Antiangiogenic Cancer Therapy

Edited by
Darren W. Davis
Roy S. Herbst
James L. Abbruzzese

CRC Press
Taylor & Francis Group

Antiangiogenic Cancer Therapy

Antiangiogenic Cancer Therapy

Edited by
Darren W. Davis
Roy S. Herbst
James L. Abbruzzese

CRC Press
Taylor & Francis Group
Boca Raton London New York

CRC Press is an imprint of the
Taylor & Francis Group, an **informa** business

CRC Press
Taylor & Francis Group
6000 Broken Sound Parkway NW, Suite 300
Boca Raton, FL 33487-2742

© 2008 by Taylor & Francis Group, LLC
CRC Press is an imprint of Taylor & Francis Group, an Informa business

No claim to original U.S. Government works
Printed in the United States of America on acid-free paper
10 9 8 7 6 5 4 3 2 1

International Standard Book Number-13: 978-0-8493-2799-5 (Hardcover)

This book contains information obtained from authentic and highly regarded sources. Reprinted material is quoted with permission, and sources are indicated. A wide variety of references are listed. Reasonable efforts have been made to publish reliable data and information, but the author and the publisher cannot assume responsibility for the validity of all materials or for the consequences of their use.

No part of this book may be reprinted, reproduced, transmitted, or utilized in any form by any electronic, mechanical, or other means, now known or hereafter invented, including photocopying, microfilming, and recording, or in any information storage or retrieval system, without written permission from the publishers.

For permission to photocopy or use material electronically from this work, please access www.copyright.com (http://www.copyright.com/) or contact the Copyright Clearance Center, Inc. (CCC) 222 Rosewood Drive, Danvers, MA 01923, 978-750-8400. CCC is a not-for-profit organization that provides licenses and registration for a variety of users. For organizations that have been granted a photocopy license by the CCC, a separate system of payment has been arranged.

Trademark Notice: Product or corporate names may be trademarks or registered trademarks, and are used only for identification and explanation without intent to infringe.

Library of Congress Cataloging-in-Publication Data

Antiangiogenic cancer therapy / edited by Darren W. Davis and Roy S. Herbst, James L. Abbruzzese
p. ; cm.
Includes bibliographical references and index.
ISBN-13: 978-0-8493-2799-5 (hardcover)
ISBN-10: 0-8493-2799-7 (hardcover)
1. Neovascularization inhibitors. I. Davis, Darren W., 1971- II. Herbst, Roy. III. Abbruzzese, James L. IV. Title.
[DNLM: 1. Neoplasms--drug therapy. 2. Angiogenesis Inhibitors--therapeutic use. QZ 267 A6283 2007]

RC271.N46.A585 2007
616.99'4061--dc22 2007000598

Visit the Taylor & Francis Web site at
http://www.taylorandfrancis.com

and the CRC Press Web site at
http://www.crcpress.com

Preface

In recent years, tremendous progress has been made in our understanding of molecular mechanisms and cellular regulation of angiogenesis in cancer. Despite this progress, clinical development of angiogenesis inhibitors for the treatment of cancer remains challenging. Given that solid tumors account for more than 85% of cancer mortality, and tumor growth and metastasis are dependent on blood vessels, targeting tumor angiogenesis is one of the most widely pursued therapeutic strategies today. Approaches to target angiogenesis in cancer include destroying the existing vasculature (antivascular) and inhibiting neovascularization (antiangiogenic). We hope that *Antiangiogenic Cancer Therapy* will stimulate the rapid translation and dissemination of basic science discoveries into novel clinical strategies that will provide more effective antiangiogenic therapies for cancer.

Antiangiogenic Cancer Therapy was made possible as a result of a key scientific observation made more than 40 years ago, when Drs Folkman and Becker observed that tumor growth in isolated perfused organs was limited in the absence of tumor vascularization. However, it was arguably Folkman's hypothesis that tumor growth is angiogenesis-dependent in 1971 that led to the notion that angiogenesis could be a relevant target for tumor therapy. Twenty years later, the successful treatment of an angiogenesis-dependent pulmonary hemangioma (a benign tumor) with interferon α-2a enabled physicians and scientists to recognize the potential therapeutic benefit of targeting angiogenesis for cancer therapy. Indeed, in 1999 the development of antiangiogenic therapies for cancer became a top priority of the National Cancer Institute. The first angiogenesis inhibitor, bevacizumab, was approved by the Food and Drug Administration in 2004 for the treatment of metastatic carcinoma of the colon or rectum. Subsequently in 2006, bevacizumab was approved for first-line treatment of patients with advanced nonsquamous nonsmall cell lung cancer.

Although information in the field of angiogenesis is rapidly expanding, our capacity to efficiently process and implement this knowledge has not kept pace. For example, no randomized Phase III trial has demonstrated a survival benefit with currently available antiangiogenic agents when used as a monotherapy. However, the combination of bevacizumab with cytotoxic regimens has led to survival benefit in previously untreated colorectal, lung, and breast cancer, and in previously treated colorectal cancer patients. These results raise important questions about the complexity and use of angiogenesis inhibitors in clinical practice. The thesis of *Antiangiogenic Cancer Therapy* is that by understanding the molecular and cellular regulation of angiogenesis itself, we will be able to understand and implement the most optimal therapeutic strategies. This challenge creates an overwhelming task for clinicians, scientists, teachers, and authors. We have carefully considered what facts and concepts are essential elements to include in this book. An aim of this book is to integrate the fundamental concepts of angiogenesis with therapeutic strategies specific to various cancer types. Thus, although each chapter may stand alone, the scientific details within each chapter provide strength to the overall conceptual framework of the book.

We are deeply grateful to the many people who have helped us compose this book. The experts who contributed to each chapter are the most authoritative in their respective fields. However, their contributions would not be possible without many years of laborious experimental failures and successes by many investigators throughout the world. Therefore, we are also indebted to the many scientists whose contributions have led to remarkable scientific advances, which are cited within each chapter. Finally, we are thankful to the outstanding staff at Taylor & Francis who oversaw the final production of this book.

Since the initial discovery that tumors are angiogenesis-dependent was made four decades ago, this edition is a celebration of the remarkable scientific progress made during that time, and we hope an even better indication of the future to come.

ABSTRACT

Antiangiogenic Cancer Therapy brings together basic scientists and oncologists to provide the most authoritative, up-to-date, and encyclopedic volume currently available on this subject. Part I of this book introduces a series of concepts and topics regarding the role of angiogenesis in cancer. These topics include strategies to prolong the nonangiogenic dormant state of human tumors, molecular mechanisms and cellular regulation of angiogenesis in solid tumors and hematologic malignancies, and the regulation of angiogenesis by the tumor microenvironment. Part II of the book covers specific molecular targets for inhibiting angiogenesis in cancer therapy. Part III discusses clinical trial design and translational research approaches essential for identifying and developing effective angiogenesis inhibitors. These discussions include noninvasive imaging methods and direct analysis of tissue biopsies. Part IV of the book covers antiangiogenic treatment for specific cancer types. These chapters are introduced by state-of-the-art discussions outlining the current understanding of the molecular biology of each cancer type followed by discussions that examine strategies for targeting angiogenesis. Organizing the chapters in this format will allow the reader to easily find the information necessary to understand the fundamental concepts of angiogenesis and the complexities associated with targeting angiogenesis for specific types of cancer. This book will serve to provide information useful to scientists and physicians engaged in the study and development of antiangiogenic agents, as well as medical professionals, medical and graduate students, and allied health professionals interested in learning more about the biology and clinical use of angiogenesis inhibitors.

Editors

Dr Darren W. Davis is president and chief executive officer of ApoCell, Inc., an innovative molecular diagnostic company located near the world famous Texas Medical Center, Houston, Texas. Dr Davis has a BS in biochemical and biophysical sciences and earned his PhD in cancer biology and toxicology at the University of Texas Graduate School of Biomedical Sciences and the University of Texas M.D. Anderson Cancer Center in Houston, Texas. Dr Davis continued his postgraduate training at M.D. Anderson Cancer Center where he developed several methods to analyze the effects of molecular-targeted therapies, including angiogenesis inhibitors, to support clinical drug development. He was shortly promoted to junior faculty and served as one of six investigators of the Goodwin Molecular Monitoring Laboratory for clinical biomarker development, Department of Translational Research, before founding ApoCell in 2004, an M.D. Anderson Cancer Center spin-off company. Dr Davis serves as the principal investigator for numerous biological correlative studies to support clinical trials. His research interests center on molecular mechanisms, apoptosis, and signal transduction of molecular-targeted therapies. Dr Davis has evaluated the pharmacodynamic effects of both conventional and drug-targeted therapies in a wide variety of animal and clinical specimens. Dr Davis has frequently been invited to speak at both national and international conferences and scientific advisory meetings. Dr Davis serves as a consultant for both basic scientists and clinicians and helps identify and select critical end points for clinical trials with leading pharmaceutical companies. Dr Davis is author or coauthor of more than 50 publications, including peer-reviewed journal articles, abstracts, book chapters, and has served as an editor. He has contributed his work to many prominent journals, such as, *Journal of Experimental Medicine*, *Cancer Research*, *Journal of Clinical Oncology*, *Clinical Cancer Research*, *Lung Cancer*, *Cancer*, and *Seminars in Oncology*. His abstracts have been presented at the annual meetings of the American Society of Clinical Oncology, the American Association for Cancer Research, and the European Organization for Research and Treatment of Cancer. Dr Davis is the inventor of four pending patents.

Dr Roy S. Herbst is professor and chief of the Section of Thoracic Medical Oncology in the Department of Thoracic/Head and Neck Medical Oncology, at the University of Texas M.D. Anderson Cancer Center in Houston, Texas. He also serves as professor in the Department of Cancer Biology and codirector of the Phase I working group. Dr Herbst earned his MD at Cornell University Medical College and his PhD in molecular cell biology at the Rockefeller University in New York City, New York. His postgraduate training included an internship and residency in medicine at Brigham and Women's Hospital in Boston, and a chief residency at West Roxbury Veterans Administration Hospital in Dedham, Massachusetts. His clinical fellowships in medicine and hematology were completed at the Dana-Farber Cancer Institute and Brigham and Women's Hospital, respectively. Subsequently, Dr Herbst completed the MS degree in clinical translational research at Harvard University in Cambridge, Massachusetts. Dr Herbst serves as the principal investigator for numerous trials and has conducted research primarily in the treatment of lung cancer, head and neck cancer, and Phase I studies. His Laboratory and Clinical work has focused on the clinical development of molecular-targeted therapies. Dr Herbst has frequently been invited to speak at both national and international conferences. Dr Herbst is author or coauthor of more than 200 publications, including peer-reviewed journal articles, abstracts, and book chapters. He has contributed his work to many prominent journals, such as *Journal of Clinical Oncology*, *Clinical Cancer Research*, *Clinical Lung Cancer*, *Lung Cancer*,

Cancer, *Annals of Oncology*, and *Seminars in Oncology*. His abstracts have been presented at the annual meetings of the American Society of Clinical Oncology, the American Association for Cancer Research, the World Conference on Lung Cancer, the Society of Nuclear Medicine Conference, and the European Organization for Research and Treatment of Cancer. Dr Herbst is an active member of the American Society of Clinical Oncology, the American Association for Cancer Research, the International Association for the Study of Lung Cancer, the Radiation Therapy Oncology Group, and the Southwest Oncology Group Lung Committee. He served as chairman of the American Society of Clinical Oncology—Lung Cancer Program Subcommittee (2001–2002), vice chairman of the Radiation Therapy Oncology Group—Lung Committee, vice chairman of the Southwest Oncology Group—Lung Committee, guest planner of the Annual Meeting Education Program (2003), chairman of the International Association for the Study of Lung Cancer—Targeted Therapy Division of the Translational Research and Targeted Therapy Subcommittee (2003 and 2005), and chairman of the American Society of Clinical Oncology—Cancer Communication Committee (2005–2006). Notably Dr Herbst is the recipient of the American Society of Clinical Oncology Young Investigator Award, the American Society of Clinical Oncology Career Development Award (1999, 2000), and the M.D. Anderson Cancer Center Physician Scientist Program Award (1999–2002).

Dr James L. Abbruzzese is the M.G. and Lillie A. Johnson chair for cancer treatment and research and chairman of the Department of Gastrointestinal Medical Oncology at the University of Texas M.D. Anderson Cancer Center in Houston, Texas. Dr Abbruzzese is a member of numerous scientific advisory boards including the external scientific advisory board for the University of Massachusetts, the Arizona Cancer Center, the Lustgarten Foundation for Pancreatic Cancer Research, and the Pancreatic Cancer Action Network. Born in Hartford, Connecticut, Dr Abbruzzese graduated medical school with honors from the University of Chicago, Pritzker School of Medicine, Chicago, Illinois. He completed residency in internal medicine at the Johns Hopkins Hospital in Baltimore, Maryland, and fellowship in medical oncology at the Dana-Farber Cancer Center, Harvard Medical School in Boston, Massachusetts. He is married and has one child. Dr Abbruzzese has published over 200 peer-reviewed articles, numerous chapters, and reviews. In 2004, he coedited a book entitled *Gastrointestinal Oncology* published by Oxford University Press. His research group was recently awarded a SPORE in pancreatic cancer and U54 grant on angiogenesis. In 2001, Dr Abbruzzese served as a cochair of the American Association for Cancer Research Program Committee. He is a member of the American Association for Cancer Research Fellowships Committee, the American Society of Clinical Oncology Grant Awards and Nominating Committees, and has many other board memberships. Dr Abbruzzese is a deputy editor of *Clinical Cancer Research* and member of several other editorial boards in the past including the *Journal of Clinical Oncology*. His clinical interests center on pancreatic cancer, new drug development, and noninvasive assessment of anticancer drug effects.

