THE MOLECULAR BASIS OF CANCER

MENDELSOHN
HOWLEY
ISRAEL
GRAY
THOMPSON

EDITION 4

ELSEVIER
SAUNDERS

The Molecular Basis of **Cancer**

John Mendelsohn, MD

Director, Khalifa Institute for Personalized Cancer Therapy
and Past President
The University of Texas M. D. Anderson Cancer Center
Houston, Texas

Joe W. Gray, PhD

Gordon Moore Endowed Chair
Chairman, Department of Biomedical Engineering
Director, OHSU Center for Spatial Systems Biomedicine
Associate Director for Translational Research
Knight Cancer Institute
Oregon Health and Science University (OHSU)
Portland, Oregon

Peter M. Howley, MD

Shattuck Professor of Pathological Anatomy
Department of Microbiology and Immunobiology
Division of Immunology
Harvard Medical School
Boston, Massachusetts

Mark A. Israel, MD

Director, Norris Cotton Cancer Center
Dartmouth-Hitchcock Medical Center
Lebanon, New Hampshire
Professor of Pediatrics and Genetics
Geisel School of Medicine at Dartmouth
Hanover, New Hampshire

Craig B. Thompson, MD

President and CEO, Memorial Sloan-Kettering Cancer Center
New York, New York

THE MOLECULAR BASIS OF CANCER   ISBN: 978-1-4557-4066-6
Copyright © 2015, 2008, 2001, 1995 by Saunders, an imprint of Elsevier Inc.

Notices

This book is dedicated to our wives:

Anne C. Mendelsohn
Jane E. Gray
Ann Howley
Susan J. Israel
Tullia Lindsten

Contributors

Stuart A. Aaronson, MD

Professor and Chair
Department of Oncological Sciences
Professor of Medicine
Mount Sinai School of Medicine
New York, New York

James L. Abbruzzese, MD

Chief
Division of Medical Oncology
Associate Director for Clinical Research
Duke Cancer Institute
Durham, North Carolina

Erika L. Abel, PhD

Lecturer/Advisor of Biology
Department of Biology
College of Arts and Sciences
Baylor University
Waco, Texas

Swarnali Acharyya, PhD

Research Fellow
Sloan-Kettering Institute
Memorial Sloan-Kettering Cancer Center
New York, New York

Bachir Alobeid, MD

Professor of Clinical Pathology and Cell Biology
Program Director, Hematopathology Fellowship Training Program
Department of Pathology and Cell Biology
Columbia University Medical Center–College of Physicians and Surgeons
The New York Presbyterian Hospital
New York, New York

Jennifer Amengual, MD

Assistant Professor of Medicine and Experimental Therapeutics
Center for Lymphoid Malignancies
Department of Medicine
Division of Hematopathology
Department of Pathology and Cell Biology
Columbia University Medical Center–College of Physicians and Surgeons
The New York Presbyterian Hospital
New York, New York

Kenneth C. Anderson, MD

Chief
Jerome Lipper Multiple Myeloma Center
Dana-Farber Cancer Institute
Boston, Massachusetts

Olena Barbash, PhD

Investigator
Oncology Research and Development
GlaxoSmithKline
Philadelphia, Pennsylvania

Robert C. Bast, Jr., MD

Vice President for Translational Research
Harry Carothers Wiess Distinguished University Chair for Cancer Research
Internist and Professor of Medicine
Departments of Experimental Therapeutics and Gynecologic Medical Oncology
Division of Cancer Medicine
The University of Texas M. D. Anderson Cancer Center
Houston, Texas

Stephen B. Baylin, MD

Professor of Oncology

Johns Hopkins University

The Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins School of Medicine

Baltimore, Maryland

Joseph R. Bertino, MD

Professor

Medicine and Pharmacology

University of Medicine and Dentistry of New Jersey

Robert Wood Johnson Medical School

Chief Scientific Officer

American Cancer Society Professor

Rutgers Cancer Institute of New Jersey

New Brunswick, New Jersey

Govind Bhagat, MD

Professor of Pathology and Cell Biology

Department of Medicine

Division of Hematopathology

Department of Pathology and Cell Biology

Columbia University Medical Center–College of Physicians and Surgeons

The New York Presbyterian Hospital

New York, New York

Mina J. Bissell, PhD

Distinguished Scientist

Life Sciences Division

Lawrence Berkeley National Laboratory

Berkeley, California

Scott A. Boerner, MS

Department of Internal Medicine

Wayne State University

Barbara Ann Karmanos Cancer Institute

Detroit, Michigan

Jessica E. Bolden, PhD

Gene Regulation Laboratory

Peter MacCallum Cancer Centre

Melbourne, Australia

Ernest C. Borden, MD

The Cleveland Clinic

Taussig Cancer Institute

Case Comprehensive Cancer Center

Cleveland, Ohio

Malcolm V. Brock, MD

Associate Professor of Surgery

Associate Professor of Oncology

Director of Clinical and Translational Research in Thoracic Surgery

Johns Hopkins University School of Medicine

The Johns Hopkins Hospital

Baltimore, Maryland

Nelson E. Brown, MD, PhD

Instructor of Medicine, Molecular Oncology Research Institute

Tufts Medical Center

Boston, Massachusetts

Andrea Califano, Laureate in Physics

Clyde and Helen Wu Professor of Chemical Systems Biology

Columbia University

Director

JP Sulzberger Columbia Genome Center

Columbia University

New York, New York

George Adrian Calin, MD, PhD

Associate Professor

Department of Experimental Therapeutics

Division of Cancer Medicine

The University of Texas M. D. Anderson Cancer Center

Houston, Texas

Peter R. Carroll, MD

Professor and Chair

Department of Urology

Ken and Donna Derr-Chevron Distinguished Professor

Director of Clinical Services and Strategic Planning

University of California–San Francisco

Helen Diller Family Comprehensive Cancer Center

Associate Dean

University of California–San Francisco School of Medicine

San Francisco, California

Chakra Chennubhotla, PhD

Assistant Professor

Department of Computational and Systems Biology

University of Pittsburgh Drug Discovery Institute

University of Pittsburgh

Pittsburgh, Pennsylvania

Toni K. Choueiri, MD

Associate Professor of Medicine

Harvard Medical School

Attending Physician

Dana-Farber Cancer Institute

Boston, Massachusetts

Christopher L. Corless, MD, PhD

Professor of Pathology

Oregon Health and Science University

Chief Medical Officer

Knight Diagnostic Laboratories

Knight Cancer Institute

Portland, Oregon

Ana Luísa Correia, PhD

Life Sciences Division

Lawrence Berkeley National Laboratory

Berkeley, California

Rebecca Critchley-Thorne, PhD

Director

Biomarker and Diagnostic Development

Cernostics, Inc.

Pittsburgh, Pennsylvania

Carlo Maria Croce, MD

The John W. Wolfe Chair in Human Cancer Genetics

Chair

Department of Molecular Virology, Immunology, and Medical Genetics

Director

Human Cancer Genetics Program

The Ohio State University, Comprehensive Cancer Center

Columbus, Ohio

Alan D. D'Andrea, MD

Alvan T. and Viola D. Fuller American Cancer Society Professor

Harvard Medical School

Department of Radiation Oncology

Dana-Farber Cancer Institute

Boston, Massachusetts

Nancy E. Davidson, MD

Hillman Professor of Oncology

Director

University of Pittsburgh Cancer Institute and UPMC Cancer Center

Associate Vice Chancellor for Cancer Research

University of Pittsburgh

Pittsburgh, Pennsylvania

April Davis, BS

Research Technician

Comprehensive Cancer Center

Department of Internal Medicine

University of Michigan

Ann Arbor, Michigan

Changchun Deng, MD, PhD

Assistant Professor of Medicine and Experimental Therapeutics

Center for Lymphoid Malignancies

Department of Medicine

Division of Hematopathology

Department of Pathology and Cell Biology

Columbia University Medical Center–College of Physicians and Surgeons

The New York Presbyterian Hospital

New York, New York

J. Alan Diehl, PhD

J. Samuel Staub, MD, Endowed Professor

Department of Cancer Biology

Perelman School of Medicine

University of Pennsylvania

Philadelphia, Pennsylvania

John DiGiovanni, PhD

Professor

Division of Pharmacology and Toxicology

College of Pharmacy

The University of Texas at Austin

Professor

Department of Nutritional Sciences

College of Natural Sciences, The University of Texas at Austin

Austin, Texas

Joseph Paul Eder, MD

Director of Experimental Therapeutics and the Phase I
Research Group
Yale Cancer Center and Smilow Cancer Hospital
Yale University School of Medicine
New Haven, Connecticut

Suhendan Ekmekcioglu, PhD

Associate Professor
Department of Melanoma Medical Oncology
Division of Cancer Medicine
The University of Texas M. D. Anderson Cancer Center
Houston, Texas

Marc S. Ernstoff, MD

O. Ross McIntyre Professor of Medicine
Norris Cotton Cancer Center
Dartmouth-Hitchcock Medical Center
Lebanon, New Hampshire
Geisel School of Medicine at Dartmouth
Hanover, New Hampshire

