries from preexisting vessels (usually venules), and vasculogenesis, in which blood vessels are formed de novo by the assembly of endothelial cell precursors called angioblasts. A landmark paper by Clark and Clark in 1932[1] included camera lucida drawings of blood vessels growing in response to a wound. Interest in the process of angiogenesis began in the 1930s and early 1940s. The first demonstration of blood vessel growth in response to a tumor was by Ide and coworkers,[2] using an ear chamber model. This was followed by a series of papers from Algire and colleagues,[3] who in the 1940s studied tumor-induced vessel growth in wound chambers. However, the lack of tools for studying angiogenesis limited further progress.

This changed in the 1970s with the introduction of a number of important reagents and assays, including the culture of capillary endothelial cells and the development of the chick chorioallantoic membrane and corneal pocket assays by Judah Folkman and his coworkers at Children's Hospital Boston and Harvard Medical School. Initially, it was anticipated that tumors might produce a "tumor angiogenesis factor" that would be unique to tumors and that could be purified due to the availability of new bioassays. The first efforts led to the purification of acidic and basic fibroblast growth factors (FGFs). These were potent endothelial cell mitogens and angiogenic factors and were shown to be produced by a wide range of normal and tumor tissues, challenging the concept that these angiogenic factors would be unique to tumors. Simultaneously, many groups were searching for inhibitors of angiogenesis. A surprising finding was the observation of endogenous inhibitors of angiogenesis in the form of fragments of highly expressed matrix and serum proteins, such as plasminogen activator (angiostatin) and collagen XVIII (endostatin).

A major breakthrough in the field came with the identification of vascular endothelial growth factor (VEGF), which was isolated from both normal and tumor cells. Interest in VEGF was heightened by the finding that its expression was regulated by hypoxia, which was long thought to be a driving force for angiogenesis in both normal and pathologic tissues, particularly tumors and the retina. Early work analyzing glioblastoma in mice demonstrated that VEGF was produced by tumors that were adjacent to the necrotic/hypoxic tumor center and that blocking VEGF signaling could suppress tumor growth. The central role of VEGF in developmental vasculogenesis and angiogenesis came with the use of molecular genetic methods that allowed targeted gene deletions; mice null for VEGF failed to develop a vascular system. With this demonstration, it was clear that VEGF regulated vasculogenesis, developmental angiogenesis, wound healing, and a wide range of

[1]Clark ER, Clark EL. 1932. Observations on living preformed blood vessels as seen in a transparent chamber inserted into the rabbit's ear. *Am J Anat* **49:** 441–477.

[2]Ide AG, Baker NH, Warren SL. 1939. Vascularization of the brown Pearce rabbit epithelioma transplant as seen in the transparent ear chamber. *Am J Roentgenol* **42:** 891–899.

[3]Algire GH, Chalkley HW, Legallais FY, Park HD. 1945. Vascular reactions of normal and malignant tumors in vivo. I. Vascular reactions of mice to wounds and to normal and neoplastic transplants. *J Natl Cancer Inst* **6:** 73–85.

pathologic angiogenesis (tumors and retinopathies, to name two). At about the same time, the VEGF receptor family of tyrosine kinases, including VEGFR-1, VEGFR-2, and VEGFR-3, was identified, along with naturally occurring soluble forms of the receptors (sFlt and sFlk). Neuropilins (NRPs) were identified as non–tyrosine kinase VEGF coreceptors. To complicate the story, the kinase activity of VEGFR-1 was shown not to be required, leading to the concept of VEGFR-1 as a decoy receptor. Moreover, the identification of a variety of markers for lymphatic endothelial cells facilitated dramatic progress in the characterization of lymphatic development and pathology, a process also regulated by members of the VEGF family: VEGFC and its receptor VEGFR-3. The use of transgenic mice has led to the identification of novel regulators of angiogenesis, such as the tie-2 receptor and its angiopoietin ligands, and the demonstration that known factors, such as PDGF B, had a role in the remodeling process. More recently, conditional and inducible systems of gene expression in mice have permitted more precise understanding of the role of these various factors in specific tissues and at particular time points.