Contributors

Abebe Akalu
Departments of Radiation Oncology
and Cell Biology
New York University School of Medicine
Cancer Institute
New York, New York

Kenneth C. Anderson
Jerome Lipper Multiple Myeloma Center
Department of Medical Oncology
Dana-Farber Cancer Institute and Harvard
Medical School
Boston, Massachusetts

Khalid Bajou
Division of Hematology–Oncology
Departments of Pediatrics and Biochemistry
and Molecular Biology
University of Southern California Keck
School of Medicine and Saban Research
Institute of Children's Hospital
Los Angeles, California

Cheryl H. Baker
Department of Biomedical Sciences
University of Central Florida
Orlando, Florida

and

Cancer Research Institute
M.D. Anderson Cancer Center–Orlando
Orlando, Florida

Pablo M. Bedano
Division of Hematology–Oncology
Indiana University School of Medicine
Indianapolis, Indiana

Peter C. Brooks
Departments of Radiation Oncology
and Cell Biology
New York University School of Medicine
Cancer Institute
New York, New York

Thomas R. Burkard
Institute for Genomics and Bioinformatics
and Christian Doppler
Laboratory for Genomics and
Bioinformatics
Graz University of Technology
Graz, Austria

and

Research Institute of Molecular Pathology
Vienna, Austria

David J. Chaplin
Oxigene, Inc.
Waltham, Massachusetts

Dharminder Chauhan
Jerome Lipper Multiple Myeloma Center
Department of Medical Oncology
Dana-Farber Cancer Institute and Harvard
Medical School
Boston, Massachusetts

Ramzi N. Dagher
Center for Drug Evaluation
and Research
U.S. Food and Drug Administration
Silver Spring, Maryland

Angus G. Dalgleish
Division of Oncology
Cell and Molecular Sciences
St. Georges University of London
London, United Kingdom

Darren W. Davis
ApoCell, Inc.
Houston, Texas

S. Davis
Regeneron Pharmaceuticals, Inc.
Tarrytown, New York

Yves A. DeClerck
Departments of Pediatrics, Biochemistry, and Molecular Biology
Keck School of Medicine
University of Southern California

and

Saban Research Institute
Childrens Hospital Los Angeles
Los Angeles, California

Bruce J. Dezube
Division of Hematology/Oncology
Beth Israel Deaconess Medical Center
Harvard Medical School
Boston, Massachusetts

Graeme J. Dougherty
Department of Radiation Oncology
University of Arizona
Tucson, Arizona

Keith Dredge
Progen Industries Ltd.
Brisbane, Australia

Dan G. Duda
Department of Radiation Oncology
Massachusetts General Hospital and Harvard Medical School
Boston, Massachusetts

Frank Eisenhaber
Research Institute of Molecular Pathology
Vienna, Austria

Heinrich Elinzano
Neuro-Oncology Branch
National Cancer Institute and National Institutes of Health
Bethesda, Maryland

Napoleone Ferrara
Genetech, Inc.
San Francisco, California

Isaiah J. Fidler
Department of Cancer Biology
University of Texas M.D. Anderson Cancer Center
Houston, Texas

Howard A. Fine
Neuro-Oncology Branch
National Cancer Institute and National Institutes of Health
Bethesda, Maryland

Judah Folkman
Karp Family Research Laboratories
Boston, Massachusetts

Nicholas W. Gale
Regeneron Pharmaceuticals, Inc.
Tarrytown, New York

Francis J. Giles
Department of Leukemia
University of Texas M.D. Anderson Cancer Center
Houston, Texas

Ramaswamy Govindan
Department of Medicine
Washington University School of Medicine
St. Louis, Missouri

Hubert Hackl
Institute for Genomics and Bioinformatics and Christian Doppler Laboratory for Genomics and Bioinformatics
Graz University of Technology
Graz, Austria

Christian Hafner
Department of Dermatology
University of Regensburg
Regensburg, Germany

Kristin Hennenfent
Division of Pharmacy Practice
St. Louis College of Pharmacy
St. Louis, Missouri

John V. Heymach
Departments of Cancer Biology and Thoracic/Head and Neck Oncology
University of M.D. Anderson Cancer Center
Houston, Texas

Daniel J. Hicklin
ImClone Systems, Inc.
New York, New York

Paulo M. Hoff
Department of Gastrointestinal Medical Oncology
University of Texas M.D. Anderson Cancer Center
Houston, Texas

Sakina Hoosen
Clinical R&D
Pfizer, Inc.
New York, New York

Mark A. Horsfield
Cardiovascular Sciences
University of Leicester
Leicester, United Kingdom

Rakesh K. Jain
Department of Radiation Oncology
Massachusetts General Hospital and Harvard Medical School
Boston, Massachusetts

Henry B. Koon
Division of Hematology/Oncology
Beth Israel Deaconess Medical Center
Harvard Medical School
Boston, Massachusetts

Hans-Georg Kopp
Department of Hematology-Oncology
Eberhard-Karls University Tubingen
Tubingen, Germany

Shaji Kumar
Department of Internal Medicine
Mayo Clinic and Foundation
Rochester, Minnesota

Mijung Kwon
Tumor Angiogenesis Section
Surgery Branch
National Cancer Institute
Bethesda, Maryland

Janessa J. Laskin
Division of Medical Oncology
University of British Columbia
Vancouver, British Columbia

Walter E. Laug
Departments of Pediatrics, Biochemistry, and Molecular Biology
Keck School of Medicine
University of Southern California

and

Saban Research Institute
Childrens Hospital Los Angeles
Los Angeles, California

Steven K. Libutti
Tumor Angiogenesis Section
Surgery Branch
National Cancer Institute
Bethesda, Maryland

Glenn Liu
The University of Wisconsin Carbone Comprehensive Cancer Center
Madison, Wisconsin

Kathy D. Miller
Division of Hematology–Oncology
Indiana University School of Medicine
Indianapolis, Indiana

Bruno Morgan
Departments of Cancer Studies and Molecular Medicine
Radiology Department
University of Leicester
Leicester, United Kingdom

Daniel Morgensztern
Washington University School of Medicine
St. Louis, Missouri

Robert J. Motzer
Memorial Sloan-Kettering Cancer Center
New York, New York

George N. Naumov
Department of Surgery
Harvard Medical School and
Vascular Biology Program
Children's Hospital Boston
Boston, Massachusetts

Maria Novatchkova
Research Institute of Molecular Pathology
Vienna, Austria

Liron Pantanowitz
Department of Pathology
Baystate Medical Center
Tufts University School of Medicine
Springfield, Massachusetts

Nicholas Papadopoulos
Regeneron Pharmaceuticals, Inc.
Tarrytown, New York

Richard Pazdur
Center for Drug Evaluation and Research
U.S. Food and Drug Administration
Silver Spring, Maryland

Klaus Podar
Jerome Lipper Multiple Myeloma Center
Department of Medical Oncology
Dana-Farber Cancer Institute and Harvard
Medical School
Boston, Massachusetts

Marco Presta
Department of Biomedical Sciences
and Biotechnology
University of Brescia
Brescia, Italy

Shahin Rafii
Division of Vascular Hematology–Oncology
Department of Genetic Medicine
Cornell University Medical College
New York, New York

Carlos Almeida Ramos
Department of Stem Cell Transplantation
and Cellular Therapy
University of Texas M.D. Anderson
Cancer Center
Houston, Texas

Albrecht Reichle
Department of Hematology and Oncology
University Hospital of Regensburg
Regensburg, Germany

John S. Rudge
Regeneron Pharmaceuticals, Inc.
Tarrytown, New York

Marco Rusnati
Department of Biomedical Sciences
and Biotechnology
University of Brescia
Brescia, Italy

Everardo D. Saad
Multidisciplinary Oncology Group
Federal University of Sao Paulo
Sao Paulo, Brazil

Alan B. Sandler
Division of Hematology and Oncology
Thoracic Oncology Vanderbilt-Ingram
Cancer Center
Nashville, Tennessee

Brian P. Schneider
Division of Hematology–Oncology
Indiana University School of Medicine
Indianapolis, Indiana

Dietmar W. Siemann
Department of Radiation Oncology
University of Florida Shands Cancer Center
Gainesville, Florida

George W. Sledge, Jr.
Division of Hematology–Oncology
Indiana University School of Medicine
Indianapolis, Indiana

David J. Stewart
Department of Thoracic/Head & Neck
Medical Oncology
University of Texas M.D. Anderson
Cancer Center
Houston, Texas

Anita Tandle
Tumor Angiogenesis Section
Surgery Branch
National Cancer Institute
Bethesda, Maryland

Gavin Thurston
Regeneron Pharmaceuticals, Inc.
Tarrytown, New York

Zlatko Trajanoski
Institute for Genomics and Bioinformatics and Christian Doppler Laboratory for Genomics and Bioinformatics
Graz University of Technology
Graz, Austria

Thomas Vogt
Department of Dermatology
University of Regensburg
Regensburg, Germany

Leslie K. Walker
The University of Wisconsin Carbone Comprehensive Cancer Center
Madison, Wisconsin

Stanley J. Wiegand
Regeneron Pharmaceuticals, Inc.
Tarrytown, New York

George Wilding
The University of Wisconsin Carbone Comprehensive Cancer Center
Madison, Wisconsin

Christopher G. Willett
Department of Radiation Oncology
Duke University Medical Center
Durham, North Carolina

Hua-Kang Wu
Department of Cancer Biology
University of Texas M.D. Anderson Cancer Center
Houston, Texas

George D. Yancopoulos
Regeneron Pharmaceuticals, Inc.
Tarrytown, New York

Karen W.L. Yee
Department of Medical Oncology and Hematology
University Health Network—Princess Margaret Hospital
Toronto, Ontario

Zhenping Zhu
ImClone Systems, Inc.
New York, New York

Amado J. Zurita
Department of Genitourinary Medical Oncology
University of Texas M.D. Anderson Cancer Center
Houston, Texas

Table of Contents

Part I

Angiogenesis in Cancer

1 Strategies to Prolong the Nonangiogenic Dormant State of Human Cancer

George N. Naumov and Judah Folkman

CONTENTS

1.1 CLINICAL "LATENCY" IN CANCER RECURRENCE FOLLOWING A PRIMARY TUMOR TREATMENT

Cancer recurrence after treatment of the primary tumor is a major cause of mortality among cancer patients. It may take years to decades before local or distant (i.e., metastatic) recurrence becomes clinically detectable as cancer. This "disease-free" period is a time of uncertainty for patients who appear "cured." For example, Demicheli et al.[1] have demonstrated two hazardous peaks of breast cancer recurrence in patients undergoing mastectomy alone without adjuvant therapy. In a group of 1173 patients, the first peak of cancer recurrence occurred at ~18 months after surgery. A second peak in cancer recurrence developed at ~5 years after surgery and was associated with a plateau-like tail extending up to 15 years.[1] Patients experiencing cancer recurrences within 5 years following surgery have a shorter overall survival than those with recurrences occurring at a later time point.[2]

Similar "latency periods" in cancer recurrence have been documented since the beginning of the twentieth century. Rupert A. Willis has summarized the "time elapsing between the excision of a human malignant tumor and the appearance of a clinically recognizable recurrence" for a variety of human cancers. For example, the latency period in breast cancer patients can be from 6 to 20 years; cutaneous and ocular melanoma, 14 to 32 years; kidney carcinoma,

6 to 8 years; and stomach and colon carcinoma, 5 to 6 years.[3] Moreover, Willis was the first to realize that these latency periods do not correspond with the natural progression of cancer, and he introduced the concept of a "dormant cancer cell" as a possible explanation.

Over the past few years, several hypotheses have been proposed in an attempt to explain the phenomenon of human tumor dormancy. Initially, it was proposed that tumor cells enter a prolonged state of mitotic arrest.[4,5] Others hypothesized that tumor size is controlled by the immune system[6–11] or hormonal deprivation in hormone-dependent tumors.[11–13] In 1972, Folkman and Gimbrone demonstrated that dormancy in human tumors could be due to blocked angiogenesis. In the following years, Folkman and colleagues have presented evidence supporting the concept that most human tumors arise without angiogenic activity and exist in a microscopic dormant state for months to years without neovascularization.[14] Such protection may be attributed in part to host-derived factors, which prevent microscopic tumors from switching to the angiogenic phenotype.