James R. Faeder, PhD

Associate Professor
Department of Computational and Systems Biology
University of Pittsburgh Drug Discovery Institute
Pittsburgh, Pennsylvania

Eric R. Fearon, MD, PhD

Maisel Professor of Oncology
Division of Molecular Medicine and Genetics
Departments of Internal Medicine, Human Genetics, and
Pathology
University of Michigan School of Medicine
Ann Arbor, Michigan

Lydia W. S. Finley, PhD

Postdoctoral Fellow
Cancer Biology and Genetics Program
Memorial Sloan-Kettering Cancer Center
New York, New York

Susan Fisher, PhD

Professor
Department of Obstetrics, Gynecology and Reproductive Sciences
Eli and Edythe Broad Center of Regenerative Medicine and
Stem Cell Research
University of California–San Francisco
San Francisco, California

Christopher D. M. Fletcher, MD, FRC Path

Professor
Department of Pathology
Harvard Medical School
Brigham and Women's Hospital
Boston, Massachusetts

Cyrus M. Ghajar, PhD

Life Sciences Division
Lawrence Berkeley National Laboratory
Berkeley, California

Adam B. Glick, PhD

Associate Professor
Center for Molecular Toxicology and Carcinogenesis
Department of Veterinary and Biomedical Sciences
The Pennsylvania State University
University Park, Pennsylvania

Albert Gough, PhD

Associate Professor
Department of Computational and Systems Biology
University of Pittsburgh Drug Discovery Institute
Pittsburgh, Pennsylvania

Ramaswamy Govindan, MD

Division of Oncology
Department of Medicine
Alvin J. Siteman Cancer Center
Washington University School of Medicine
St. Louis, Missouri

Jennifer R. Grandis, MD, FACS

Vice Chair for Research
Distinguished Professor of Otolaryngology and Pharmacology
University of Pittsburgh School of Medicine
Program Leader
Head and Neck Cancer Program
University of Pittsburgh Cancer Institute
Pittsburgh, Pennsylvania

Joe W. Gray, PhD

Gordon Moore Endowed Chair

Chairman

Department of Biomedical Engineering

Director

OHSU Center for Spatial Systems Biomedicine

Associate Director for Translational Research

Knight Cancer Institute

Oregon Health and Science University (OHSU)

Portland, Oregon

Douglas R. Green, PhD

Peter C. Doherty Endowed Chair of Immunology

St. Jude Children's Research Hospital

Memphis, Tennessee

Kirsten L. Greene, MD, MS

Associate Professor of Urology

Vice Chair, Education

Department of Urology

University of California–San Francisco

Helen Diller Family Comprehensive Cancer Center

Veterans Affairs Medical Center

San Francisco, California

Elizabeth A. Grimm, PhD

Professor

Department of Melanoma Medical Oncology

Deputy Head for Research Affairs

Division of Cancer Medicine

Frank McGraw Memorial Endowed Chair in Cancer Research

The University of Texas M. D. Anderson Cancer Center

Houston, Texas

Luca Grumolato, PhD

Professor

Chair of Excellence INSERM

Université de Rouen

Rouen, France

Instructor, Department of Oncological Sciences

Mount Sinai School of Medicine

New York, New York

Jian Gu, PhD

Assistant Professor

Department of Epidemiology

The University of Texas M. D. Anderson Cancer Center

Houston, Texas

David A. Guertin, PhD

Assistant Professor

Program in Molecular Medicine

University of Massachusetts Medical Center

Worcester, Massachusetts

William C. Hahn, MD, PhD

Associate Professor of Medicine

Harvard Medical School

Chief

Division of Molecular and Cellular Oncology

Dana-Farber Cancer Institute

Senior Associate Member

Boston, Massachusetts

Cancer Program, Broad Institute of MIT and Harvard

Cambridge, Massachusetts

William N. Hait, MD, PhD

Global Head

Janssen Research and Development

Raritan, New Jersey

Matthew C. Havrda, PhD

Instructor in Pediatrics

Geisel School of Medicine at Dartmouth

Hanover, New Hampshire

Norris Cotton Cancer Center

Dartmouth-Hitchcock Medical Center

Lebanon, New Hampshire

Matthew L. Hedberg, MD/PhD Candidate

University of Pittsburgh School of Medicine

Pittsburgh, Pennsylvania

Roy S. Herbst, MD, PhD

Professor of Medicine and Pharmacology

Chief of the Medical Oncology

Associate Director for Translational Science

Yale University School of Medicine

Yale Cancer Center

New Haven, Connecticut

Philip W. Hinds, PhD

Professor

Molecular Oncology Research Institute

Tufts Medical Center

Boston, Massachusetts

Daniela Hoehn, MD, PhD

Assistant Professor of Clinical Pathology and Cell Biology
Department of Medicine
Division of Hematopathology
Department of Pathology and Cell Biology
Columbia University Medical Center–College of Physicians and Surgeons
The New York Presbyterian Hospital
New York, New York

Peter M. Howley, MD

Shattuck Professor of Pathological Anatomy
Department of Microbiology and Immunobiology
Division of Immunology
Harvard Medical School
Boston, Massachusetts

Andrew C. Hsieh, MD

Helen Diller Family Comprehensive Cancer Center
University of California–San Francisco Medical Center
San Francisco, California

Patrick Hwu, MD

Department of Melanoma Medical Oncology
Center for Cancer Immunology Research
The University of Texas M. D. Anderson Cancer Center
Houston, Texas

Mark A. Israel, MD

Director
Norris Cotton Cancer Center
Dartmouth-Hitchcock Medical Center
Lebanon, New Hampshire
Professor of Pediatrics and Genetics
Geisel School of Medicine at Dartmouth
Hanover, New Hampshire

Tyler Jacks, PhD

Director
Koch Institute for Integrative Cancer Research
David H. Koch Professor of Biology
Investigator
Howard Hughes Medical Institute
Massachusetts Institute of Technology
Cambridge, Massachusetts

Johanna A. Joyce, PhD

Cancer Biology and Genetics Program
Memorial Sloan-Kettering Cancer Center
New York, New York

William G. Kaelin, Jr., MD

Professor of Medicine
Harvard Medical School
Dana-Farber Cancer Institute
Boston, Massachusetts

Hagop Kantarjian, MD

Department of Leukemia
The University of Texas M. D. Anderson Cancer Center
Houston, Texas

Brian Keith, PhD

Associate Investigator and Director of Education
Abramson Family Cancer Research Institute
Adjunct Professor
Department of Cancer Biology
University of Pennsylvania
Philadelphia, Pennsylvania

Ronan Kelly, MD, MBA

Assistant Professor of Oncology
Johns Hopkins University School of Medicine
The Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins School of Medicine
Baltimore, Maryland

Hasan Korkaya, PhD

Research Investigator
Comprehensive Cancer Center
Department of Internal Medicine
University of Michigan
Ann Arbor, Michigan

W. Michael Korn, MD

Associate Professor in Residence
Department of Medicine
(Gastroenterology and Medical Oncology)
University of California–San Francisco
San Francisco, California

Razelle Kurzrock, MD
Murray Professor of Medicine
Senior Deputy Center Director
Clinical Science Director
Center for Personalized Therapy and Clinical Trials Office
Vice Chair
Division of Hematology and Oncology
The University of California–San Diego
Moores Cancer Center
San Diego, California

Jill E. Larsen, PhD
Hamon Center for Therapeutic Oncology Research
Simmons Cancer Center
University of Texas Southwestern Medical Center
Dallas, Texas

Adrian V. Lee, PhD
Professor
Department of Pharmacology and Chemical Biology, and Department of Human Genetics
Director
Women's Cancer Research Center
University of Pittsburgh Cancer Institute
Magee-Womens Research Institute
University of Pittsburgh
Pittsburgh, Pennsylvania

J. Jack Lee, PhD
Professor
Department of Biostatistics
Division of Quantitative Sciences
The University of Texas M. D. Anderson Cancer Center
Houston, Texas

Anthony G. Letai, MD, PhD
Associate Professor in Medicine
Dana-Farber Cancer Institute
Harvard Medical School
Boston, Massachusetts

Timothy Lezon, PhD
Assistant Professor
Department of Computational and Systems Biology
University of Pittsburgh Drug Discovery Institute
University of Pittsburgh
Pittsburgh, Pennsylvania

Long-Cheng Li, MD
Department of Urology
University of California–San Francisco
Helen Diller Family Comprehensive Cancer Center
University of California
San Francisco, California

Yan Li, PhD
Postdoctoral Fellow
Abramson Family Cancer Research Institute
Perelman School of Medicine
University of Pennsylvania
Philadelphia, Pennsylvania

Scott M. Lippman, MD
Director
Moores Cancer Center
University of California–San Diego
San Diego, California

Yong-Jun Liu, MD, PhD
The Baylor Institute for Immunology Research
Baylor Research Institute
Dallas, Texas

Gregory Lizée, PhD
Associate Professor
Departments of Immunology and Melanoma Medical Oncology
Center for Cancer Immunology Research
The University of Texas M. D. Anderson Cancer Center
Houston, Texas