More than 40 years ago, Judah Folkman[4] predicted the existence of angiogenesis factors that were active in promoting tumor growth, and he postulated the corollary that inhibitors of angiogenesis factor would stop tumor growth. This concept—and all of the work that was conducted by Judah Folkman, his coworkers, and the members of the field that he inspired—launched the development by academia and by industry of a large number of antiangiogenesis agents for the treatment of tumors and ocular pathology. Many of these drugs worked by blocking the activity of VEGF, either by neutralizing VEGF itself or by blocking its receptors. Recent observations have led to some controversy regarding the modest effect of antiangiogenesis agents in tumor treatment and the possibility that this is caused by a compensatory increase in expression of other angiogenic factors. Although these questions are being resolved, the use of antiangiogenesis drugs has revolutionized the treatment of wet macular degeneration—retaining and even restoring vision for a pathology for which there was previously no effective treatment. Results of recent clinical trials indicate that anti-VEGF will also have a major impact on the treatment of other ocular pathologies, including diabetic macular edema, proliferative diabetic retinopathy, branch vein occlusions, and the retinopathy of prematurity.

This volume contains 30 chapters covering many different aspects of angiogenesis, both normal and pathological. In light of the fact that a PubMed search of the term "angiogenesis" lists 53,323 citations, no such collection of chapters can be inclusive. Rather, we have attempted to highlight the most important advances. Chapters on the biology of endothelial cells review the vast extent of endothelial cell (EC) heterogeneity and function. Chapters on vessel formation and patterning cover the role of tip and stalk cells in vessel sprouting and how these cell phenotypes are regulated, as well as how lumens are formed. Developmental angiogenesis in zebrafish is described. Relatively new and rapidly evolving areas of endothelial stem cells, microRNAs, and endothelial–neural interactions are addressed, as are differences between tumor endothelial cells and normal cells. There is also a comparison of the regulation of lymphatic and blood vessel development. A section on molecular regulators of angiogenesis covers factors involved in the formation of blood vessels, including VEGF, VPF, PIGF, and the angiopoietins, along with a discussion of the angiogenesis inhibitors thrombospondin and semaphorin. The contribution of the tumor microenvironment to tumor angiogenesis is considered. In terms of treatment, the concept of blood vessel normalization for facilitating drug delivery is described. Vascular pathologies are addressed through chapters focusing on a number of examples and sources of vascular dysfunction including hemangiomas, preeclampsia, and arteriovenous malformation, as well as the role of polymorphisms, integrins, and disruption of EC-EC junctions. The book closes with a section critiquing anti-VEGF therapy,

[4]Folkman J. 1971. Tumor angiogenesis: Therapeutic implications. *N Engl J Med* **285:** 1182–1186.

which deals with an important evaluation of anti-VEGF drugs in the clinic based on clinical trial information. It is hoped that the vast amount of basic knowledge about angiogenesis that has been acquired over the last four decades and reported in this volume will result in improved therapies for angiogenesis-dependent disease.

We would like to thank the people at Cold Spring Harbor Laboratory Press, including Barbara Acosta and Richard Sever, for their assistance and patience, and Melissa Anderson and Kristin Johnson at Children's Hospital Boston for their help with administrative issues and cover art, respectively.

We thank Cold Spring Harbor Laboratory Press for the opportunity to work on this project together. As mentees of Judah Folkman, this was a labor of love in his memory. As friends and colleagues for more than 30 years, it was a pleasure for the two of us to work together and to see the remarkable strides that have been made in this field.

Michael Klagsbrun
Patricia A. D'Amore

Dedication

M. Judah Folkman (1933–2008)

Scientist, surgeon and creator of the field of angiogenesis research.[1]

Dr. Folkman (courtesy of K. Johnson)

Judah Folkman often said "Science goes where you imagine it." Few imagined more boldly or pushed science further than he did. As director of the Vascular Biology Program and a former surgeon-in-chief at the Children's Hospital Boston in Massachusetts, Folkman brought both a scientist's and a surgeon's perspectives to finding solutions to medical problems. His research observation that some tumours grow whereas others remain dormant, fused with his clinical experience in removing hot, bloody malignancies, produced a profound insight—that the recruitment of a dedicated blood supply, a process known as angiogenesis, is essential to tumour growth.

Folkman's single-mindedness in demonstrating this principle redefined our understanding of cancer biology. Furthermore, it established angiogenesis as a fundamental biological process operating in health, embryonic development and many diseases besides cancer—macular degeneration, heart disease, diabetic retinopathy, endometriosis and obesity among them. Peter Carmeliet has predicted that treatments to stimulate or repress angiogenesis will eventually benefit half a billion people worldwide (P. Carmeliet *Nature* **438**, 932–936; 2005). Today, thousands of researchers around the world, many trained in Folkman's lab, are working to make this promise a reality. But when Folkman embarked on the scientific odyssey that would establish the field of angiogenesis, he sailed alone.