1.2 ANGIOGENESIS DEPENDENCE OF TUMOR GROWTH

Cancer progression is a multistep process (Figure 1.1). With each step, the genetic and epigenetic events in the process become increasingly complex and may be more difficult to

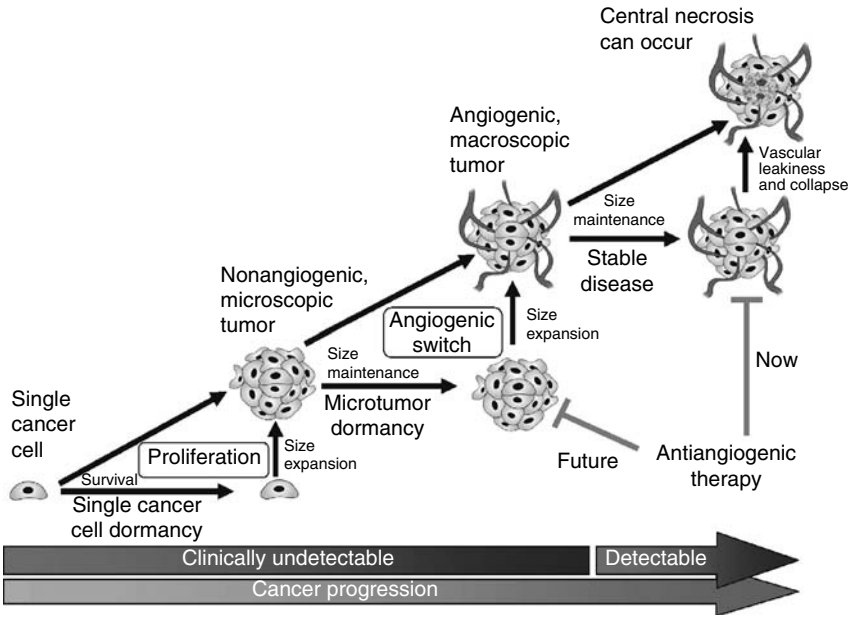

FIGURE 1.1 (See color insert following page 558.) Rate-limiting steps in the tumor progression. Solitary nonproliferating, dormant cancer cells can persist for long periods of time, until they come out of G_0 arrest and start to proliferate. Tumor mass can expand only to a microscopic mass without the recruitment of new blood vessels. Human cancers can remain nonangiogenic and dormant for long periods of time, delaying the tumor progression process. During this microscopic dormant state, nonangiogenic tumors are actively proliferating and undergoing apoptosis. Nonangiogenic tumors can expand in mass after undergoing the angiogenic switch and recruitment of new blood vessels. Angiogenic macroscopic tumors that do not expand in mass are known as "stable disease," although angiogenic tumors can remain at a constant size for prolonged periods of time. Current antiangiogenic therapy targets angiogenic microscopic and macroscopic tumors. However, future antiangiogenic therapy will target nonangiogenic microscopic tumors with the aim of keeping them in a dormant state by preventing the angiogenic switch.

treat. As the cancer progresses from a single neoplastic cell to a large, lethal tumor, it acquires a series of mutations, becoming: (1) self-sufficient in growth signaling, by oncogene activation and loss of tumor suppressor genes, (2) insensitive to antigrowth signaling, (3) unresponsive to apoptotic signaling, (4) capable of limitless cell replications, and (5) tumorigenic and metastatic.[15] Current experimental and clinical evidence indicates that these neoplastic properties may be necessary, but not sufficient, for a cancer cell to progress into a population of tumor cells, which becomes clinically detectable, metastatic, and lethal. For a tumor to develop a highly malignant and deadly phenotype, it must first recruit and sustain its own blood supply, a process known as tumor angiogenesis.[16,17]

For more than a century, it has been observed that surgically removed tumors are hyperemic compared to normal tissues.[18,19] Generally, this phenomenon was explained as simple dilation of existing blood vessels induced by tumor factors. However, Ide et al.[20] demonstrated that tumor-associated hyperemia could be related to new blood vessel growth, and vasodilation may not be the sole explanation for this phenomenon. They showed that when a wound induced in a transparent rabbit ear chamber completely regressed, the implantation of a tumor in the chamber resulted in the growth of new capillary blood vessels.[20] These initial observations were later confirmed by Algire et al.,[21,22] demonstrating that new vessels in the periphery of a tumor implant arose from preexisting host vessels, and not from the tumor implant itself. At the time, this novel concept of tumor-induced neovascularization was generally attributed to an inflammatory reaction, thought to be a side effect of tumor growth, and it was not perceived as a requirement for tumor growth.[23]

In the early 1960s, Folkman and Becker observed that tumor growth in isolated perfused organs was severely restricted in the absence of tumor vascularization.[24–28] In 1971, Folkman proposed the hypothesis that tumor growth is angiogenesis-dependent.[16] This hypothesis suggested that tumor cells and vascular endothelial cells within a neoplasm may constitute a highly integrated, two-compartment system, which dictates tumor growth. This concept indicated that endothelial cells may switch from a resting state to a rapid growth phase because of ''diffusible'' signals secreted from the tumor cells. Moreover, Folkman proposed that angiogenesis could be a relevant target for tumor therapy (i.e., antiangiogenic therapy).

We now know that angiogenesis plays an important role in numerous physiologic and pathologic processes. The hallmark of pathologic angiogenesis is the persistent growth of blood vessels. Sustained neovascularization can continue for months or years during the progression of many neoplastic and nonneoplastic diseases.[29,30] However, tumor angiogenesis is rarely, if ever, downregulated spontaneously. The fundamental objective of antiangiogenic therapy is to inhibit the progression of pathologic angiogenesis. In contrast, the goal of antivascular therapy is to rapidly occlude new blood vessels so that the blood flow stops. Both therapeutic approaches target the ability of tumors to progress from the nonangiogenic to the angiogenic phenotype, a process termed the ''angiogenic switch.''[31,40]

Cancer usually becomes clinically detectable only after tumors have become angiogenic and have expanded in mass. Failure of a tumor to recruit new vasculature or to reorganize the existing surrounding vasculature results in a nonangiogenic tumor, which is microscopic in size and unable to increase in mass (Figure 1.1). Without new blood supply, microscopic tumors are usually restricted to a size of $<$1–2 mm in diameter and are highly dependent on surrounding blood vessels for oxygen and nutrient supply. At sea level, the diffusion limit of oxygen is ~100 μm.[32] Therefore, all mammalian cells, including neoplastic cells, are required to be within 100–200 μm of a blood vessel. As nonangiogenic tumors attempt to expand in mass, attributed to uncontrolled cancer cell proliferation, some tumor cells fall outside the oxygen diffusion limit and become hypoxic. It is well known that hypoxic conditions induce a set of compensatory responses within cancer cells, such as increased transcription of the

hypoxia-inducible factor (HIF). Subsequently, this hypoxic signaling leads to upregulation of proangiogenic proteins, such as vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), and nitric oxide synthase (NOS).[33] The angiogenic switch in tumors is presumed to be closely regulated by the presence of pro- and antiangiogenic proteins in the tumor microenvironment. An increase in the local concentration of proangiogenic proteins allows angiogenesis to occur and ultimately permits a tumor to expand in mass. Antiangiogenic therapy offers a fourth anti-cancer modality, in addition to conventional therapeutic approaches, which target well-established and genetically unstable tumors.

1.3 EXPERIMENTAL MODELS OF HUMAN TUMOR DORMANCY

As early as the 1940s, experimental systems involving the transplantation of tumor pieces in isolated perfused organs and in the anterior chamber of the eyes of various species of animals have demonstrated the effects of neovascularization on tumor growth. Greene et al.,[34] observed that H-31 rabbit carcinoma tumor implanted into the eyes of guinea pigs did not vascularize and failed to grow for 16–26 months. During this period, the transplants measured ~2.5 mm in diameter. However, when the same tumors were reimplanted into their original host (i.e., rabbit eyes), they vascularized and grew to fill the anterior chamber within 50 days. Similarly, Folkman et al.[25] showed that in isolated perfused thyroid and intestinal segment tumors, implants grew and arrested at a small size (2–3 mm diameter). This inability of neoplasms to evoke a new blood supply was later attributed to endothelial cell degeneration in the perfused organs that are perfused with platelet-free hemoglobin solution.[35] In 1972, Gimbrone et al.[36] provided in vivo evidence that the progressive growth of a homologous solid tumor can be deliberately arrested at a microscopic size when neovascularization is prevented. In these experiments, two comparable tumor pieces were implanted in each eye of the same animal: one directly on the iris (i.e., angiogenic milieu) and the other suspended in the anterior chamber (i.e., avascular milieu) in the opposing eye. The vascularized tumor implanted on the iris grew to a size 15,000 times the initial volume and filled the anterior chamber of the rabbit eye within 14 days. In contrast, the tumor implant in the avascular anterior chamber remained avascular, and by day 14 after implantation, had only increased by 4 times its initial volume. These ''dormant'' tumors remained at a size of ~1 mm in diameter for up to 44 days. During this period, the tumors developed a central necrotic core surrounded by a layer of viable tumor cells, in which mitotic figures were observed. Overall, these microscopic tumors remained avascular, as demonstrated through microscopic and histological analyses and fluorescein tests. The malignant growth potential of these microscopic tumors was demonstrated in vivo by reimplanting the tumors directly on the irises of fresh animals. In the irises, the dormant tumors became vascularized and grew rapidly until the anterior chambers of the eyes were filled with tumor, in a manner similar to the control iris implants. These fundamental observations established the relationship between tumor growth and angiogenesis. Moreover, they provided an in vivo experimental model for further investigations of tumor dormancy.

One of the most pressing questions at that time was whether tumor-induced angiogenesis could be inhibited, preventing dormant tumors from progressing to the angiogenic phenotype. Using a V2 rabbit carcinoma in the corneal implant animal model, Brem et al.,[37] demonstrated that tumor angiogenesis could be blocked by diffusible proteins from the cartilage of newborn rabbits. The coimplants of tumor and cartilage pieces completely prevented vascularization in 28% of tumors and significantly delayed the vascularization in the remaining tumors, which eventually became vascularized. In addition, cartilage pieces inhibited vessel formation around a tumor implant in the chorioallantoic membrane (CAM) of chick embryos. The inhibitory proteins found in cartilage were later identified, isolated, and characterized as tissue inhibitors of metalloproteinases (TIMPs).[37a] Subsequently, other

angiogenesis inhibitors were identified. Endostatin and angiostatin were discovered as internal peptide fragments of plasminogen and 20 kDa C-terminal fragments of collagen XVIII, respectively.[38,39]

A spontaneous tumor dormancy model in transgenic mice was described by Hanahan and Folkman,[40] in which autochthonous tumors arise in the pancreatic islets as a result of simian virus 40 T antigen (Tag) oncogene expression. In this experimental model, only 4% of tumors become angiogenic after 13 weeks. In contrast, the remaining 96% of pancreatic islet tumors remain microscopic and nonangiogenic.[40,41] The spontaneous progression of non-angiogenic lesions to the angiogenic phenotype in these transgenic tumor-bearing mice led to the development of the ''angiogenic switch'' concept.[40]

More recently, Achilles et al.[42] reported that human cancers contain subpopulations that differ in their angiogenic potential. These findings suggested that the angiogenic phenotype of a human tumor cell may be controlled by genetic and epigenetic mechanisms. Therefore, human tumors can contain both angiogenic and nonangiogenic tumor cell populations, characterized by their in vivo ability to recruit new blood vessels to a tumor. However, the factors involved in the proportional regulation of these two tumor cell populations are still unknown. The observed heterogeneity of angiogenic activity among human tumor cells allowed for the isolation of these two populations of cancer cells and the development of new and fruitful human tumor dormancy experimental models.

Single-cell cloning of a human tumor cell line was employed as a strategy for the isolation of angiogenic and nonangiogenic tumor cell populations.[42] Achilles et al. established and selected subclones from a human liposarcoma cell line (SW-872) based on high, intermediate, or low proliferation rates in vitro. These clones were expanded in vitro into a population of tumor cells and were then inoculated into immunodeficient (SCID) mice. Three different growth patterns were observed: (1) highly angiogenic and rapidly growing tumors, (2) weakly angiogenic and slowly growing tumors, and (3) nonangiogenic and dormant tumors. In a subsequent experiment, Almog et al.[43] demonstrated that the nonangiogenic tumors spontaneously switch to the angiogenic phenotype and initiate exponential growth ~130 days after subcutaneous inoculation.[43] During the 130 day dormancy period, microscopic (~1–2 mm in diameter) tumors remain avascular and are virtually undetectable by palpation. Because this animal dormancy model was based on the in vitro tumor cell proliferation differences between the angiogenic and nonangiogenic liposarcoma clones, it raised two fundamental questions: (1) Is there a correlation between tumor cell proliferation and angiogenic potential? (2) Can the observed differences in tumor growth be recapitulated using populations of human tumor cells that have not been cloned?