A. Thomas Look, MD
Professor of Pediatrics
Harvard Medical School
Vice Chair for Research
Department of Pediatric Oncology
Dana-Farber Cancer Institute
Boston, Massachusetts

Patricia M. LoRusso, DO
Professor of Medicine, Director
Phase I Clinical Trials Program
Wayne State University
Barbara Ann Karmanos Cancer Institute
Detroit, Michigan

Scott W. Lowe, PhD
Chair
Geoffrey Beene Cancer Research Center
Cancer Biology and Genetics Program
Memorial Sloan-Kettering Cancer Center
New York, New York

David Malkin, MD
POGO Chair in Childhood Cancer Control
Medical Director
Pediatric Oncology Group of Ontario
Professor of Pediatrics
University of Toronto
Staff Oncologist
Division of Hematology/Oncology
Senior Scientist
Genetics and Genomic Biology Program
The Hospital for Sick Children
Toronto, Canada

Elaine R. Mardis, PhD
Co-Director
The Genome Institute
Professor of Genetics
Washington University School of Medicine
St. Louis, Missouri

Judith Margolin, MD
Associate Professor of Pediatric Hematology/Oncology
Texas Children's Cancer Center
Baylor College of Medicine
Houston, Texas

Joan Massagué, PhD
Chair
Cancer Biology and Genetics Program
Sloan-Kettering Institute
Memorial Sloan-Kettering Cancer Center
New York, New York

Lynn Matrisian, PhD
Vice President
Scientific and Medical Affairs
Pancreatic Cancer Action Network
Manhattan Beach, California

Frank McCormick, PhD, FRS
Director
Helen Diller Family Comprehensive Cancer
Center
University of California–San Francisco
San Francisco, California

John Mendelsohn, MD
Director
Khalifa Institute for Personalized Cancer Therapy
and Past President
The University of Texas M. D. Anderson Cancer Center
Houston, Texas

Gordon B. Mills, MD, PhD
Department Chair
Department of Systems Biology, Division of Cancer
Medicine
Olga Keith Weiss Distinguished University Chair for Cancer
Research
Professor of Medicine and Immunology
Department of Systems Biology
Division of Cancer Medicine
The University of Texas M. D. Anderson Cancer
Center
Houston, Texas

John D. Minna, MD
Hamon Center for Therapeutic Oncology Research
Simmons Cancer Center
Department of Pharmacology
University of Texas Southwestern Medical Center
Dallas, Texas

Mehdi Mollapour, PhD
Departments of Urology, Biochemistry, and Molecular Biology
SUNY Upstate Medical University
Syracuse, New York

Daniel Morgensztern, MD
Assistant Professor of Medical Oncology
Yale University School of Medicine
New Haven, Connecticut

Robert F. Murphy, PhD

Professor

Departments of Biological Sciences, Biomedical Engineering, and Machine Learning

Director

Ray and Stephanie Lane Center for Computational Biology

Carnegie Mellon University

Pittsburgh, Pennsylvania

Len Neckers, PhD

Urologic Oncology Branch

National Cancer Institute

Bethesda, Maryland

Owen A. O'Connor, MD, PhD

Professor of Medicine and Experimental Therapeutics

Center for Lymphoid Malignancies

Department of Medicine

Division of Hematopathology

Department of Pathology and Cell Biology

Columbia University Medical Center–College of Physicians and Surgeons

The New York Presbyterian Hospital

New York, New York

Peter H. O'Donnell, MD

Assistant Professor of Medicine

The University of Chicago

Chicago, Illinois

Steffi Oesterreich, PhD

Professor

Department of Pharmacology and Chemical Biology

University of Pittsburgh Cancer Institute

Magee-Womens Research Institute

University of Pittsburgh

Pittsburgh, Pennsylvania

Drew Pardoll, MD, PhD

Seraph Professorship in Oncology

Martin D. Abeloff Professorship

Co-Director

Cancer Immunology and Hematopoiesis Program

Professor of Oncology, Medicine, Pathology, and Molecular Biology and Genetics

The Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins School of Medicine

Baltimore, Maryland

Elspeth Payne, BSc, MB, ChB

Research Department of Haematology

School of Life and Medical Sciences

University College London Cancer Institute

London, United Kingdom

David G. Poplack, MD

Elise C. Young Professor of Pediatric Oncology

Head, Hematology/Oncology Section

Texas Children's Cancer Center and Hematology Service

Houston, Texas

Sean M. Post, PhD

Department of Leukemia

The University of Texas M. D. Anderson Cancer Center

Houston, Texas

Daniela F. Quail, MD

Cancer Biology and Genetics Program

Memorial Sloan-Kettering Cancer Center

New York, New York

Alfonso Quintás-Cardama, MD

Department of Leukemia

The University of Texas M. D. Anderson Cancer Center

Houston, Texas

Karen R. Rabin, MD, PhD

Assistant Professor of Pediatric Hematology/Oncology

Texas Children's Cancer Center

Baylor College of Medicine

Houston, Texas

Mark J. Ratain, MD

Leon O. Jacobson Professor of Medicine

The University of Chicago

Director

Center for Personalized Therapeutics

Associate Director for Clinical Sciences

Comprehensive Cancer Center

Chicago, Illinois

Julie D. R. Reimann, MD, PhD

Miraca Life Sciences

Tufts University School of Medicine

Newton, Massachusetts

Ignacio Romero, MD

Area Clinica Oncologia Ginecologica

Instituto Valenciano de Oncologia

Valencia, Spain

Eric Rubin, MD

Associate Director Clinical Science

The Cancer Institute of New Jersey

Professor of Medicine and Pharmacology

University of Medicine and Dentistry of New Jersey

Robert Wood Johnson Medical School

New Brunswick, New Jersey

Davide Ruggero, PhD

Associate Professor of Urology

Helen Diller Family Comprehensive Cancer Center

University of California–San Francisco

San Francisco, California

David M. Sabatini, MD, PhD

Professor of Biology

Whitehead Institute for Biomedical Research

Koch Center for Integrative Cancer Research at MIT

Investigator

Howard Hughes Medical Institute

Cambridge, Massachusetts

Ahmed Sawas, MD

Instructor of Clinical Medicine

Center for Lymphoid Malignancies

Department of Medicine

Division of Hematopathology

Department of Pathology and Cell Biology

Columbia University Medical Center–College of Physicians and Surgeons

The New York Presbyterian Hospital

New York, New York

Eric S. Schafer, MD, MHS

Assistant Professor of Pediatric Hematology/Oncology

Texas Children's Cancer Center

Baylor College of Medicine

Houston, Texas

Rachna T. Schroff, MD

Assistant Professor

Department of Gastrointestinal Medical Oncology

Division of Cancer Medicine

The University of Texas M. D. Anderson Cancer Center

Houston, Texas

Steven I. Sherman, MD

Professor and Chair

Naguib Samaan Distinguished Professor of Endocrinology

Department of Endocrine Neoplasia and Hormonal Disorders

The University of Texas M. D. Anderson Cancer Center

Houston, Texas

Sabina Signoretti, MD

Associate Professor of Pathology

Harvard Medical School

Associate Pathologist

Brigham and Women's Hospital

Dana-Farber Cancer Institute

Boston, Massachusetts

Branimir I. Sikic, MD

Professor of Medicine (Oncology)

Director

Stanford Clinical and Translational Research Unit

Co-director

Stanford University Center for Clinical and Translational Education and Research (Spectrum)

Stanford, California

M. Celeste Simon, PhD

Investigator

Howard Hughes Medical Institute

Professor

Department of Cell and Developmental Biology and Abramson Family Cancer Research Institute

University of Pennsylvania

Philadelphia, Pennsylvania

Paul T. Spellman, PhD

Associate Professor

Department of Medical and Molecular Genetics

Knight Cancer Institute

Oregon Health and Science University

Portland, Oregon

Josh Stuart, PhD
Associate Professor
Biomolecular Engineering Department
Associate Director
Center for Biomolecular Science and Engineering
Baskin School of Engineering
University of California–Santa Cruz
Santa Cruz, California

Rishi Surana, MD/PhD Candidate
Tumor Biology Training Program
Lombardi Comprehensive Cancer Center
Georgetown University
Washington, District of Columbia

Tanja Meyer Tamguney, PhD
Director of Research
SillaJen
Postdoctoral Scholar–McCormick Laboratory
Helen Diller Family Comprehensive Cancer Center
University of California–San Francisco
San Francisco, California

D. Lansing Taylor, PhD
Professor of Computational and Systems Biology
Director
University of Pittsburgh Drug Discovery Institute
University of Pittsburgh Cancer Institute
Pittsburgh, Philadelphia

Mario D. Terán, MD
Postdoctoral Fellow
Johns Hopkins University School of Medicine
Division of Thoracic Surgery
Baltimore, Maryland

Craig B. Thompson, MD
President and CEO
Memorial Sloan-Kettering Cancer Center
New York, New York

Giovanni Tonon, MD, PhD
Head
Functional Genomics of Cancer Unit
Division of Molecular Oncology
San Raffaele Scientific Institute
Milan, Italy