Folkman was born in 1933, in Cleveland, Ohio, and was educated at Ohio State University and Harvard Medical School. He followed this with a surgical residency at Massachusetts General Hospital. His angiogenesis hypothesis had its roots in the late 1960s. On leave from his surgical residency to complete two years of military service at the National Naval Medical Center in Bethesda, Maryland, he was developing blood substitutes and testing them by perfusing rabbit thyroid glands in the lab. Out of curiosity, he seeded the glands with mouse tumour cells. The tumours grew to about 1 millimetre, then stopped. Yet, *in vivo*, the same cells formed large, lethal cancers.

After completing his surgical training and establishing his lab at the Children's Hospital, Folkman pursued the puzzle of dormant versus active tumours. Watching thread-like capillaries grow straight

[1]First published in *Nature* **451:** 781 (14 February 2008) | doi:10.1038/451781a, and reprinted with express permission from the authors, Michael Klagsbrun and Marsha A. Moses.

towards dormant tumours, he concluded that the tumours secreted an angiogenesis stimulator. Because not all tumours become vascularized, however, he further proposed a corollary process of angiogenesis inhibition. Blocking angiogenesis, he argued, would provide a new approach to controlling certain cancers.

Folkman published his ideas about angiogenesis in 1971 (J. Folkman *N. Engl. J. Med.* **285**, 1182–1186; 1971). This landmark paper initially met with scepticism. Undaunted, he and his small research group developed the cell-culture methods, bioassays and drug-delivery systems they needed to validate the theory. Notably, the technology for slow-release, drug-delivery polymers emerged from this work.

It took a decade for Folkman's lab to isolate the first angiogenesis stimulator—basic fibroblast growth factor (bFGF)—and yet another for it to identify the inhibitors angiostatin and endostatin. These breakthroughs launched an era of discovery and validation, during which Folkman's group and others uncovered additional pro- and antiangiogenic factors, began mapping the molecular pathways of pathological angiogenesis, and started to develop antiangiogenic drugs. Today, more than 50 angiogenesis inhibitors are approved or in clinical trials around the globe. Although most target cancer, drugs to treat a condition called wet age-related macular degeneration have had the most stunning success so far, reversing blindness in many patients.

Folkman never stopped pushing his own imagination or the bounds of scientific understanding. In 2004, he proposed that we may someday control cancer by "treating a biomarker" with angiogenesis inhibitors (J. Folkman & R. Kalluri *Nature* **427**, 787; 2004), just as we treat heart disease by prescribing statins in response to high cholesterol levels. Folkman foresaw biomarkers for the 'angiogenic switch', the point at which the balance of angiogenesis stimulators and inhibitors shifts in favour of stimulation, generating the web of blood vessels that turns cancer into a killer. The switch happens years before a tumour can be imaged or felt. Folkman speculated that if cancer were detected this early and treated with low-toxicity antiangiogenic drugs, it would remain dormant. Patients would have cancer but not disease.

At the time of his sudden death on 14 January, Folkman had been testing this theory in patients at risk of recurrent cancer, using biomarkers for early angiogenesis developed in his Vascular Biology Program. He had also begun to view angiogenesis as an organizing principle of biology, essential for the growth of any mass from a cancerous tumour to an atherosclerotic plaque. He had proposed that angiogenesis biomarkers could potentially detect a range of blood-vessel-dependent diseases, and that one day a single, broad-spectrum angiogenesis inhibitor or a combination of antiangiogenic drugs might be used to treat them all. This was another bold theory, one left for others to pursue.

Folkman's contributions to vision and cardiovascular research, as well as to cancer biology, were widely recognized. Apart from being elected to the National Academy of Sciences and Institute of Medicine, and appointed to the President's Cancer Advisory Board, he received more than 150 awards and prizes, including scientific honours from 11 nations. But neither the awards nor accounts of his scientific accomplishments capture what those who knew him valued most in Folkman—his humanity and generosity. He was legendary for sharing unpublished data, and masterly at balancing guidance and creative freedom to nurture young investigators' careers.

Nowhere was Folkman's compassion and generosity more evident, however, than with patients. He would leave the office late in the evening, a briefcase slung over each shoulder, a notebook of to-do's in his breast pocket, wheeling his laptop behind him. After dinner with his wife of 47 years, Paula, he would retire to his study, take out the notebook, and begin calling patients who had left messages that day. Most were people he had never met, desperate for hope and advice. He called them all.

Folkman never used his first name, Moses, but he shared much in common with his Biblical namesake. He was a teacher, leader and iconoclast. Although he did not live to see the full promise of antiangiogenic therapy realized, he trained and inspired many who will carry forward his dream.