To address these questions, human tumor cell lines were obtained from the American Type Culture Collection (ATCC, Manassas, VA) based on their ''no take'' phenotype in immunodeficient mice. These cell lines were assessed for in vivo tumor growth over extended time periods. Following a subcutaneous inoculation of a tumor cell suspension, mice were monitored for palpable tumors at the site of inoculation for more than a year (i.e., about half the normal life span of a mouse) and, sometimes, for the life of the animal. Some of the mice inoculated with the ''no take'' tumor cells spontaneously formed palpable tumors after a dormancy period, which varied from months to more than a year, depending on cancer type (Figure 1.2). With time, tumors became angiogenic, and palpable, expanded in mass exponentially, and within ~50 days of first detection, killed the host animal. Stable cell lines were established from representative angiogenic tumors. When reinoculated into SCID mice, these angiogenic tumor cells formed large (>1 cm in diameter) tumors within a month following inoculation (i.e., without a dormancy period), in 100% of the inoculated mice. It was found that each cancer type had a characteristic and predictable dormancy period and generated a consistent proportion of tumors that switched to the angiogenic phenotype. However, once nonangiogenic tumors switched to the angiogenic phenotype, they escaped

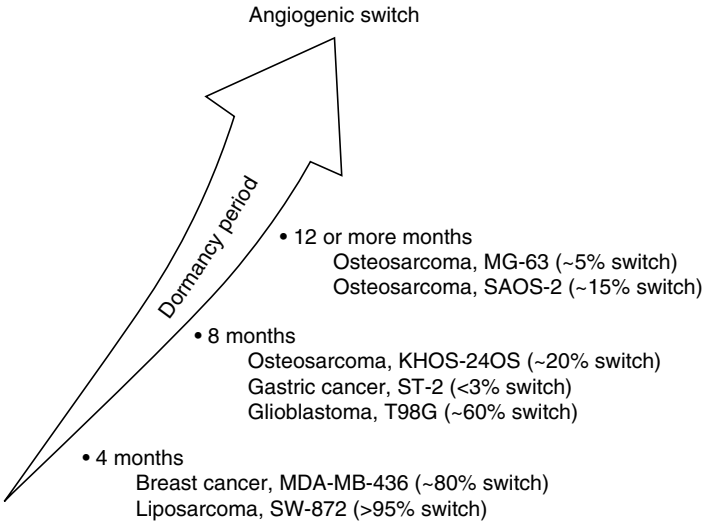

FIGURE 1.2 Human tumor cell lines that spontaneously switch to the angiogenic phenotype after a prolonged dormancy period in immunodeficient mice. The percent of mice that switch to the angiogenic phenotype is shown in brackets and varies between human tumor types.

from the dormancy state and formed lethal tumors in 100% of the mice, regardless of cancer type. At this time, tumor cell population–based animal dormancy models have been developed and characterized for breast cancer, osteosarcoma, and glioblastoma (Figure 1.2).[44] In contrast to the single-cell-derived human liposarcoma animal model, the angiogenic and the nonangiogenic tumor cell populations of the rest of the animal models were derived by in vivo selection for the angiogenic and nonangiogenic phenotypes. These in vivo models of nonangiogenic human tumors permit analysis of the switch to the angiogenic phenotype, and make it possible to address the question of whether the switch can be bi-directional.

1.4 DORMANT TUMORS HAVE BALANCED PROLIFERATION AND APOPTOSIS

After inoculation in animals, human tumor cells can remain dormant for more than a year. However, this does not mean that the tumor cells are in G_0 arrest. Although some tumor cells might be in mitotic arrest, as demonstrated in some tumor dormancy models,[45–47] we reported that the majority of tumor cells are proliferating or undergoing apoptosis. The tumor cell proliferation index in nonangiogenic tumors can be as high as that of large, vascularized tumors. In a human breast cancer (MDA-MB-436) animal model, more than 50% of tumor cells were proliferating and more than 10% were undergoing apoptosis in all microscopic tumors analyzed at various time points during dormancy, as well as in all macroscopic angiogenic tumors.[44] In a different human osteosarcoma (MG-63 and SAOS-2) and gastric (ST-2) cancer dormancy models, microscopic tumors were unable to grow beyond a threshold size of ~1–2 mm in diameter. Within these nonangiogenic tumors, there appears to be a balance between proliferating cells and cells undergoing apoptosis.[48] Tumor cell proliferation in these tumors was ~12%, and tumor cell apoptosis ranged from 4% to 7.5%.

1.5 DEFINITION OF A HUMAN DORMANT TUMOR BASED ON EXPERIMENTAL ANIMAL MODELS

Based on xenograft models of various human tumors inoculated or surgically implanted into immunodeficient animals, a ''dormant'' tumor can be defined by its microscopic size and nonexpanding mass. In contrast, a ''stable'' tumor is macroscopic and expanding in mass. In more detail, we define human nonangiogenic tumors as:

1. Unable to induce angiogenic activity, by repulsion of existing blood vessels in the local stroma and/or relative absence of intratumoral microvessels.
2. Remain harmless to the host until they switch to the angiogenic phenotype (i.e., may remain harmless for 1 year or more, which is half the life span of a mouse).
3. Express equal or more antiangiogenic (i.e., thrombospondin-1) than angiogenic (i.e., VEGF, bFGF) proteins.
4. Grow in vivo to ~1 mm in diameter or less, at which time further expansion ceases.
5. Only visible with a hand lens or a dissecting microscope (5–10 × magnification).
6. White or transparent by gross examination.
7. Unable to spontaneously metastasize from the microscopic dormant state.
8. Show active tumor cell proliferation and apoptosis in mice and remain metabolically active during the dormancy period.
9. Can be cloned from a human angiogenic tumor, because human tumors are heterogeneous and contain a mixture of nonangiogenic and angiogenic tumor cells.

In contrast, angiogenic human tumors (as observed in our animal models) are defined as:

1. Able to induce angiogenic activity, by recruiting blood vessels form the surrounding stroma and/or forming new blood vessels within the tumor tissue.
2. Lethal to the host in only few weeks.
3. Express significantly more angiogenic than antiangiogenic proteins.
4. Grow along an exponential curve until they kill the host.
5. Visible and easily detectable based on their macroscopic size.
6. Red by gross examination.
7. Can spontaneously metastasize to various organs.
8. Can be cloned from a human angiogenic tumor, because human tumors are heterogeneous and contain a mixture of nonangiogenic and angiogenic tumor cells.

1.6 IN VIVO IMAGING OF HUMAN DORMANT TUMORS

Traditionally, various in vivo imaging techniques have been used for the detection and quantification of tumors implanted orthotopically or ectopically (i.e., outside their orthotopic site). However, some of these techniques can be employed for the in vivo detection of microscopic dormant tumors. By definition, nonangiogenic, dormant tumors are microscopic in size. Therefore, they are usually undetectable by palpation (limited to tumor sizes 50 mm^3 and smaller) when located in the subcutaneous space or mammary fat pad. It is an even greater challenge to detect microscopic tumors located in internal organs. In the originally published dormancy model of osteosarcoma (MG-63), the presence of dormant tumors in a fraction of the inoculated mice was revealed through careful examination of the hair growth overlying the original tumor inoculation site.[48] The inner side of the skin in the area associated with hair growth contained a microscopic white lesion, from which a histology section showed a viable tumor. Although this detection method clearly reveals this interesting phenomenon, it is terminal (i.e., the animal has to be euthanized) and does not provide longitudinal quantitative information about the tumor size.

Stable infection of tumor cells with fluorescent proteins (such as green fluorescent protein [GFP] and red fluorescent protein [RFP]) or luciferase allows for in vivo longitudinal detection of tumors even at a microscopic size. GFP-expressing tumor cells can be visualized noninvasively from the skin surface by directed blue light (488 nm) epi-illumination. Submillimeter tumors can be localized using this method. The utility of fluorescence visualization of dormant tumors has been reported by Udagawa et al.,[48] using osteosarcoma (MG-63 and SAOS-2) and gastric cancer (ST-2) dormancy models. Tumor-associated blood vessels appear dark against the background of a fluorescent tumor tissue, allowing for morphological (e.g., vessel diameters, tortuousity, branching) and even functional (e.g., red blood cell velocity) quantification of angiogenesis.[49] More recently, this labeling technique was used to determine the minimum number of human tumor cells necessary to form a nonangiogenic, dormant microscopic tumor in mice (Naumov et al., unpublished). However, detecting fluorescently labeled tumors has its limitations. Microscopic tumors in internal organs can only be visualized ex vivo. Certain procedures, such as in vivo videomicroscopy, can be used for visualization of liver and lung metastases.[50,51] However, in the brain, excitation or emission of fluorescently labeled tumor cells is not only limited by tissue depth, but also by light penetration through the skull.

Infection of tumor cells with the luciferase reporter gene allows for the reliable detection in mice of a signal from tumors that are <1 mm in diameter (as verified by histology) in all internal organs, including the brain. Almog et al.[43] has used the luciferase method for the detection of dormant human liposarcoma tumors in the renal fat pad of mice. The method can also be used to monitor the growth of microscopic human glioblastomas stereotactically after tumor cells are inoculated in the brains of mice (Naumov et al., unpublished work). Following intravenous injection of the luciferine substrate, the enzymatic activity of luciferase is rapid and transient. Only viable and metabolically active tumor cells can be detected by luminescence. The transient effect of the enzymatic reaction allows for real-time detection of tumor cells and for monitoring their viability during the dormancy period, as well as at times throughout the angiogenic switch. The persistent luciferase signal during the dormancy period of microscopic human tumors confirms the previous conclusion (based on histology) that dormancy does not result from tumor cell cycle arrest or eradication. Although the intensity of the luciferase signal directly correlates with the size of tumor, this imaging modality does not provide a clear tumor boundary or an anatomical outline of the tumor. However, small animal magnetic resonance imaging (MRI) provides a clear anatomical definition of a microscopic tumor, and it can be effectively used in combination with luciferase imaging (Naumov et al., unpublished work). Recent reports have demonstrated that single cancer cells can be detected in a mouse brain using MRI.[52] Individual tumor cells trapped within the brain microcirculation were detected using MRI and validated using high-resolution confocal microscopy. Graham et al.[53] demonstrated that three-dimensional, high-frequency ultrasound can quantitatively monitor the growth of liver micrometastases as small as 0.5 mm in diameter.

Collectively, these recent advances in animal imaging modalities enable, in most cases, noninvasive, real-time, longitudinal observations of single cancer cell trafficking and detection of nonangiogenic microscopic tumors in vivo during the dormancy period and as they switch to the angiogenic phenotype. Quantitative imaging of tumors throughout their progression to the angiogenic phenotype can be used for evaluating the efficacy of antiangiogenic therapy in primary and metastatic tumors.

1.7 MOLECULAR MECHANISMS OF THE HUMAN ANGIOGENIC SWITCH

Transfection of human osteosarcoma (MG-63 and SAOS-2) and gastric cancer (ST-2) cells with activated c-*Ha-ras* oncogene induces loss of dormancy in otherwise nonangiogenic human cell lines.[48] When inoculated in immunosuppressed mice, wild-type (or control

vector-transfected) tumor cells did not form palpable tumors for more than 8 months. White tumor foci, which were avascular or contained sparse vessels, were found throughout the dormancy period at the site of inoculation. However, *ras*-transfected human osteosarcoma (MG-63 and SAOS-2) and gastric cancer (ST-2) cells formed vascularized large tumors within 1 month. The in vivo growth of *ras*-transfected tumor cells was associated with significantly increased angiogenic response, increased proliferation, and decreased apoptosis when compared with wild-type tumor cells. Loss of the dormant phenotype induced by activated *ras* correlated with increased levels (1.5 to 2.5-fold) of $VEGF_{165}$, as assessed in the conditioned media relative to the control tumor cells.[48] Overexpression of $VEGF_{165}$ in the tumor cells also resulted in a loss of dormancy and induced a robust angiogenic response in 30% of animals inoculated with gastric cancer and 40% of animals inoculated with osteosarcoma. In contrast to *ras*-transfected tumor cells, loss of dormancy in $VEGF_{165}$-transfected tumor cells was not associated with an increase in tumor cell proliferation, but was associated with reduced apoptosis. Therefore, the angiogenic response induced by $VEGF_{165}$ was found to be sufficient for the induction of a loss of dormancy by reducing apoptosis. Activation of *ras* can directly induce tumor cell proliferation and confer resistance to apoptosis.[54,55] In addition, *ras* activation can indirectly stimulate an angiogenic response in tumors by inducing proangiogenic proteins, such as VEGF,[56] and by downregulating angiogenesis inhibitors, such as thrombospondin.[57–60] Similar to *ras*, other oncogenes and tumor suppressor genes can indirectly affect tumor growth via an angiogenic mechanism. For example, *p53*, *PTEN*, and *Smad 4* have been shown to increase thrombospondin-1 expression by upregulation of *Tsp-1* gene or by increased mRNA expression.[12,61–63] Thrombospondin-1 expression can be decreased by *Myc*, *Ras*, *Id1*, *WT1*, c-*jun*, and v-*stc* via transcriptional repression, myc phosphorylation, or regulation of mRNA turnover and stability.[59,60,64–69] The inherently low toxicity of natural angiogenesis inhibitors, in addition to their selective effect on pathological neovascularization without harm to normal vasculature, makes them attractive therapeutic agents.

In addition to thrombospondin-1 regulation, the *p53* tumor suppressor gene regulates other currently unidentified inhibitors of angiogenesis.[70] Teodoro et al.[71] recently reported that wild-type *p53* mobilizes endostatin through a specific α(II) collagen prolyl-4-hydroxylase (α(II)PH gene product), which binds to *p53*. *p53* is inactivated in over 50% of all human tumors. Reintroduction of wild-type *p53* into mouse fibrosarcoma (T241) cells correlates with increased thrombospondin-1 expression and induces angiogenesis-restricted dormancy.[72] Inoculation of parental T241 fibrosarcoma cells into a mouse ear resulted in vascularized, visible tumors within 2 weeks. In contrast, when wild-type *p53* was introduced in the same cells, only 12% of the tumors became angiogenic 2 months after inoculation. Therefore, expression of wild-type *p53* resulted in the loss of an angiogenic phenotype. Loss of the angiogenic phenotype was also correlated with the upregulation of the mRNA-encoding thrombospondin-1. This experimental model demonstrated that *p53* can act as a tumor suppressor, independent of its direct effects on cell proliferation and survival. Moreover, *p53* had an indirect antitumor effect by inhibiting angiogenesis and increasing the rate of apoptosis.