Robert A. Weinberg, PhD
Daniel K. Ludwig Professor for Cancer Research
Whitehead Institute for Biomedical Research
Department of Biology
Massachusetts Institute of Technology
Cambridge, Massachusetts

Louis M. Weiner, MD
Director
Lombardi Comprehensive Cancer Center
Professor
Department of Oncology
Francis L. and Charlotte G. Gragnani Chair
Department of Oncology
Georgetown University
Washington, District of Columbia

Danny R. Welch, PhD
Professor and Chair
Adjunct Faculty of Department of Molecular and Integrative Physiology
The University of Kansas Medical Center
Kansas City, Kansas

Eileen White, PhD
Associate Director for Basic Science
Rutgers Cancer Institute of New Jersey
Professor, Molecular Biology and Biochemistry
Rutgers University
New Brunswick, New Jersey

Max S. Wicha, MD
Professor
Department of Internal Medicine
Director
University of Michigan Comprehensive Cancer Center
Distinguished Professor of Oncology
University of Michigan
Ann Arbor, Michigan

Monte M. Winslow, PhD
Assistant Professor
Department of Genetics
Stanford University School of Medicine
Stanford, California

H. Ewa Witkowska, PhD

Adjunct Professor

Department of Obstetrics, Gynecology, and Reproductive Sciences

University of California–San Francisco

San Francisco, California

Xifeng Wu, MD, PhD

Professor and Chair

Department of Epidemiology

The University of Texas M. D. Anderson Cancer Center

Houston, Texas

Stuart H. Yuspa, MD

Head

In Vitro Pathogenesis Section

Laboratory Co-Chief

Laboratory of Cancer Biology and Genetics

Center for Cancer Research

Bethesda, Maryland

Preface

Research in molecular genetics and cancer biology and advances in analytical technologies have revolutionized our understanding of cancer. Over the past three decades, there has been a massive acceleration in discoveries and observations that explains the genetic basis of cancer, a disease that until recently was thought about primarily in purely descriptive terms. Conversely, the study of malignancy has transformed our understanding of the molecular and genetic processes that govern the growth and proliferation of normal cells.

By 1995, our knowledge had expanded to the point that we felt it worthwhile to write a textbook describing *the molecular basis of cancer* for students, researchers, and providers of clinical care from a variety of disciplines. The aim in this fourth edition of the textbook continues to explain, rather than to merely recount.

Five editors, selected for their diverse expertise and their reputations as educators, met to design a sequence of sections and chapters that would lead the reader from the basic genetic and molecular mechanisms of carcinogenesis, to the molecular and biological features of cancer cell growth and metastasis, then to advances in sequencing technologies and bioinformatics that enable personalized risk assessment and diagnostics, followed by a description of molecular and genetic abnormalities that drive the common types of cancer, and finally to the molecular basis for new, targeted approaches to cancer therapy.

A purpose of this textbook is to describe the scientific underpinnings that will enable clinicians and other professionals who manage cancer patients to better understand the disease and its therapy. This book will be of equal, or possibly greater, interest to laboratory and clinical investigators in biomedical research and to advanced students and trainees, who need to understand the molecular mechanisms that govern the functioning and malfunctioning of malignant cells. Although the chapters follow a sequence that moves from pathogenesis to therapy, each chapter stands alone in its treatment of the subject matter.

Cancer arises as a result of genetic and epigenetic alterations that either enhance or diminish the activities of critical pathways that mediate normal cellular activities. Impaired capacity to repair genetic alterations can contribute to the likelihood that cells accumulate these genetic abnormalities, leading to malignant transformation. The disease is not merely a disorder of individual transformed cells. These cells grow into tumor masses and attract a blood supply, and they invade through surrounding tissues and metastasize. Molecular influences from the environment around the cancer cells contribute importantly to the capacity of genetically altered cells to produce malignant tumors.

A remarkable lesson gained from cancer research is that the strategies utilized by widely divergent cell lineages to regulate growth and differentiation share common molecular pathways. The accumulation of mutations and altered expression of genes critical for these pathways is a recurrent theme observed in many different types of cancer. Cancers also appear to select for genetic abnormalities that may be most advantageous for escape from normal regulatory mechanisms in their particular microenvironments.

What is most exciting today is the active dialogue between clinical investigators and laboratory scientists who share an interest in applying the new knowledge of genetics and molecular biology to the early diagnosis, targeted treatment, and improved prevention of disease. Today we have the opportunity to select treatments for clinical administration from among hundreds of new biological and chemical anticancer agents targeting pathways altered by specific molecular irregularities that result from aberrant genes. It is only recently that we can detect the genetic aberrations in cancer specimens from individual patients in a reasonable time frame and at a reasonable cost. This means that genomic assays can be used to select therapies that target the products of the aberrant genes in a patient's cancer and are more likely to provide benefit for that patient. The knowledge we present in this textbook should supply a basis upon which these new approaches to cancer therapy can be evaluated and implemented by those interested in understanding and critically assessing the many new products of the biotechnology revolution.

The editors are delighted that we were able to recruit as contributing authors outstanding investigators who are excited about the challenge of presenting their areas of expertise in a textbook format. In many cases this has required more time and effort than they initially anticipated, and we are grateful for their dedication. We hope that we have come at least part of the way toward achieving what we set out to do. We have been assisted and encouraged by the professionals at Elsevier, as well as the patient and ever-essential help of the secretaries in our offices.

John Mendelsohn, MD
Joe W. Gray, PhD
Peter M. Howley, MD
Mark A. Israel, MD
Craig B. Thompson, MD

William C. Hahn and Robert A. Weinberg

Cancer: A Genetic Disorder

Our understanding of the origins of cancer has changed dramatically over the past three decades, due in large part to the revolution in molecular biology that has altered the face of all biomedical research. Powerful experimental tools have been thrust into the hands of cancer biologists. These tools, including newly devised and implemented technologies that permit the interrogation of entire genomes, have made it possible to uncover and dissect the complex molecular machinery operating inside the single cell, normal and malignant, to understand its operations, and to pinpoint the defects that cause cancer cells to proliferate abnormally.

Three decades ago, at least three rival models of cancer's origins had substantial following among those interested in the roots of cancer. One model portrayed cancer as a disease of abnormal differentiation. According to this thinking, the changes in cell behavior that occur during the process of development run awry during tumor progression, causing cells to make inappropriate choices in moving up or down differentiation pathways. This concept of cancer's origins had important implications for the molecular origins of cancer: because the process of differentiation involves changes in cell phenotype without underlying changes in the genome, this model suggested that cancer was essentially an epigenetic process—a change in cell behavior without an underlying change in its genetic constitution.

An alternative model was advanced by the virologists. By the early 1970s, a number of distinct cancer-causing viruses had been catalogued in various animal species and in humans. These ranged from the Rous sarcoma virus, whose discovery reached back to the first decade of the century, to Shope papillomavirus, Epstein-Barr virus, papovaviruses such as SV40 and polyomavirus, and a variety of retroviruses that infected various mammals and birds. The existence of these viruses suggested that similar agents operated to trigger human tumors. Such hypothetical human tumor viruses were thought capable of insinuating themselves into human cells and transforming them from a normal to a malignant growth state.[1]

Yet another way of explaining cancer's origins was advanced by those who were impressed by the increasing connections being forged between carcinogens and mutagens. More than half a century of experiments had demonstrated the abilities of radiation as well as a vast array of chemicals to induce tumors in animals and occasionally in humans. Independent of this research, *Drosophila* and bacterial geneticists had documented the abilities of some of these carcinogenic agents to act as mutagens. The most influential of these experiments was to come from the laboratory of Bruce Ames. In the mid-1970s, Ames described a correlation between the mutagenic potencies of various chemical compounds and their respective potencies to induce tumors in laboratory animals.[2]

Ames' correlation (Figure 1-1) yielded the inference that the carcinogenic powers of agents derive directly from their abilities to damage genes and thus the DNA of cells. This strengthened the convictions of those who had long embraced the notion that cancer cells were really mutants and that their abnormal behavior derived from mutant genes that they carried in their genomes. This model implied that such mutant genes arose through somatic mutations, i.e., mutations that occur in somatic tissues during the lifetime of an organism and alter genes that were pristine at the moment of conception.

