# 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.

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