In a recent report, Naumov et al.[44] compared the tumor cell secretion and intracellular levels of thrombospondin-1 in nonangiogenic and angiogenic tumor cell populations isolated from a human breast cancer cell line (MDA-MB-436). Angiogenic cells contain 2.5-fold higher levels of c-Myc and p-Myc than their nonangiogenic counterparts, as assessed by Western blot. In contrast, angiogenic tumor cells contain significantly lower levels of thrombospondin-1 than nonangiogenic tumor cells. Moreover, nonangiogenic human breast cancer cells secrete at least 20-fold higher levels of thrombospondin-1 than angiogenic cells. Similar findings were reported using a different human breast cancer cell line (MDA-MB-435).[60] Watnick et al.[60] reported that phosphoinositide 3-kinase (PI3K) can induce a signal transduction cascade leading to the phosphorylation of c-Myc and the subsequent repression of

thrombospondin-1. Treatment with LY294002 (a PI3K inhibitor) caused thrombospondin-1 levels within angiogenic cells to increase but had no effect on levels in nonangiogenic cells.[44] Therefore, the PI3K signaling pathway is responsible for the repression of thrombospondin-1, and it is regulated differently in angiogenic and nonangiogenic human tumor cells.

1.8 INDUCTION OF TUMOR DORMANCY USING ANTIANGIOGENIC THERAPY

Tumor progression is highly dependent on the surrounding stroma, including the endothelial cells, fibroblasts, local basement membrane factors, macrophages, platelets, T cells, and other cellular compartments. Antiangiogenic therapy can target the endothelial cell compartment in atleast two distinct ways: directly or indirectly (Figure 1.3).[73]

Direct angiogenesis inhibitors block vascular endothelial cells from proliferating, migrating, or increasing their survival. For example, SU 11248 directly blocks VEGF receptors (among other receptors involved with angiogenic signaling) on endothelial cells (Figure 1.3). Direct angiogenesis inhibitors include: (1) synthetic inhibitors or peptides designed to interfere with specific steps in the angiogenic process (e.g., inhibitors of metalloproteinases, antagonists of the $\alpha_V\beta_3$ or $\alpha_5\beta_1$ integrins), (2) low molecular weight molecules (e.g., TNP-470, caplostatin, thalidomide, 2-methoxyestradiol), and (3) endogenous (i.e., natural) angiogenesis inhibitors (e.g., TSP-1, platelet factor 4, interferon-α, IL-12, angiostatin, endostatin, arrestin, canstatin, tumstatin).[5,39,74–92]

Bouck et al.[93] were the first to demonstrate that a tumor can generate angiogenesis inhibitor (i.e., thrombospondin-1). They subsequently suggested that the angiogenic phenotype was a result of a net imbalance of endogenous angiogenesis stimulators and inhibitors.

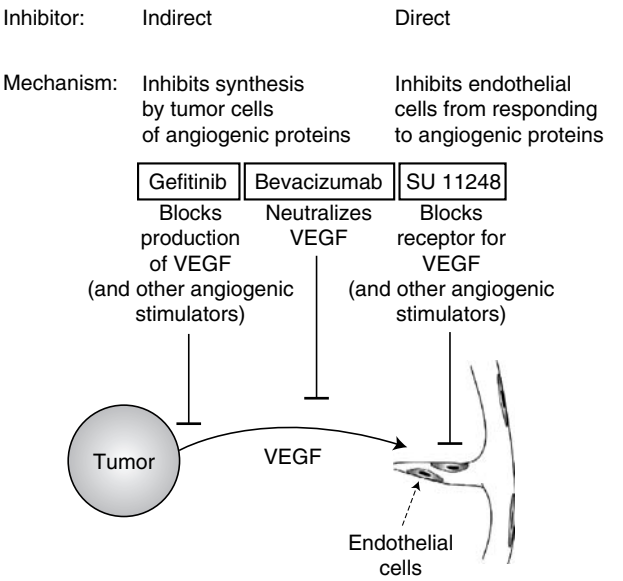

FIGURE 1.3 Examples of direct and indirect angiogenesis inhibitors that can block production of a tumor cell angiogenic protein (Gefitinib), or neutralize a systemic proangiogenic protein (Bevacizumab), or block a receptor for a tumor cell produced angiogenic protein (SU 11248). (Adapted from Folkman, J. et al., *Cancer Medicine*, 7th edn., 2006. With permission.)

In a series of experiments, Folkman and colleagues reported that the surgical removal of a primary Lewis lung carcinoma tumor in mice results in the exponential growth of lung metastases.[94,95] In these experimental animal models, the presence of a primary tumor generated increased circulating angiostatin levels. Angiostatin is a potent antiangiogenic plasminogen fragment,[96] which inhibits the in vivo growth of Lewis lung metastases by preventing neovascularization.[77] However, gene transfer of a cDNA coding for mouse angiostatin into murine T241 fibrosarcoma cells successfully suppressed lung metastatic tumor growth after the removal of the primary tumor.[97] Cao et al.[97] demonstrated that pulmonary micrometastases, expressing angiostatin, remain in a dormant and avascular state for 2–5 months after removal of primary tumors. These dormant micrometastases were characterized as having a high rate of apoptosis counterbalanced by a high proliferation rate.

Holmgren et al.[95] investigated whether treatment with an exogenous angiogenesis inhibitor could replace the endogenous angiogenesis suppressive ability of a primary tumor. In animals with surgically removed primary Lewis lung carcinoma, or T241 mouse sarcomas, treatment with TNP-470 resulted in the suppression of metastases comparable to that observed in the presence of the primary tumor. Therefore, exogenous treatment can be used to replace the endogenous angiogenesis inhibition of a primary tumor. Moreover, it can be used for the systemic suppression of angiogenesis-maintained micrometastases of both Lewis lung cancer and T241 fibrosarcoma in a dormant state. These dormant micrometastases were characterized as having high tumor cell proliferation counterbalanced by high cell death rate (i.e., apoptosis), indicating that inhibition of angiogenesis limits tumor growth by elevating tumor cell apoptosis. Exogenous angiogenesis inhibitors, such as TNP-470, mimic the primary tumor suppression by maintaining high apoptosis in the lung micrometastases, but without having an effect on tumor cell proliferation. A similar mechanism of sustained micrometastatic dormant state has been demonstrated using angiostatin, which maintains a high apoptotic index in lung metastases after the removal of a primary tumor without affecting tumor cell proliferation.[38]

Indirect angiogenesis inhibitors target tumor cell proteins created by oncogenes that drive the angiogenic switch. In general, their mechanism of action is by decreasing or blocking the expression of other tumor cell products, neutralizing the tumor cell product itself, or by blocking receptors on endothelial cells. The impact of oncogenes on tumor angiogenesis has been reviewed by Rak and Kerbel.[59,98,99] For example, gefitinib (Iressa) blocks VEGF production from tumor cells. However, even systemically available VEGF can be neutralized by bevacizumab (Avastin) before it binds to VEGF receptors on endothelial cells (Figure 1.3). There is an emerging group (e.g., thyrosine kinase inhibitors) of anticancer drugs originally developed to target oncogenes, which also have "indirect" antiangiogenic activity. For example, the *ras* farnesyl transferase inhibitors block oncogene signaling pathways, which upregulate tumor cell production of VEGF and downregulate production of Tsp-1.[100] Trastuzumab, an antibody that blocks *HER2/neu* receptor tyrosine kinase signaling, suppresses tumor cell production of angiogenic proteins, such as TGF-α, angiopoietin 1, plasminogen activator inhibitor-1 (PAI-1), and VEGF.[101,102] At the same time, trastuzumab has been shown to upregulate the expression of Tsp-1 (endogenous angiogenesis inhibitor), which may be an important mechanism of its antiangiogenic activity.[102] Upregulation of endogenous antiangiogenic proteins, using direct or indirect angiogenesis inhibitors, can be a useful approach for preventing the angiogenic switch and keeping human tumors in a microscopic dormant state (Figure 1.4).

Acquired drug resistance is a major obstacle in the treatment of cancer. Genetic instability, heterogeneity, and high mutational rates of tumor cells are the major causes of drug resistance.[103] In contrast, antiangiogenic therapy targets endothelial cells, which are genetically stable and have a low mutational rate. In an experimental animal model,

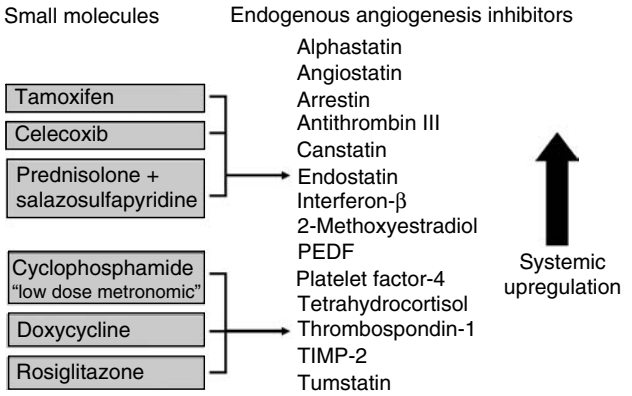

FIGURE 1.4 Summary of a few small molecules and the corresponding increase in endogenous angiogenesis inhibitors. Examples of small molecules that are orally available and may induce increased systemic levels of endogenous angiogenesis inhibitors. Chronic systemic increase of antiangiogenic proteins can prevent the angiogenic switch and delay the progression of cancer. (Adapted from Folkman, J., *Exp. Cell Res.*, 312(5), 594, 2006. With permission.)

Boehm et al.[104] reported that antiangiogenic therapy targeted against tumor-associated endothelial cells does not result in drug resistance. In this study, Lewis lung cancer, T241 fibrosarcoma, and B16F10 melanoma were repeatedly treated with endostatin. When tumors reached the size of ~350–400 mm^3, endostatin treatment was initiated until the tumor became undetectable. Endostatin therapy was then stopped, and the tumor was allowed to regrow. Endostatin therapy was resumed when tumors reached a mean volume of 350–400 mm^3. After the second (for melanoma), fourth (for fibrosarcoma), and sixth (for Lewis lung carcinoma) cycles of endostatin treatment, all tumors remained as barely visible subcutaneous nodules (size 5–50 mm^3) for up to 360 days. In contrast, endostatin resistance developed rapidly when Lewis lung carcinomas were treated with conventional cytotoxic chemotherapy. These studies demonstrated that repeated cycles of endostatin therapy induced tumor dormancy, which persisted indefinitely after therapy.

1.9 METASTATIC DORMANCY

Metastasis, the spread of cancer from a primary tumor to secondary organs, is the major cause of cancer-related deaths. Hematogenous or lymphatic spread of only a few cancer cells from a primary tumor can successfully form a macroscopic tumor at a secondary site.[50,105,106] However, for a macrometastasis to become lethal, it must successfully complete a number of steps (Figure 1.1).[107] Two different types of tumor dormancy have been identified in the metastatic process: (1) solitary dormant cancer cells, which are in G_0 cell cycle arrest and (2) dormant nonangiogenic tumors, in which tumor cells are actively proliferating and dying, but the tumor fails to recruit blood vessels. Both of these steps can contribute to a latency period associated with metastatic growth of human cancer (Figure 1.1).[108]

Previous studies by Holmgren and colleagues[95,109] have identified nonangiogenic micrometastases as a potential contributor to metastatic dormancy. These studies showed that dormant micrometastases did not grow in size beyond 200 μm, but they remained metabolically active. This size limitation was associated with a steady-state balance between the rates of tumor cell proliferation and apoptosis, with no net growth of the metastases. Changes in the intrinsic properties of these dormant micrometastases, or their microenvironment at a later time, triggered metastatic growth associated with a disturbance of the proliferation and apoptosis balance. Progressive growth in such micrometastases was restricted due to suppression of tumor angiogenesis.

Naumov et al.[110] have identified another possible source of metastatic dormancy: viable, solitary dormant tumor cells that are neither proliferating nor undergoing apoptosis after arriving at a metastatic site. These studies showed that more than 50%–80% of breast cancer cells, distributed to mouse liver via the circulation, can remain in the tissue for extended periods of time (up to 77 days) as solitary nonproliferating dormant cells. This surprising phenomenon was observed in populations of breast cancer cells of high and low metastatic ability. In the case of the highly metastatic cell line (D2A1 cells), lethal macrometastases grew from a very small subset of cells (~0.006%), with the majority (~80% cell loss) of injected cells undergoing apoptosis or destroyed by leukocytes. However, ~20% of the injected cells persisted as nonproliferating dormant cells. In contrast, ~80% of poorly metastatic breast cancer cells remained as nonproliferating dormant solitary cells in the mouse liver. A subset of these cells could be recovered and grown under in vitro culture conditions 11 weeks after injection into mice. The recovered tumor cells retained their ability to form primary tumors in the mammary fat pad of mice. These solitary dormant tumor cells may be a potential source of an occasional nonangiogenic metastasis, and of an even rarer, but lethal, angiogenic metastasis.

Taken together, these studies demonstrated that metastasis is a dynamic process, where solitary, nonproliferating dormant cancer cells, nonangiogenic micrometastases, and angiogenic macrometastases can coexist at each stage of the metastatic process. While nonangiogenic micrometastases could be vulnerable to antiangiogenic and cytotoxic chemotherapeutic agents (administered in a metronomic, low-dose regimen, as described by Browder et al.[111] and Kerbel and colleagues[112,113]), solitary dormant cells could remain unaffected because of their inability to proliferate.

Naumov et al.[114] showed that nonproliferating solitary dormant breast cancer cells remained unaffected by doxorubicin treatment. However, the same treatment successfully inhibited actively growing macrometastases in the same mice. Therefore, doxorubicin chemotherapy, which successfully reduced the metastatic burden, failed to affect the number of solitary dormant cells. These findings have important clinical implications for patients undergoing adjuvant chemotherapy. It is possible that dormant nonproliferating tumor cells can remain unaffected by standard chemotherapy and may retain their potential to initiate growth at a later date. Both solitary cancer cells and nonangiogenic metastases can remain dormant and undetectable for months or years, leading to an uncertainty in the prognosis for patients who have already been treated for the primary cancer.