This last model of cancer's origins would eventually dominate thinking; the other two models largely fell by the scientific wayside. As the 1970s progressed, the search for tumorigenic viruses associated with most types of common human cancers bogged down. Human papillomavirus (HPV) clearly had strong associations with cervical carcinomas, Epstein-Barr virus (EBV) with Burkitt's lymphomas in Africa and nasopharyngeal carcinomas in southeast Asia, and hepatitis B and C viruses (HBV, HCV) with hepatocellular carcinomas in east Asia. Together, these accounted for as much as 20% of tumors worldwide.[3] However, the remaining types of cancers, and thus the vast majority of human cancers arising in the Western world, had no obvious viral associations in spite of extensive attempts to uncover them.
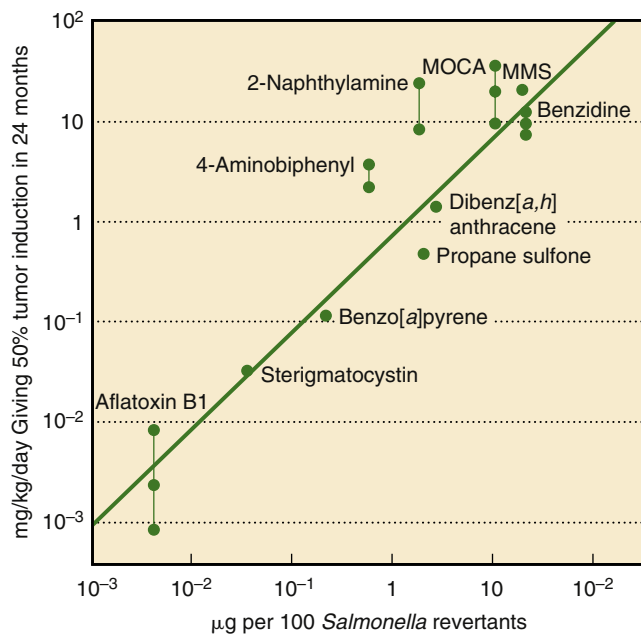
Figure 1-1 Relationship of carcinogenic to mutagenic potencies of chemical compounds The ability to quantify both the mutagenic potencies of a variety of chemical compounds, measured in the Ames mutagenesis test, and to relate this to their carcinogenic potencies, as measured in laboratory rodents, allowed this graph and correlation to be made between the two mechanisms of action. *(Adapted from Meselson M et al. In: Hiatt HH et al., eds.*, Origins of Human Cancer, Book C: Human Risk Assessment. *Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, 1977.)*

The epigenetic model of cancer lost its attractiveness largely because an extensive array of mutant growth-controlling genes was discovered in the genomes of human tumor cells. So the focus shifted increasingly to genes, more specifically the genomes of cancer cells. Cancer genetics in the 1970s and early 1980s became a branch of somatic cell genetics—the genetics of cells and their somatically mutated genes. Indeed, advances in the technology of DNA sequencing have now enabled the enumeration of mutations present in specific cancer genomes and will eventually lead to a compendium of recurrent genetic alterations in human cancers.

The Discovery of Cellular Oncogenes

The notion that cancer cells were mutants should have motivated a systematic search for genes that suffered mutation during the development of tumors. Moreover, these mutant genes should possess another property: they needed to specify some of the aberrant phenotypes ascribed to tumor cells, including alterations in cell shape, decreased dependence on external mitogenic stimuli, and an ability to grow without tethering to a solid substrate (anchorage independence). The fact that viruses were not important causative agents of most types of human tumors generated another conclusion about

these cancer-causing genes: they were likely to be endogenous to the cell rather than being imported into the cell from some external source. Stated differently, it seemed likely that these cancer genes were mutant versions of preexisting normal cellular genes.

In the 1970s, when this line of thinking matured, the experimental opportunities to test its validity were limited. The human genome, which harbored these hypothetical cancer genes, represented daunting complexity. Its vastness precluded any simple, systematic survey strategy designed to locate mutant growth-controlling genes within cancer cells. Indeed, it is only now, three decades later, that the means, deep sequencing of cancer genomes, for conducting effective systematic surveys for cancer genes has been developed. Thus the discovery of cancer-causing genes—oncogenes as they came to be called—depended on a circuitous, indirect experimental strategy.

Ironically, it was tumor viruses, in the midst of being discredited as important etiologic agents of human cancer, that led the way to finding the elusive cancer genes. Varmus and Bishop's study of the Rous sarcoma virus (RSV) broke open the puzzle. Their initial agenda was to understand the replication strategy of this chicken virus. However, in the years after 1974, they focused their attentions to unraveling the mechanism used by RSV to transform an infected normal cell into a tumor cell.

Earlier work of others had indicated that a single gene, named *src*, carried the vital cancer-causing information present in the viral genome. Accordingly, the Varmus and Bishop laboratory launched a research program to trace the origins of this virus-associated *src* oncogene. In fact, the origins of most viral genes were obscure, shrouded in the deep evolutionary past. It seemed that most viruses and thus their genes originated hundreds of millions of years ago, perhaps as derivatives of the cells that they learned to parasitize.

However, as this team reported in 1976, the *src* gene behaved differently: it was a recent acquisition by the Rous virus. Many closely related retroviruses shared with RSV an ability to replicate in chicken cells and a very similar set of genes needed for viral replication. However, these other viruses lacked the *src* gene and the ability to transform infected cells into cancer cells, suggesting that the *src* oncogene carried by RSV was a relatively recent genetic acquisition. The Varmus-Bishop group soon traced the origins of the *src* gene to an unexpected source—a closely related gene that resided in the genome of normal chickens and, by extension, in the genomes of all vertebrates. They named this gene c-*src* (cellular *src*) to distinguish it from the v-*src* (viral *src*) oncogene carried by the virus.[4]

The Varmus-Bishop evidence converged on a simple conceptual model. It explained all their observations and ultimately much more. The progenitor of RSV lacked the
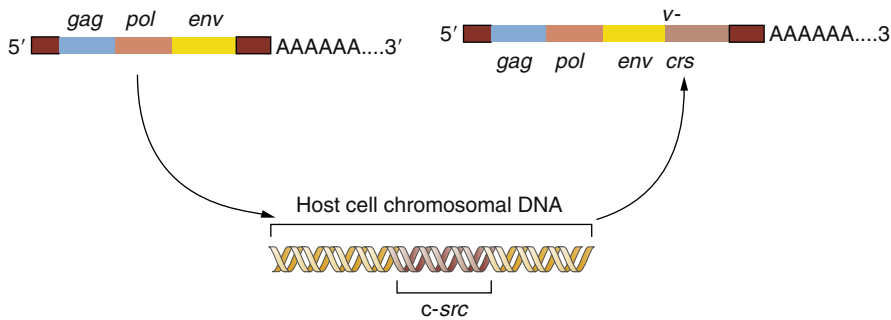
FIGURE 1-2 THE ORIGIN OF THE ROUS SARCOMA VIRUS *src* ONCOGENE The acquisition of the v-*src* oncogene by a precursor of Rous sarcoma virus apparently occurred when an avian leukosis virus (ALV) lacking this oncogene infected a chicken cell and appropriated the cellular c-*src* proto-oncogene, thereafter carrying this acquired gene and exploiting it to transform subsequently infected cells.

v-*src* gene but grew well in chicken cells. During one of its periodic forays into a chicken cell, this ancestor virus picked up a copy of the c-*src* gene and incorporated it into its own viral genome. Once *src* was present within the viral genome, this slightly remodeled gene—now v-*src*—was exploited by RSV to transform cells it encountered in subsequent rounds of infection.

This provided a testimonial to the cleverness and plasticity of retroviruses, which seemed able to capture and then exploit normal cellular genes to do their bidding. But another implication was even more important: the Varmus-Bishop work pointed to the existence of a normal cellular gene, the c-*src* gene, that seemed to possess a latent ability to induce cancer. This cancer-causing ability was unmasked when the c-*src* gene was abducted by the chicken retrovirus that became the progenitor of RSV (Figure 1-2).

The c-*src* gene was named a *proto-oncogene* to indicate its inherent potential to become activated into a cancer-causing oncogene. Within several years, it became clear that as many as a dozen other tumorigenic retroviruses also carried oncogenes, each of which had been abstracted from the genome of an infected vertebrate cell.[5,6] Hence, there were many proto-oncogenes in the normal cell genome, not just c-*src*. Each seemed to be present in the DNA of a normal mammalian or avian host species, and by extension, present as well in the genomes of all vertebrates.

These discoveries were momentous because they demonstrated that normal cellular genes had the ability to induce cancer if removed from their normal chromosomal context and placed under the control of one or another retrovirus. Still, a key piece was missing from this puzzle. Retroviruses seemed to be absent from most, indeed from almost all, human tumors. Could proto-oncogenes ever become activated without direct intervention by a marauding retrovirus?

An obvious response was that proto-oncogenes might be altered by mutational events that did not remove these genes from their normal chromosomal roosts. Instead, these mutations would alter proto-oncogenes in situ in the chromosome by affecting either the control sequences or the protein-encoding sequences of these genes. This notion led to another question: If some proto-oncogenes could become

activated by somatic mutations, such as those inflicted by chemical or physical carcinogens, would these be the same proto-oncogenes that were the targets of mobilization and activation by retroviruses?

In 1979 and 1980, answers came, once again from unexpected quarters. These newer experiments depended on the use of gene transfer, also known as *transfection*. The transfection procedure could be used to convey DNA, and thus genes, from tumor cells into normal recipient cells. The goal here was to see whether the transferred tumor cell DNA could induce some type of malignant transformation in the recipient cells. Success in such an experiment would indicate that the transferred gene(s) previously operated in the donor tumor cell to induce its transformation.

These transfection experiments succeeded (Figure 1-3). DNA extracted from chemically transformed mouse fibroblasts was able to induce normal mouse fibroblasts to undergo transformation.[7] Retroviruses were clearly absent from both the donor tumor cells and the recipients that underwent transformation and so could not be invoked to explain the cancer-causing powers of the transferred DNA. Soon the identity of these transferred genes, which functioned as oncogenes, became apparent. They were members of the *ras* family of oncogenes, which had initially been discovered through their association with rodent sarcoma viruses.[5,8] These rodent retroviruses had acquired *ras* proto-oncogenes from normal rodent cells, much like RSV, which had stolen a copy of the *src* proto-oncogene from a chicken cell.

Unanswered by this was the genetic mechanism that imparted oncogenic powers to the tumor-associated *ras* oncogene, more specifically an H-*ras* oncogene. It soon became clear that the tumor-associated H-*ras* oncogene was closely related to, indeed virtually indistinguishable from, a normal H-*ras* proto-oncogene that was present in the genomes of all vertebrates. Still, the tumor-associated *ras* oncogene carried different information than did the precursor proto-oncogene: the oncogene caused the malignant transformation of cells into which it was introduced, whereas the counterpart proto-oncogene had no obvious effects on cell phenotype. This particular puzzle was solved in 1982 with the finding that an H-*ras* oncogene cloned from a human bladder
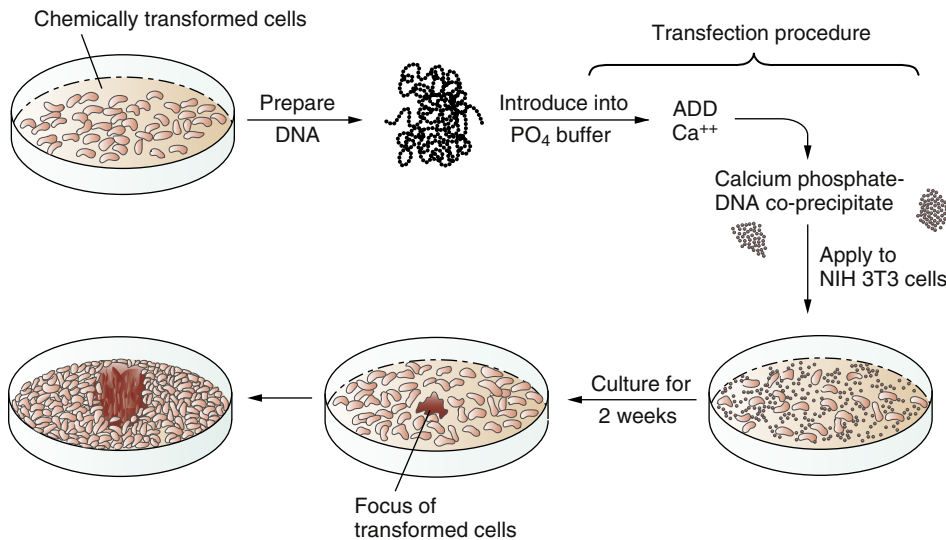
Figure 1-3 Transfection of a cellular oncogene The fact that the carcinogenicity of various chemical compounds was correlated with their mutagenicity suggested that cancer cells often carry mutant, cancer-inducing genes, i.e., oncogenes, in their genomes. This could be proven by an experiment in which DNA was extracted from chemically transformed mouse fibroblasts and introduced, via the procedure of transfection, into untransformed mouse fibroblasts. The appearance of foci of transformed cells in the latter indicated the transmission of a transforming gene from the donor to the recipient cells, indicating that chemical carcinogens could indeed generate a mutant, cancer-causing gene.

carcinoma carried a point mutation—a single nucleotide substitution—that distinguished it from its counterpart proto-oncogene.[9-11] This genetic alteration, clearly a somatic mutation, sufficed to convert a normally benign proto-oncogene into a virulent oncogene.

Within months, yet other activated oncogenes were found in human tumors by using DNA probes prepared from a variety of retrovirus-associated oncogenes. The *myc* oncogene, initially associated with avian myelocytomatosis virus, was found to be present in increased gene copy number (i.e., amplified) in some human hematopoietic tumors[12]; in yet others, *myc* was activated through a chromosomal translocation that juxtaposed its coding sequences with those of immunoglobulin genes, thereby placing the expression of the *myc* gene under the control of these antibody genes rather than its own normal transcriptional control elements.[13] These discoveries extended and solidified a simple point: a common repertoire of proto-oncogenes could be activated either by retroviruses (usually in animal tumors) or by somatic mutations (in human tumors). The activating mutations involved either base substitution, amplification in gene copy number, or chromosomal translocation.

Multistep Tumorigenesis

The discoveries of mutant, tumor-associated oncogenes in human tumors led to a simple model of cancer formation. Mutagenic carcinogens entered into cells of a target tissue and mutated a proto-oncogene. The resulting oncogene then induced the now-mutant cell to initiate a program of malignant growth. Eventually, years later, the progeny of this mutant founder cell formed a large enough mass to become a macroscopically apparent tumor.

While satisfying conceptually, this simple model of cancer formation clearly conflicted with a century's worth of histopathologic analyses, which had indicated that tumor formation is really a multistep process, in which initially normal cell populations pass through a succession of intermediate stages on their way to becoming frankly malignant. Each of these intermediate stages contains cells that were more aberrant than those seen in the preceding steps. This body of observations persuaded many that the formation of a malignancy depended on a succession of phenotypic changes in the cells forming these various growths. Quite possibly, each of these shifts in cell phenotype reflected a change in the underlying genetic makeup of the evolving pre-malignant cell population. Such a multistep genetic model of tumor progression stood in direct conflict with the single-hit model of transformation that was suggested by the discovery of the point-mutated *ras* oncogene.

By 1983, one solution to this dilemma became apparent. In that year, experiments showed that a single introduced oncogene could not transform fully normal rat cells into ones that were tumorigenic. Two and maybe even more oncogenes seemed to be required to effect this conversion.[14,15] For example, whereas an introduced *ras* oncogene could not transform normal embryo cells into tumor cells, the co-introduction of a *ras* plus a *myc* oncogene, or a *ras* plus an adenovirus E1A oncogene, succeeded in doing so. It appeared that such pairs of oncogenes collaborated with one another to induce the full malignant transformation of normal cells (Figure 1-4, *A*). Moreover, this experiment suggested that human tumors carried two or more mutant oncogenes that collaborated with one another to orchestrate the many aberrant phenotypes associated with highly malignant cells.

Observations such as these pointed to a new way of conceptualizing the multistep tumorigenesis long studied by the pathologists. It seemed plausible that each of the
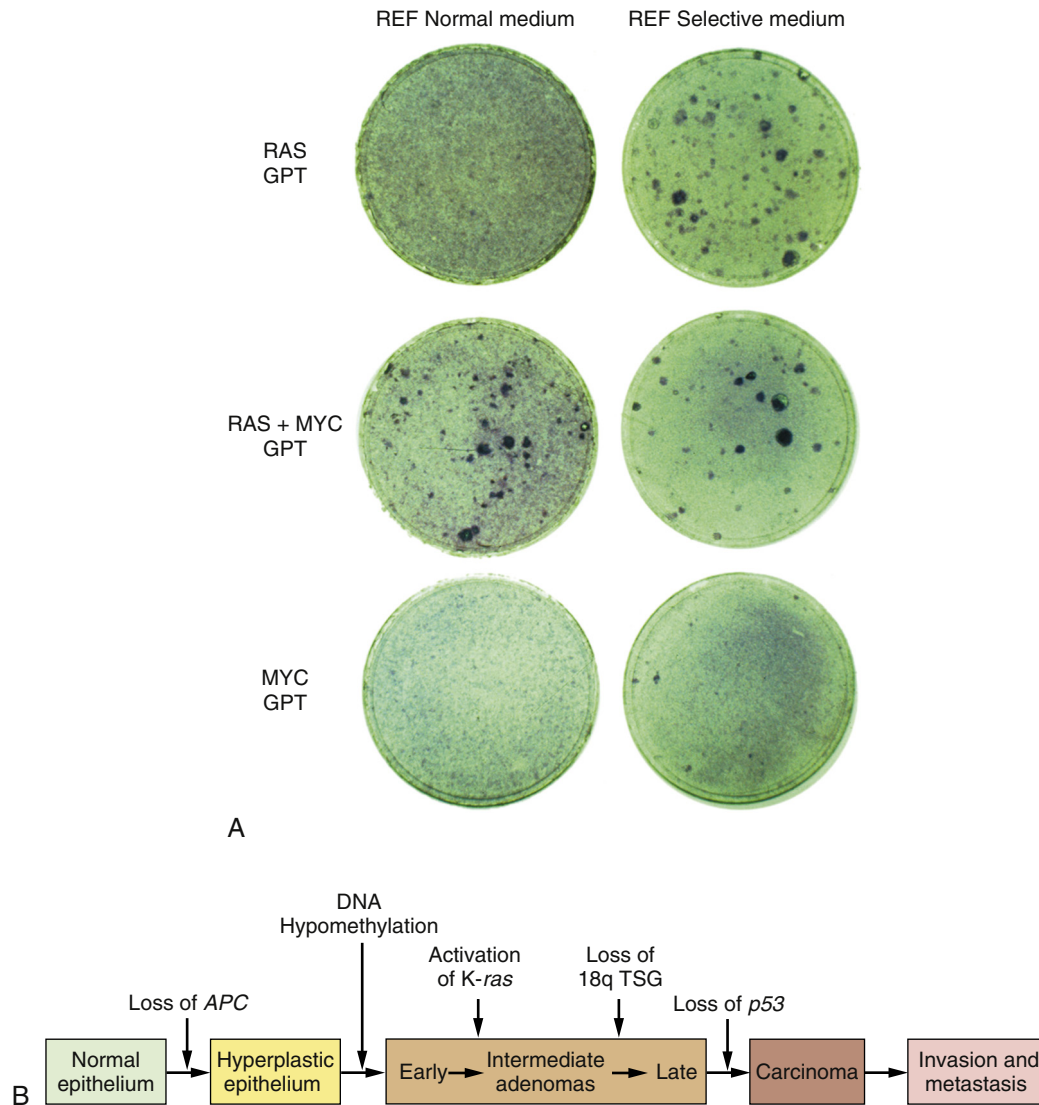
FIGURE 1-4 MULTISTEP TUMORIGENESIS IN VITRO AND IN VIVO (A) The ability of oncogenes to collaborate to transform cells in vitro was illustrated in this 1983 experiment in which neither a *ras* nor a *myc* oncogene was found able to induce foci when introduced into early passage rat embryo fibroblasts (REFs). However, when the two were introduced concomitantly, transformation ensued, as indicated by the appearance of foci. This suggested that tumor progression in vivo might involve a succession of mutations that created multiple collaborating cellular oncogenes. **(B)** By 1989, analyses of the genomes of colonic epithelial cells at various stages of tumor progression revealed that the more progressed the cells were, the more mutations they had acquired. In fact, some of the indicated mutations involved inactivation of tumor suppressor genes, to be discussed later. *(A, from Land H, Parada LF, Weinberg RA. Nature. 1983;304:596-602; B, courtesy B. Vogelstein.)*