1.10 ANGIOGENIC SWITCH-RELATED BIOMARKERS FOR DETECTION OF DORMANT TUMORS

Even with recent advances in the clinical detection of human cancer, a tumor that is microscopic in size (~1 mm in diameter) remains undetectable. A panel of angiogenic switch-related biomarkers is under development using the human tumor dormancy models. These biomarkers include circulating endothelial progenitor cells (CEPs) and platelets in the blood, as well as matrix metalloproteinases (MMPs) in the urine. The detection of a single microscopic human tumor in existing animal models can be achieved using each one of these biomarkers alone or in combination.[115–117]

We compared the in vivo ability of angiogenic and nonangiogenic human breast tumors (MDA-MB-436 cells) to mobilize mature circulating endothelial cells (CECs) (CD45−, Flk+, CD31+, CD117−) and CEPs (CD45−, Flk+, CD31+, CD117+).[115] The number of blood-borne CECs and CEPs was quantified using a flow cytometer. There was little difference in the percent of mature CECs in the blood of mice inoculated with angiogenic and nonangiogenic cells. However, mice inoculated with nonangiogenic cells had approximately fourfold decrease in CEPs when compared with control mice. Mice inoculated with angiogenic cells

had levels of CEPs comparable to those in the control mice. Previous reports[44] have shown that these nonangiogenic breast cancer cells (MDA-MB-436 cells) secrete at least 20-fold higher levels of thrombospondin-1 (Tsp-1) than their angiogenic counterparts. Other studies have suggested that endogenous inhibitors of angiogenesis, such as Tsp-1 and endostatin, may inhibit the mobilization of CEPs.[118] These observations suggest that microscopic dormant (nonangiogenic) tumors may suppress the mobilization of CEPs from the bone marrow via systemic thrombospondin-1.

Klement et al.[117] recently reported that blood platelets can sequester both pro- and antiangiogenic proteins. It is estimated that at least 100 billion platelets are produced per day by megakaryocytes in the bone marrow of an average 70 kg person.[119,120] With a life span of 7–8 days in humans, it is estimated that there are approximately a trillion platelets in constant circulation.[119,121] Folkman and colleagues proposed that the platelet compartment of the blood stream can potentially accumulate angiogenesis-related proteins and possibly release them at a later time.[117] Using a novel "platelet angiogenic proteome," as quantitatively assessed by SELDI-ToF technology (Ciphergen, Freemont, CA), the presence of microscopic human tumors in mice can be detected.[117] Using this technology, the accumulation and reduction in angiogenesis-related proteins sequestered in platelets can be quantitatively followed throughout the angiogenic switch. The identification of proteins that are associated with the angiogenic switch and that may be used as angiogenic switch-related biomarkers is currently under investigation.

In summary, the tumor dormancy animal models presented here permit further clarification of the role of CEC/CEPs, platelets, and MMPs as participants in the "angiogenic switch." Moreover, this angiogenic switch-related biomarker panel may prove to be a useful diagnostic method for the presence of microscopic cancers at primary and metastatic sites long before detection by conventional methods. It may be feasible to develop a panel of angiogenesis-associated biomarkers that can identify the presence of a microscopic human tumor, predict its switch to the angiogenic phenotype, and possibly serve as a guide for antiangiogenic therapy. In the future, it may be possible for a patient who is at risk for cancer recurrence to take an oral drug that can elevate endogenous platelet-associated antiangiogenic proteins and delay, if not prevent, the formation of recurrent tumors.

1.11 CONCLUSION

We speculate that the development of more specific and sensitive biomarkers may permit the very early detection of recurrent cancer, possibly years before symptoms or anatomical location. If this concept can be validated, then relatively nontoxic angiogenesis inhibitors may be used to "treat the biomarkers" without ever seeing the recurrent tumor (i.e., cancer without disease).[14]

ACKNOWLEDGMENTS

The authors thank Ms. Jenny Grillo for critical reading and editing of this chapter. We also thank Kristin Johnson for help with graphics. This work was supported by the Breast Cancer Research Foundation, NIH Program Project (grant #P01CA45548), and an Innovator Award from the Department of Defense.

REFERENCES

1. Demicheli R, Abbattista A, Miceli R, Valagussa P, Bonadonna G. Time distribution of the recurrence risk for breast cancer patients undergoing mastectomy: further support about the concept of tumor dormancy. *Breast Cancer Res Treat* 1996;41(2):177–185.

2. Karrison TG, Ferguson DJ, Meier P. Dormancy of mammary carcinoma after mastectomy. *J Natl Cancer Inst* 1999;91(1):80–85.
3. Willis RA. *Pathology of tumours*. London: Butterworth; 1948.
4. Hadfield G. The dormant cancer cell. *Br Med J* 1954;4888:607–610.
5. Rastinejad F, Polverini PJ, Bouck NP. Regulation of the activity of a new inhibitor of angiogenesis by a cancer suppressor gene. *Cell* 1989;56(3):345–355.
6. Wheelock EF, Weinhold KJ, Levich J. The tumor dormant state. *Adv Cancer Res* 1981;34:107–140.
7. Saudemont A, Jouy N, Hetuin D, Quesnel B. NK cells that are activated by CXCL10 can kill dormant tumor cells that resist CTL-mediated lysis and can express B7-H1 that stimulates T cells. *Blood* 2005;105(6):2428–2435.
8. Stewart TH. Immune mechanisms and tumor dormancy. *Medicina (B Aires)* 1996;56(Suppl 1): 74–82.
9. Saudemont A, Quesnel B. In a model of tumor dormancy, long-term persistent leukemic cells have increased B7-H1 and B7.1 expression and resist CTL-mediated lysis. *Blood* 2004;104(7):2124–2133.
10. Marches R, Scheuermann R, Uhr J. Cancer dormancy: from mice to man. *Cell Cycle* 2006;5(16): 1772–1778.
11. Jain RK, Safabakhsh N, Sckell A et al. Endothelial cell death, angiogenesis, and microvascular function after castration in an androgen-dependent tumor: role of vascular endothelial growth factor. *Proc Natl Acad Sci USA* 1998;95(18):10820–10825.
12. Dameron KM, Volpert OV, Tainsky MA, Bouck N. Control of angiogenesis in fibroblasts by p53 regulation of thrombospondin-1. *Science* 1994;265(5178):1582–1584.
13. O'Reilly MS, Holmgren L, Shing Y et al. Angiostatin: a circulating endothelial cell inhibitor that suppresses angiogenesis and tumor growth. *Cold Spring Harb Symp Quant Biol* 1994;59:471–482.
14. Folkman J, Kalluri R. Cancer without disease. *Nature* 2004;427(6977):787.
15. Folkman J, Heymach J, Kalluri R. *Tumor angiogenesis*. 7th edn. Hamilton, Ontario: B.C. Decker; 2006.
16. Folkman J. Tumor angiogenesis: therapeutic implications. *N Engl J Med* 1971;285(21):1182–1186.
17. Folkman J. What is the evidence that tumors are angiogenesis dependent? *J Natl Cancer Inst* 1990;82(1):4–6.
18. Coman D, Sheldon WF. The significance of hyperemia around tumor implants. *Am J Pathol* 1946;22:821–831.
19. Warren B. The vascular morphology of tumors. In: Peterson H-I, ed. *Tumor blood circulation: angiogenesis, vascular morphology and blood flow of experimental human tumors*. Florida: CRC Press; 1979:1–47.
20. Ide A, Baker NH, Warren SL. Vascularization of the Brown–Pearce rabbit epithelioma transplant as seen in the transparent ear chamber. *Am J Roentgenol* 1939;42:891–899.
21. Algire G, Legallais, FY. Growth rate of transplanted tumors in relation to latent period and host vascular reaction. *Cancer Res* 1947;7:724.
22. Algire G, Chalkely HW, Legallais FY, Park H. 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* 1945;6:73–85.
23. Folkman J. Toward an understanding of angiogenesis: search and discovery. *Perspect Biol Med* 1985;29(1):10–36.
24. Folkman J, Long DM, Jr., Becker FF. Growth and metastasis of tumor in organ culture. *Cancer* 1963;16:453–467.
25. Folkman J, Cole P, Zimmerman S. Tumor behavior in isolated perfused organs: in vitro growth and metastases of biopsy material in rabbit thyroid and canine intestinal segment. *Ann Surg* 1966;164(3):491–502.
26. Folkman J. Anti-angiogenesis: new concept for therapy of solid tumors. *Ann Surg* 1972;175(3):409–416.
27. Folkman J. The vascularization of tumors. *Sci Am* 1976;234(5):58–64, 70–73.
28. Folkman J. The intestine as an organ culture. In: Burdette W, ed. *Carcinoma of the colon and antecedent epithelium*. Springfield (IL): CC Thomas; 1970:113–127.
29. Folkman J, Brem H. Angiogenesis and inflammation. In: Gallin JI, Goldstein IM, Snyderman R, eds. *Inflammation: basic principles and clinical correlates*. 2nd edn. New York: Raven Press; 1992:821–839.

30. Folkman J. Angiogenesis in arthritis. In: Smolen J, Lipsky P, eds. *Targeted therapies in rheumatology*. London: Martin Dunitz; 2003:111–131.
31. Folkman J, Watson K, Ingber D, Hanahan D. Induction of angiogenesis during the transition from hyperplasia to neoplasia. *Nature* 1989;339(6219):58–61.
32. Torres Filho IP, Leunig M, Yuan F, Intaglietta M, Jain RK. Noninvasive measurement of microvascular and interstitial oxygen profiles in a human tumor in SCID mice. *Proc Natl Acad Sci USA* 1994;91(6):2081–2085.
33. North S, Moenner M, Bikfalvi A. Recent developments in the regulation of the angiogenic switch by cellular stress factors in tumors. *Cancer Lett* 2005;218(1):1–14.
34. Greene HSN. Heterologous transplantation of mammalian tumors. *J Exp Med* 1941;73:461–486.
35. Gimbrone MA, Jr., Aster RH, Cotran RS, Corkery J, Jandl JH, Folkman J. Preservation of vascular integrity in organs perfused in vitro with a platelet-rich medium. *Nature* 1969;222(188):33–36.
36. Gimbrone MA, Jr., Leapman SB, Cotran RS, Folkman J. Tumor dormancy in vivo by prevention of neovascularization. *J Exp Med* 1972;136(2):261–276.
37. Brem H, Folkman J. Inhibition of tumor angiogenesis mediated by cartilage. *J Exp Med* 1975;141(2):427–439.
37a. Moses MA, Sudhalter J, Langer R. Identification of an inhibitor of neovascularization from the cartilage. *Science* 1990;248:1408–1410.
38. O'Reilly MS, Holmgren L, Chen C, Folkman J. Angiostatin induces and sustains dormancy of human primary tumors in mice. *Nat Med* 1996;2(6):689–692.
39. O'Reilly MS, Boehm T, Shing Y et al. Endostatin: an endogenous inhibitor of angiogenesis and tumor growth. *Cell* 1997;88(2):277–285.
40. Hanahan D, Folkman J. Patterns and emerging mechanisms of the angiogenic switch during tumorigenesis. *Cell* 1996;86(3):353–364.
41. Hanahan D, Christofori G, Naik P, Arbeit J. Transgenic mouse models of tumour angiogenesis: the angiogenic switch, its molecular controls, and prospects for preclinical therapeutic models. *Eur J Cancer* 1996;32A(14):2386–2393.
42. Achilles EG, Fernandez A, Allred EN et al. Heterogeneity of angiogenic activity in a human liposarcoma: a proposed mechanism for "no take" of human tumors in mice. *J Natl Cancer Inst* 2001;93(14):1075–1081.
43. Almog N, Henke V, Flores L et al. Prolonged dormancy of human liposarcoma is associated with impaired tumor angiogenesis. *FASEB J* 2006;20(7):947–949.
44. Naumov GN, Bender E, Zurakowski D et al. A model of human tumor dormancy: an angiogenic switch from the nonangiogenic phenotype. *J Natl Cancer Inst* 2006;98(5):316–325.
45. Aguirre Ghiso JA, Kovalski K, Ossowski L. Tumor dormancy induced by downregulation of urokinase receptor in human carcinoma involves integrin and MAPK signaling. *J Cell Biol* 1999;147(1):89–104.
46. Aguirre-Ghiso JA, Liu D, Mignatti A, Kovalski K, Ossowski L. Urokinase receptor and fibronectin regulate the ERK(MAPK) to p38(MAPK) activity ratios that determine carcinoma cell proliferation or dormancy in vivo. *Mol Biol Cell* 2001;12(4):863–879.
47. Aguirre-Ghiso JA, Estrada Y, Liu D, Ossowski L. ERK(MAPK) activity as a determinant of tumor growth and dormancy; regulation by p38(SAPK). *Cancer Res* 2003;63(7):1684–1695.
48. Udagawa T, Fernandez A, Achilles EG, Folkman J, D'Amato RJ. Persistence of microscopic human cancers in mice: alterations in the angiogenic balance accompanies loss of tumor dormancy. *FASEB J* 2002;16(11):1361–1370.
49. Naumov GN, Wilson SM, MacDonald IC et al. Cellular expression of green fluorescent protein, coupled with high-resolution in vivo videomicroscopy, to monitor steps in tumor metastasis. *J Cell Sci* 1999;112(Pt 12):1835–1842.
50. Chambers AF, Groom AC, MacDonald IC. Dissemination and growth of cancer cells in metastatic sites. *Nat Rev Cancer* 2002;2(8):563–572.
51. Naumov GN, MacDonald IC, Chambers AF, Groom AC. Solitary cancer cells as a possible source of tumour dormancy? *Semin Cancer Biol* 2001;11(4):271–276.
52. Heyn C, Ronald JA, Mackenzie LT et al. In vivo magnetic resonance imaging of single cells in mouse brain with optical validation. *Magn Reson Med* 2006;55(1):23–29.