histopathological transitions arising during tumor development occurred as a consequence of a new mutation sustained in the genome of an evolving, premalignant cell population (Figure 1-4, *B*). According to this thinking, tumor development was a form of Darwinian evolution, in which each successive mutation in a growth-controlling gene conferred increased proliferative potential and thus selective advantage on the cells bearing the mutant gene.[16,17] Ultimately, a multiply mutated cell bearing half a dozen or more mutant genes might exhibit all of the phenotypes associated with highly malignant cancer cells.

This mechanistic model was validated through the creation of transgenic mice. Cloned copies of mutant oncogenes, such as *ras* and *myc,* were introduced into the germlines of mice. These transgenes were structured so that the oncogene was placed under the control of a transcriptional promoter that ensured expression of the resulting "transgene" in a specific tissue or developmental stage. Now the presence of a mutant oncogene in a particular tissue could be guaranteed through the actions of an appropriately engineered transgene rather than being dependent on the random actions of mutagenic carcinogens.

In one highly instructive group of experiments, a *myc* or a *ras* oncogene was placed under the control of the mouse mammary tumor virus transcriptional promoter, which guaranteed its expression in the mammary epithelium of

the pregnant female mouse.[18] As anticipated, these mice contracted breast cancer at extremely high rates. This demonstrated that mutant oncogenes were far more than markers of cancer progression; indeed, they could actually play a causal role in driving tumor pathogenesis.

Significantly, the transgenic mice did not contract cancer rapidly in their mammary tissue even though a mutant oncogene was implanted and expressed in virtually all of the epithelial cells of their mammary glands. Instead, their mammary carcinomas arose with several months' delay, indicating that a second (and perhaps third) alteration was required in addition to the activated transgene before mammary epithelial cells launched a program of malignant growth. The nature of this additional alteration(s) was not always clear, but it almost certainly involved stochastic somatic mutations striking the mammary epithelial cells, creating mutant growth-controlling genes that collaborated with the transgene to trigger the outgrowth of malignant cell clones. In the years that followed, this work was extended to many types of human tumors, the cells of which were found to possess multiple mutant genes that contributed to tumor formation.

The Discovery of Tumor Suppressor Genes

The model of multistep tumorigenesis implied that a tumor cell carries two or more mutant oncogenes, each activated by somatic mutation during one of the stages of tumor development. However, experimental validation of this model initially proved to be difficult. Most attempts at detecting mutant oncogenes in human tumor genomes yielded a *ras* or perhaps a *myc* oncogene, but rarely were two mutant oncogenes found to coexist in the genomes of human tumor cells. This left two logical alternatives. Either the genome of a typical human tumor cell did not contain multiple mutated genes, as the multistep model of cancer suggested, or there were indeed multiple mutated cancer-causing genes in tumors, but many of these were not oncogenes of the type that had been studied intensively in the 1970s and early 1980s.

In fact, there were candidate genes waiting in the wings. These others operated in a fashion diametrically opposite to that of the oncogenes: they seemed to prevent cancer rather than favoring it and came to be called "tumor suppressor genes." Several independent lines of evidence led to the discovery and characterization of these genes.

Experiments using cell hybridization initiated by Henry Harris in Oxford provided the first indication of the existence of these suppressor genes.[19] These cell hybridizations involved the physical fusion of two distinct types of cells that were propagated in mixed cultures. The conjoined cells would form a common hybrid cytoplasm and ultimately pool their chromosomes, yielding a hybrid genome.

Often these cell hybridizations involved the fusion of cells with two distinct genotypes. In some of these experiments, tumor cells were fused with normal cells. The motive here was to see which genome would dominate in determining the behavior of the resulting hybrids. Counter to the expectations of many, the resulting hybrid cells turned out, more often than not, to be nontumorigenic.[19] This indicated that the genes present in the normal genome dominated over those carried in the cancer cell. In the language of genetics, the normal alleles were dominant, whereas the cancer cell–associated alleles were recessive. (More properly, the alleles present in the cancer cell created a phenotype that was recessive to the normal cell phenotype.)

This unanticipated behavior could most easily be rationalized by assuming that normal cells carried certain growth-normalizing genes, the presence of which was needed to maintain normal proliferation. Cancer cells seemed to have lost these genes, ostensibly through mutations that resulted in inactivated versions of the genes present in normal cells. When reintroduced into the cancer cells via cell fusion, the normal alleles reimposed control on the cancer cells, restoring their behavior to that of a normal cell. In effect, these growth-normalizing genes suppressed the tumorigenic phenotype of the cancer cells and were, for this reason, termed *tumor suppressor genes* (TSGs).

In their normal incarnations, the TSGs seemed to constrain growth, unlike the proto-oncogenes, which seemed to be involved in promoting normal proliferation. Inactivated, null alleles of TSGs were found in tumor cell genomes in contrast to the hyperactivated alleles of proto-oncogenes (i.e., oncogenes) found in these genomes.

The study of retinoblastoma, the childhood eye tumor, converged on these cell hybridization studies in a dramatic way. This work had been pioneered by Alfred Knudson, who, beginning in the early 1970s, studied the genetics of this rare tumor. Knudson learned much by comparing the two forms of this cancer: sporadic retinoblastoma, which seemed to be due exclusively to accidental somatic mutations, and familial retinoblastoma, which appeared, like many familial cancers, to be due to the transmission of a mutated gene in the germline.

Knudson's analysis of the kinetics of retinoblastoma onset persuaded him that a common set of gene(s) operated to generate both kinds of tumors.[20,21] Although the nature of these genes eluded him, their number was clear. Sporadic retinoblastomas seemed to arise following two successive somatic mutations affecting a lineage of cells in the retina. The triggering of familial retinoblastomas seemed to require only a single somatic mutation. Knudson speculated that in these familial tumors, a second mutated gene was required

to trigger tumorigenesis and that this gene was already present in mutant form in all the cells of the retina, having been inherited in mutated form from a parent of the affected child.

For the cancer geneticist, Knudson's most important concept was the notion that a retinal cell needed to lose two mutant genes before it was transformed into a tumor cell. Sometimes one of the two mutant null alleles was contributed by the germline; more often, both genes arose through somatic mutation. However, the nature of these genes and the mutations that recruited them into the tumorigenic process remained elusive. Finally, in 1979, karyotypic analysis of a retinoblastoma revealed an interstitial deletion in the q14 band of chromosome 13.[22] Later work revealed that this resulted in the loss of a gene, termed *RB*. Hence, one of the two mutational events needed to make a retinoblastoma involved the inactivation of an *RB* gene copy, in this particular case through the wholesale deletion of the chromosomal region carrying the *RB* gene.

By 1983, the nature of the second mutational event became clear: it involved the loss of the second, hitherto intact copy of the *RB* gene.[23] Hence, the two mutational events hypothesized by Knudson involved the successive inactivation of the two copies of this gene. Suddenly, the need for two mutations became clear: The first mutation left the cell with a single, still-intact copy of the *RB* gene, which was able, on its own, to continue programming normal proliferation. Only when this surviving gene copy was eliminated from the cell genome did runaway proliferation begin (Figure 1-5). Thus, mutations that inactivate an *RB* gene copy create alleles that function recessively at the cellular level. Only when both wild-type alleles are lost through various mutational mechanisms does a retinal cell begin to behave abnormally.