53. Graham KC, Wirtzfeld LA, MacKenzie LT et al. Three-dimensional high-frequency ultrasound imaging for longitudinal evaluation of liver metastases in preclinical models. *Cancer Res* 2005;65(12):5231–5237.
54. Goustin AS, Leof EB, Shipley GD, Moses HL. Growth factors and cancer. *Cancer Res* 1986;46(3):1015–1029.
55. Bonni A, Brunet A, West AE, Datta SR, Takasu MA, Greenberg ME. Cell survival promoted by the Ras-MAPK signaling pathway by transcription-dependent and -independent mechanisms. *Science* 1999;286(5443):1358–1362.
56. Rak J, Mitsuhashi Y, Bayko L et al. Mutant *ras* oncogenes upregulate VEGF/VPF expression: implications for induction and inhibition of tumor angiogenesis. *Cancer Res* 1995; 55(20):4575–4580.
57. Good DJ, Polverini PJ, Rastinejad F et al. A tumor suppressor-dependent inhibitor of angiogenesis is immunologically and functionally indistinguishable from a fragment of thrombospondin. *Proc Natl Acad Sci USA* 1990;87(17):6624–6628.
58. Sheibani N, Frazier WA. Repression of thrombospondin-1 expression, a natural inhibitor of angiogenesis, in polyoma middle T transformed NIH3T3 cells. *Cancer Lett* 1996;107(1):45–52.
59. Rak J, Yu JL, Klement G, Kerbel RS. Oncogenes and angiogenesis: signaling three-dimensional tumor growth. *J Investig Dermatol Symp Proc* 2000;5(1):24–33.
60. Watnick RS, Cheng YN, Rangarajan A, Ince TA, Weinberg RA. Ras modulates Myc activity to repress thrombospondin-1 expression and increase tumor angiogenesis. *Cancer Cell* 2003;3(3):219–231.
61. Volpert OV, Dameron KM, Bouck N. Sequential development of an angiogenic phenotype by human fibroblasts progressing to tumorigenicity. *Oncogene* 1997;14(12):1495–1502.
62. Chandrasekaran L, He CZ, Al-Barazi H, Krutzsch HC, Iruela-Arispe ML, Roberts DD. Cell contact-dependent activation of alpha3beta1 integrin modulates endothelial cell responses to thrombospondin-1. *Mol Biol Cell* 2000;11(9):2885–2900.
63. Lawler J, Sunday M, Thibert V et al. Thrombospondin-1 is required for normal murine pulmonary homeostasis and its absence causes pneumonia. *J Clin Invest* 1998;101(5):982–992.
64. Volpert OV. Modulation of endothelial cell survival by an inhibitor of angiogenesis thrombospondin-1: a dynamic balance. *Cancer Metastasis Rev* 2000;19(1–2):87–92.
65. Mettouchi A, Cabon F, Montreau N et al. SPARC and thrombospondin genes are repressed by the c-*jun* oncogene in rat embryo fibroblasts. *EMBO J* 1994;13(23):5668–5678.
66. Dejong V, Degeorges A, Filleur S et al. The Wilms' tumor gene product represses the transcription of thrombospondin 1 in response to overexpression of c-*Jun*. *Oncogene* 1999;18(20):3143–3151.
67. Slack JL, Bornstein P. Transformation by v-*src* causes transient induction followed by repression of mouse thrombospondin-1. *Cell Growth Differ* 1994;5(12):1373–1380.
68. Tikhonenko AT, Black DJ, Linial ML. Viral Myc oncoproteins in infected fibroblasts down-modulate thrombospondin-1, a possible tumor suppressor gene. *J Biol Chem* 1996; 271(48):30741–30747.
69. Janz A, Sevignani C, Kenyon K, Ngo CV, Thomas-Tikhonenko A. Activation of the myc oncoprotein leads to increased turnover of thrombospondin-1 mRNA. *Nucleic Acids Res* 2000;28(11):2268–2275.
70. Van Meir EG, Kikuchi T, Tada M et al. Analysis of the *p53* gene and its expression in human glioblastoma cells. *Cancer Res* 1994;54(3):649–652.
71. Teodoro JG, Parker AE, Zhu X, Green MR. p53-mediated inhibition of angiogenesis through up-regulation of a collagen prolyl hydroxylase. *Science* 2006;313(5789):968–971.
72. Holmgren L, Jackson G, Arbiser J. p53 induces angiogenesis-restricted dormancy in a mouse fibrosarcoma. *Oncogene* 1998;17(7):819–824.
73. Kerbel R, Folkman J. Clinical translation of angiogenesis inhibitors. *Nat Rev Cancer* 2002;2(10):727–739.
74. Taylor S, Folkman J. Protamine is an inhibitor of angiogenesis. *Nature* 1982;297(5864):307–312.
75. Ingber D, Fujita T, Kishimoto S et al. Synthetic analogues of fumagillin that inhibit angiogenesis and suppress tumour growth. *Nature* 1990;348(6301):555–557.
76. Brooks PC, Montgomery AM, Rosenfeld M et al. Integrin alpha v beta 3 antagonists promote tumor regression by inducing apoptosis of angiogenic blood vessels. *Cell* 1994;79(7):1157–1164.

77. O'Reilly MS, Holmgren L, Shing Y et al. Angiostatin: a novel angiogenesis inhibitor that mediates the suppression of metastases by a Lewis lung carcinoma. *Cell* 1994;79(2):315–328.
78. O'Reilly MS, Pirie-Shepherd S, Lane WS, Folkman J. Antiangiogenic activity of the cleaved conformation of the serpin antithrombin. *Science* 1999;285(5435):1926–1928.
79. Stetler-Stevenson WG, Krutzsch HC, Liotta LA. Tissue inhibitor of metalloproteinase (TIMP-2). A new member of the metalloproteinase inhibitor family. *J Biol Chem* 1989;264(29):17374–17378.
80. Moses MA, Sudhalter J, Langer R. Identification of an inhibitor of neovascularization from cartilage. *Science* 1990;248(4961):1408–1410.
81. Kruger EA, Figg WD. TNP-470: an angiogenesis inhibitor in clinical development for cancer. *Expert Opin Investig Drugs* 2000;9(6):1383–1396.
82. D'Amato RJ, Loughnan MS, Flynn E, Folkman J. Thalidomide is an inhibitor of angiogenesis. *Proc Natl Acad Sci USA* 1994;91(9):4082–4085.
83. Folkman J. Angiogenesis-dependent diseases. *Semin Oncol* 2001;28(6):536–542.
84. Crum R, Szabo S, Folkman J. A new class of steroids inhibits angiogenesis in the presence of heparin or a heparin fragment. *Science* 1985;230(4732):1375–1378.
85. D'Amato RJ, Lin CM, Flynn E, Folkman J, Hamel E. 2-Methoxyestradiol, an endogenous mammalian metabolite, inhibits tubulin polymerization by interacting at the colchicine site. *Proc Natl Acad Sci USA* 1994;91(9):3964–3968.
86. Fotsis T, Zhang Y, Pepper MS et al. The endogenous oestrogen metabolite 2-methoxyoestradiol inhibits angiogenesis and suppresses tumour growth. *Nature* 1994;368(6468):237–239.
87. Voest EE, Kenyon BM, O'Reilly MS, Truitt G, D'Amato RJ, Folkman J. Inhibition of angiogenesis in vivo by interleukin 12. *J Natl Cancer Inst* 1995;87(8):581–586.
88. Maione TE, Gray GS, Petro J et al. Inhibition of angiogenesis by recombinant human platelet factor-4 and related peptides. *Science* 1990;247(4938):77–79.
89. Colorado PC, Torre A, Kamphaus G et al. Anti-angiogenic cues from vascular basement membrane collagen. *Cancer Res* 2000;60(9):2520–2526.
90. Kamphaus GD, Colorado PC, Panka DJ et al. Canstatin, a novel matrix-derived inhibitor of angiogenesis and tumor growth. *J Biol Chem* 2000;275(2):1209–1215.
91. Maeshima Y, Colorado PC, Torre A et al. Distinct antitumor properties of a type IV collagen domain derived from basement membrane. *J Biol Chem* 2000;275(28):21340–21348.
92. Dawson DW, Volpert OV, Gillis P et al. Pigment epithelium-derived factor: a potent inhibitor of angiogenesis. *Science* 1999;285(5425):245–248.
93. Bouck N. Tumor angiogenesis: the role of oncogenes and tumor suppressor genes. *Cancer Cells* 1990;2(6):179–185.
94. O'Reilly M, Rosenthal R, Sage HE, Smith S, Holmgren L, Moses M, Shing Y, Folkman J. The suppression of tumor metastases by a primary tumor. *Surg Forum* 1993;44:474–476.
95. Holmgren L, O'Reilly MS, Folkman J. Dormancy of micrometastases: balanced proliferation and apoptosis in the presence of angiogenesis suppression. *Nat Med* 1995;1(2):149–153.
96. Lay AJ, Jiang XM, Kisker O et al. Phosphoglycerate kinase acts in tumour angiogenesis as a disulphide reductase. *Nature* 2000;408(6814):869–873.
97. Cao Y, O'Reilly MS, Marshall B, Flynn E, Ji RW, Folkman J. Expression of angiostatin cDNA in a murine fibrosarcoma suppresses primary tumor growth and produces long-term dormancy of metastases. *J Clin Invest* 1998;101(5):1055–1063.
97a. Camphausen K, Moses MA, Beecken WD, Khan MK, Folkman J, O'Reilly MS. Radiation therapy to a primary tumor accelerates metastatic growth in mice. 2001;61(5):2207–2211.
98. Kerbel RS, Viloria-Petit A, Okada F, Rak J. Establishing a link between oncogenes and tumor angiogenesis. *Mol Med* 1998;4(5):286–295.
99. Rak J, Yu JL, Kerbel RS, Coomber BL. What do oncogenic mutations have to do with angiogenesis/vascular dependence of tumors? *Cancer Res* 2002;62(7):1931–1934.
100. Okada F, Rak JW, Croix BS et al. Impact of oncogenes in tumor angiogenesis: mutant K-*ras* up-regulation of vascular endothelial growth factor/vascular permeability factor is necessary, but not sufficient for tumorigenicity of human colorectal carcinoma cells. *Proc Natl Acad Sci USA* 1998;95(7):3609–3614.

101. Petit AM, Rak J, Hung MC et al. Neutralizing antibodies against epidermal growth factor and ErbB-2/neu receptor tyrosine kinases down-regulate vascular endothelial growth factor production by tumor cells in vitro and in vivo: angiogenic implications for signal transduction therapy of solid tumors. *Am J Pathol* 1997;151(6):1523–1530.
102. Izumi Y, Xu L, di Tomaso E, Fukumura D, Jain RK. Tumour biology: herceptin acts as an anti-angiogenic cocktail. *Nature* 2002;416(6878):279–280.
103. Moscow J, Schneider E, Sikic BI, Morrow CS, Cowan KH. Drug resistance and its clinical circumvention. In: Kufe DW, Bast RC, Jr., Hite WH, Hong WK, Pollock RE, Weichselbaum RR, Holland JF, Frei E, III, eds. *Cancer medicine*. Hamilton: B.C. Decker Inc.; 2006:630–647.
104. Boehm T, Folkman J, Browder T, O'Reilly MS. Antiangiogenic therapy of experimental cancer does not induce acquired drug resistance. *Nature* 1997;390(6658):404–407.
105. Chambers AF, Naumov GN, Vantyghem SA, Tuck AB. Molecular biology of breast cancer metastasis. Clinical implications of experimental studies on metastatic inefficiency. *Breast Cancer Res* 2000;2(6):400–407.
106. Weiss L. Metastatic inefficiency. *Adv Cancer Res* 1990;54:159–211.
107. Fidler IJ. Antivascular therapy of cancer metastasis. *J Surg Oncol* 2006;94(3):178–180.
108. Naumov GN, Akslen LA, Folkman J. Role of angiogenesis in human tumor dormancy: animal models of the angiogenic switch. *Cell Cycle* 2006;5(16).
109. Murray C. Tumour dormancy: not so sleepy after all. *Nat Med* 1995;1(2):117–118.
110. Naumov GN, MacDonald IC, Weinmeister PM et al. Persistence of solitary mammary carcinoma cells in a secondary site: a possible contributor to dormancy. *Cancer Res* 2002;62(7):2162–2168.
111. Browder T, Butterfield CE, Kraling BM et al. Antiangiogenic scheduling of chemotherapy improves efficacy against experimental drug-resistant cancer. *Cancer Res* 2000;60(7):1878–1886.
112. Kerbel RS, Viloria-Petit A, Klement G, Rak J. 'Accidental' anti-angiogenic drugs. Anti-oncogene directed signal transduction inhibitors and conventional chemotherapeutic agents as examples. *Eur J Cancer* 2000;36(10):1248–1257.
113. Klement G, Baruchel S, Rak J et al. Continuous low-dose therapy with vinblastine and VEGF receptor-2 antibody induces sustained tumor regression without overt toxicity. *J Clin Invest* 2000;105(8):R15–R24.
114. Naumov GN, Townson JL, MacDonald IC et al. Ineffectiveness of doxorubicin treatment on solitary dormant mammary carcinoma cells or late-developing metastases. *Breast Cancer Res Treat* 2003;82(3):199–206.
115. Naumov GN, Beaudry P, Bender ER, Zurakowski D, Watnick R, Almog N, Heymach J, Folkman J. Suppression of circulating endothelial progenitor cells by human dormant breast cancer cells. *Clin Exp Met* 2004;21(7):636.
116. Harper J, Naumov GN, Exarhopoulos A, Bender E, Louis G, Folkman J, Moses MA. Predicting the switch to the angiogenic phenotype in a human tumor model. In: Proceedings of the American Association for *Cancer Res* 2006:837.
117. Klement G, Kikuchi L, Kieran M, Almog N, Yip T, Folkman J. Early tumor detection using platelet uptake of angiogenesis regulators. Blood 2004;104:239a, abstract 839.
118. Schuch G, Heymach JV, Nomi M et al. Endostatin inhibits the vascular endothelial growth factor-induced mobilization of endothelial progenitor cells. *Cancer Res* 2003;63(23):8345–8350.
119. Harker LA, Finch CA. Thrombokinetics in man. *J Clin Invest* 1969;48(6):963–974.
120. Italiano JE, Hartwig JH. Megakaryocyte development and platelet formation. In: Michelson AD, ed. *Platelets*. Boston: Academic Press; 2002:21–36.
121. Kaushansky K. Lineage-specific hematopoietic growth factors. *N Engl J Med* 2006; 354(19):2034–2045.

2 Vascular Endothelial Growth Factor: Basic Biology and Clinical Implications

Napoleone Ferrara

CONTENTS

The observation that tumor growth can be accompanied by increased vascularity was reported more than one century ago [for review, see (1)]. In 1939, Ide et al. postulated for the first time the existence of a tumor-derived blood vessel growth-stimulating factor (2). In 1945, Algire et al. advanced this concept, hypothesizing that rapid tumor growth is crucially dependent on the development of a neovascular supply (3). In 1971, Folkman (4) proposed that antiangiogenesis may be a valid strategy to treat human cancer and a search for regulators of angiogenesis that may also represent therapeutic targets began.

Neovascularization is essential also for physiological processes such as embryogenesis, tissue repair, and reproductive functions (5). The development of the vascular tree initially occurs by "vasculogenesis," the in situ differentiation of endothelial cell precursors, the angioblasts, from the hemangioblasts (6). The juvenile vascular system then evolves from the primary capillary plexus by subsequent pruning and reorganization of endothelial cells in a process called "angiogenesis" (7). Recent studies suggest that incorporation of bone

marrow-derived endothelial progenitor cells (EPC) in the growing vessels complements the sprouting of resident endothelial cells (8–12). Additionally, a subset of perivascular monocytes seems to be particularly important for new vessel growth (13).

Many potential angiogenic factors have been described over the last two decades (14,15). Much evidence indicates that vascular endothelial growth factor (VEGF) is a particularly important regulator of angiogenesis (1). While new vessel growth and maturation are highly complex and coordinated processes, requiring the sequential activation of a series of receptors (e.g., Tie1, Tie2, and platelet-derived growth factor receptor (PDGFR-β)) by numerous ligands in endothelial and mural cells [for recent reviews, see (16,17)], VEGF action often represents a rate-limiting step in angiogenesis. VEGF (referred to also as VEGF-A) belongs to a gene family that includes placenta growth factor (PlGF) (18), VEGF-B (19), VEGF-C (20), and VEGF-D (21,22). VEGF-C and VEGF-D regulate lymphangiogenesis (23).

2.1 IDENTIFICATION OF VEGF

Independent lines of research contributed to the discovery of VEGF, emphasizing the biological complexity of this molecule (1).

In 1983, Senger et al. (24) described the identification in the conditioned medium of a guinea pig tumor cell line of a protein able to induce vascular leakage in the skin, which was named "tumor vascular permeability factor" (VPF). VPF was proposed to be a mediator of the high permeability of tumor blood vessels. However, these efforts did not yield the full purification of the VPF protein. Due to the lack of amino acid sequence information, VPF remained molecularly unknown and thus more definitive studies were not possible at that time.

In 1989, we reported the isolation of an endothelial cell mitogen from medium conditioned by bovine pituitary follicular cells, which we named "vascular endothelial growth factor" (VEGF) (25). NH_2-terminal amino acid sequencing proved that VEGF was distinct from the known endothelial cell mitogens and indeed did not match any known protein in available databases (25). Subsequently, Connolly et al. (26), following up on the work by Senger et al., independently reported the isolation and sequencing of VPF. cDNA cloning of VEGF (27) and VPF (28) revealed that VEGF and VPF were the same molecule. This was surprising, considering that other known endothelial cell mitogens (e.g., bFGF) do not increase vascular permeability.

2.2 BIOLOGICAL ACTIVITIES OF VEGF-A

VEGF-A stimulates the growth of vascular endothelial cells derived from arteries, veins, and lymphatics [for reviews, see (29,30)]. VEGF also induces angiogenesis in three-dimensional in vitro models (31). VEGF-A also induces angiogenesis in a variety of in vivo model systems (30).

VEGF-A is also an important survival factor for endothelial cells (32–35). VEGF prevents endothelial apoptosis induced by serum starvation. Such activity is mediated by the phosphatidylinositol (PI) 3′ kinase/Akt pathway (34,36). In addition, VEGF induces expression of the antiapoptotic proteins Bcl-2, A1 (33), XIAP (37), and survivin (38) in endothelial cells. In vivo, VEGF prosurvival effects are developmentally regulated. VEGF inhibition results in apoptotic changes in the vasculature of neonatal, but not adult mice (39). VEGF dependence has been demonstrated in endothelial cells of newly formed but not of established vessels within tumors (35,40).

Endothelial cells are the primary targets of VEGF-A, but several studies have reported mitogenic and nonmitogenic effects of VEGF-A also on certain nonendothelial cell types, including retinal pigment epithelial cells (41), pancreatic duct cells (42), and Schwann cells (43).

The earliest evidence that VEGF-A can affect blood cells was a report describing its ability to promote monocyte chemotaxis (44). Subsequently, VEGF-A was reported to have hematopoietic effects, inducing colony formation by mature subsets of granulocyte–macrophage progenitor cells (45). VEGF-deficient hematopoietic stem cells (HSCs) and bone marrow mononuclear cells fail to repopulate lethally irradiated hosts, despite coadministration of a large excess of wild-type cells (46).

As previously noted, VEGF is also known as VPF based on its ability to induce vascular leakage (24,47). Such permeability-enhancing activity underlies important roles of this molecule in inflammation and several pathological circumstances, including intraocular neovascular syndromes [reviewed in (48,49)].

2.3 VEGF ISOFORMS

Alternative exon splicing results in the generation of four different VEGF isoforms, that have respectively, 121, 165, 189, and 206 amino acids following signal sequence cleavage ($VEGF_{121}$, $VEGF_{165}$, $VEGF_{189}$, $VEGF_{206}$) (50,51). $VEGF_{165}$, the predominant isoform, lacks the residues encoded by exon 6, whereas $VEGF_{121}$ lacks the residues encoded by exons 6 and 7. Less frequent splice variants have also been reported, including $VEGF_{145}$ (52), $VEGF_{183}$ (53), $VEGF_{162}$ (54), and $VEGF_{165b}$ (55).

Native VEGF is a heparin-binding homodimeric glycoprotein of 45 kDa (25). Such properties closely correspond to those of $VEGF_{165}$, which is now recognized as the major VEGF isoform (56).

$VEGF_{121}$ is an acidic polypeptide, which fails to bind to heparin (56). $VEGF_{189}$ and $VEGF_{206}$ are highly basic and bind to heparin with high affinity (56). $VEGF_{121}$ is a freely diffusible protein. In contrast, $VEGF_{189}$ and $VEGF_{206}$ are almost completely sequestered in the extracellular matrix (ECM). $VEGF_{165}$ has intermediate properties, since it is secreted but a significant fraction remains bound to the cell surface and ECM (57). The ECM-bound isoforms may be released in a diffusible form by heparin or heparinase, which displaces them from their binding to heparin-like moieties, or by plasmin cleavage at the –COOH terminus, which generates a bioactive fragment consisting of the first 110 NH_2-terminal amino acids (56). Given the important role of plasminogen activation during physiological and pathological angiogeneisis processes (58), this proteolytic mechanism can be particularly important in regulating locally the activity and bioavailability of VEGF. More recent studies have shown that matrix metalloproteinase (MMP)-3 can also cleave $VEGF_{165}$ to generate diffusible, nonheparin binding, bioactive proteolytic fragments (59). In addition, Plouet et al. (60) have proposed a role for urokinase in the generation of bioactive VEGF.

2.4 REGULATION OF *VEGF* GENE EXPRESSION

2.4.1 Oxygen Tension

Oxygen tension plays a key role in regulating the expression of a variety of genes (61). VEGF mRNA expression is induced by exposure to low pO_2 in a variety of pathophysiological circumstances (62,63). A 28-base sequence has been identified in the 5′ promoter of the rat and human *VEGF* gene, which mediates hypoxia-induced transcription (64,65). Such a sequence represents a binding site for hypoxia-inducible factor 1 (HIF-1) (66). HIF-1 is a basic, heterodimeric, helix-loop-helix protein consisting of two subunits, HIF-1α and aryl hydrocarbon receptor nuclear translocator (ARNT), also known as HIF-1β (67). Recent studies have demonstrated the critical role of the product of the von Hippel-Lindau (*VHL*) tumor suppressor gene in HIF-1-dependent hypoxic responses [for review, see (68)]. The *VHL* gene is inactivated in patients with VHL disease, an autosomal dominant neoplasia syndrome

characterized by capillary hemangioblastomas in retina and cerebellum, and in most sporadic clear cell renal carcinomas (69). The VHL protein is known to interact with a series of proteins including elongins B and C and CUL2, a member of the Cullin family (70). More recent studies demonstrated that indeed one of the functions of VHL is to be part of a ubiquitin ligase complex, which targets HIF subunits for proteasomal degradation (71,72). Oxygen promotes the hydroxylation of HIF at a proline residue (71,72). Recently, a family of prolyl hydroxylases related to *Egl-9 Caenorhabditis elegans* gene product was identified as HIF prolyl hydroxylases (61,73,74).

2.4.2 Growth Factors, Hormones, and Oncogenes

Several growth factors, including EGF, TGF-α, TGF-β, KGF, IGF-1, FGF, and PDGF, upregulate VEGF mRNA expression (75–77), suggesting that paracrine or autocrine release of such factors cooperates with local hypoxia in regulating VEGF release in the microenvironment. In addition, inflammatory cytokines such as IL-1-α and IL-6 induce expression of VEGF in several cell types, including synovial fibroblasts (78,79).

Hormones are also regulators of *VEGF* gene expression. Thyroid-stimulating hormone has been shown to induce *VEGF* expression in several thyroid carcinoma cell lines (80). Shifren et al. (81) have also shown that ACTH is able to induce *VEGF* expression in cultured human fetal adrenal cortical cells, suggesting that *VEGF* may be a local regulator of adrenal cortical angiogenesis and a mediator of the tropic action of ACTH.

A variety of transforming events also result in induction of *VEGF* gene expression. Oncogenic mutations or amplification of ras lead to *VEGF* upregulation (82,83). Mutations in the wnt-signaling pathway, which are frequently associated with premalignant colonic adenomas, result in upregulation of *VEGF* (84). Interestingly, *VEGF* is upregulated in polyps of Apc knockout [Apc(Delta716)] mice, a model for human familial adenomatous polyposis (85).

2.5 VEGF RECEPTORS

VEGF binds two highly related receptor tyrosine kinases (RTKs), VEGF receptor-1 (VEGFR-1) and VEGFR-2. Both VEGFR-1 and VEGFR-2 have seven immunoglobulin (Ig)-like domains in the extracellular domain, a single transmembrane region, and a consensus tyrosine kinase sequence, which is interrupted by a kinase-insert domain (86–88).

A member of the same family of RTKs is VEGFR-3 (Flt-4) (89) which, however, is not a receptor for VEGF-A, but instead binds the lymphangiogenic factors VEGF-C and VEGF-D (23). In addition to these RTKs, VEGF interacts with a family of coreceptors, the neuropilins.

2.5.1 VEGFR-1 (Flt-1)

Although Flt-1 (fms-like tyrosine kinase) was the first RTK to be identified as a VEGF receptor (92), the precise function of this molecule is still a subject of debate. VEGFR-1 binds not only VEGF-A but also PlGF (90) and VEGF-B (91), which in turn fails to bind VEGFR-2 Flt-1, reveals a weak tyrosine autophosphorylation in response to VEGF (92,93). Park et al. (90) initially proposed that VEGFR-1 may not primarily be a receptor transmitting a mitogenic signal, but rather a "decoy" receptor, able to regulate in a negative fashion the activity of VEGF on the vascular endothelium, by sequestering and rendering this factor less available to VEGFR-2. Thus, the observed potentiation of the action of VEGF by PlGF could be explained, at least in part, by displacement of VEGF from VEGFR-1 binding (90). Recent studies have shown that a synergism exists between VEGF and PlGF in vivo,