The *RB* gene became the paradigm for a large cohort of similarly acting TSGs that suffer inactivation during tumor progression. These TSGs are scattered throughout the cell genome and act through a variety of cell-physiologic mechanisms to control cell proliferation.[24] They are united only by the fact that they control proliferation in a negative way, so that their loss permits uncontrolled cell multiplication to proceed.

The discovery of the *RB* gene gave substance and specificity to the genes that Harris had postulated from his cell fusion experiments. Equally important, they opened the door to understanding a variety of familial cancer syndromes. In the case of *RB*, inheritance of a mutant, defective allele predisposes to retinoblastoma early in life with more than 90% probability. Inheritance of a defective allele of the *APC* TSG predisposes with high frequency to adenomatous polyposis coli syndrome and thus to colon cancer. The presence of a mutant *TP53* gene in the germline leads to increased rates of tumors in a number of organ sites, including sarcomas and
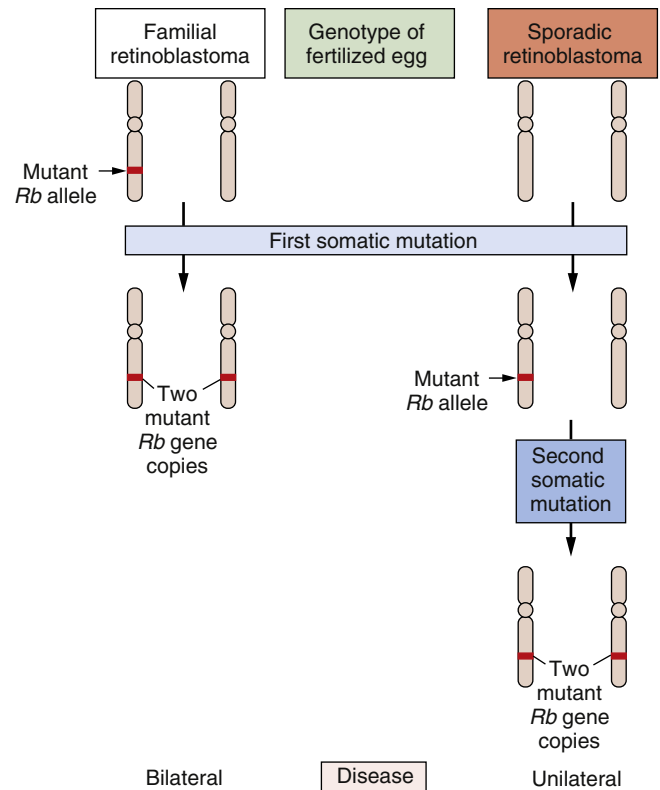

FIGURE 1-5 GENETICS OF RETINOBLASTOMA DEVELOPMENT The development of retinoblastomas requires the successive inactivation of two copies of the chromosomal *RB* gene. In the case of familial retinoblastomas, one of the two copies of this gene is already mutated in one or another gamete and is transmitted to the offspring, who is therefore heterozygous at this locus in all cells of the body; subsequent loss, through somatic alterations, of the surviving wild-type gene copy leaves a retinal cell with no functional copies of this gene, enabling tumor formation to begin. In sporadic retinoblastomas, the conceptus is genetically wild type; however, two successive somatic mutations occurring in a lineage of retinal precursor cells leaves some of these cells, once again, without functional *RB* gene copies, and as before permits retinoblastoma tumorigenesis to begin.

carcinomas, yielding the Li-Fraumeni syndrome. More than two dozen heritable cancer syndromes have been associated with germline inheritance of defective TSGs.[25,26]

In each case, the inheritance of a mutant, functionally defective TSG allele obviates one of two usually required somatic mutations. Because an inactivating somatic mutation represents a low-probability event per cell generation, the presence of an already-mutant inherited TSG allele enormously accelerates the overall kinetics of tumor formation. As a consequence, the likelihood of a tumor arising during the course of a normal lifespan is enormously increased.

The search for TSGs has been difficult, as their existence only becomes apparent when they are absent from a cellular genome. However, one peculiarity of TSG genetics has greatly aided the discovery of these genes. This involves the genetic mechanisms by which the second copy of a TSG is lost. In principle, two independent somatic mutations could successively inactivate the two copies of a TSG,

thereby liberating a cell from the growth-constraining influences of this gene. However, each of these mutations normally occurs with a low probability—perhaps 10^{-6} per cell generation. The likelihood of both mutations occurring is therefore roughly 10^{-12} per cell generation, an extremely low probability. (Actually, because cancer cell genomes become progressively destabilized as tumors develop, this probability is usually higher.)

In fact, evolving premalignant cell populations carrying a single, already-inactivated TSG copy often resort to another genetic mechanism to eliminate the second, still-intact copy of this TSG. They discard the chromosomal arm (or chromosomal region) carrying the still-intact TSG copy and replace it with a duplicated copy of the chromosomal region carrying the mutant, already-inactivated TSG copy. All this is achieved via the exchange of genetic material between paired homologous chromosomes.

The end result of these genetic gymnastics is the duplication of the mutant TSG copy. Thus, the TSG goes from a heterozygous state (involving one mutant and one wild-type gene allele) to a homozygous state (involving two mutant gene copies). Almost always, the chromosomal region flanking the TSG suffers the same fate. Consequently, known genes as well as other genetic markers within this flanking region that were initially present in a heterozygous configuration now become reduced to a homozygous configuration. This genetic behavior has motivated cancer geneticists to analyze the genomes of human tumor cells, looking for chromosomal regions that repeatedly suffer loss of heterozygosity (LOH) during tumor progression. Such LOHs represent presumptive evidence for the presence of TSGs in these regions whose second wild-type copies have been eliminated by LOH during the course of tumor development. Once such a region is localized to a chromosomal region, several currently available gene molecular strategies can be exploited to further narrow the chromosomal domain carrying the TSG and ultimately to isolate the TSG through molecular cloning.

The existence of many dozen still-unknown TSGs is suspected because of the documented LOH affecting specific chromosomal regions of various types of human tumor cells. The effort to identify and clone these genes is being greatly facilitated by efforts such as those included in the International Cancer Genome Consortium and the Cancer Genome Anatomy Project (TCGA). Nonetheless, the successful identification and cloning of a significant cohort of TSGs has already provided one solution to a major puzzle posed earlier. As mentioned, although human tumor cells were hypothesized to carry a number of distinct, mutated growth-controlling genes, most tumors appeared to carry only a single activated oncogene. We now realize that many of the other targets of mutation during tumor progression are TSGs. Their inactivation collaborates with the activated

oncogenes to create malignant cells and thus tumors. In the widely cited study of human multistep tumor progression—that described in colonic tumors by Vogelstein and his coworkers—the mutation of a K-*ras* oncogene is accompanied by mutations of the *APC* and *TP53* TSGs and a third TSG that maps to chromosome 18.[27] This evidence, together with a wealth of genetic studies reported subsequently, indicates that TSGs are inactivated even more frequently than oncogenes are activated during the course of forming many types of human tumors. Importantly, the inactivation of TSGs often phenocopies the cell-biological effects of oncogenes. This means that the inactivation of TSGs is as important to the biology of tumor progression as oncogene activation.

Unexpectedly, the discovery of TSGs also made it possible to understand how a variety of DNA tumor viruses succeed in transforming the cells that they infect. Unlike retroviruses, these DNA viruses carry oncogenes that have resided in their genomes for millions, and likely hundreds of millions, of years. Any connections with antecedent cellular genes, to the extent they once existed, were obscured long ago by the extensive remodeling that these oncogenes underwent while being carried in the genomes of the various DNA tumor viruses. Independent of their ultimate origins, it was clear in the 1980s that the oncogenes (and encoded oncoproteins) were deployed by DNA viruses to perturb key components of the normal cellular growth-controlling circuitry. However, the precise control points targeted by these viral oncoproteins remained obscure.

In the late 1980s, it was learned that a number of DNA tumor virus oncoproteins bind to the products of two centrally important TSGs, pRB and p53.[28,29] For example, the large T oncoprotein of SV40 binds and sequesters both the p53 and pRB proteins of infected host cells; the E6 and E7 oncoproteins of human papillomaviruses target p53 and pRB, respectively. As a consequence, a virus-infected cell is deprived of the services of these two key negative regulators of its proliferation. Indeed, these virus-mediated inactivations closely mimic the state seen in many nonviral tumors that have been deprived of pRB and p53 function by somatic mutations striking the TSGs specifying these two proteins. So the transforming mechanisms used by these viruses could be rationalized by referring to the same genes and proteins that were known to be inactivated by mutational mechanisms in many types of spontaneous, nonviral human tumors. Importantly, these findings reinforced the notion that a single, central growth-regulating machinery operating in all types of cells suffers disruption by a variety of ostensibly unrelated genetic mechanisms, leading eventually to the formation of cancers.

The activation of oncogenes and the loss of TSGs together explain many of the phenotypes that one associates with cancer cells. These cells are able to grow without attachment to solid substrate, the aforementioned phenotype of anchorage