

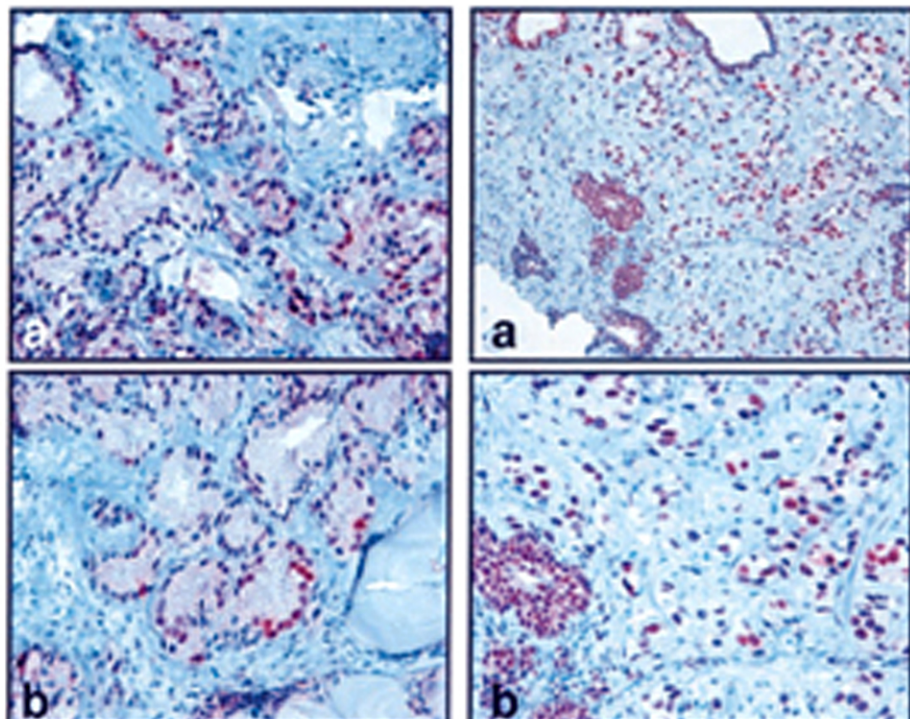
IMMUNOHISTOCHEMISTRY AND *IN SITU* HYBRIDIZATION OF HUMAN CARCINOMAS

MOLECULAR PATHOLOGY, COLORECTAL CARCINOMA,
AND PROSTATE CARCINOMA

Carcinoma low-grade

Carcinoma high-grade

P53



Edited by **M.A. HAYAT**

**Handbook of Immunohistochemistry
and *in situ* Hybridization of
Human Carcinomas, Volume 2**

Handbook of Immunohistochemistry and *in situ* Hybridization of Human Carcinomas

Edited by M. A. Hayat

Volume 1

Molecular Genetics; Lung and Breast Carcinomas

Volume 2

Molecular Pathology, Colorectal Carcinoma, and Prostate Carcinoma

**Handbook of
Immunohistochemistry and
in situ Hybridization of
Human Carcinomas,
Volume 2**

**Molecular Pathology, Colorectal
Carcinoma, and Prostate Carcinoma**

Edited by

M.A. Hayat


Distinguished Professor
Department of Biological Sciences
Kean University
Union, New Jersey



Amsterdam • Boston • Heidelberg • London • New York • Oxford
Paris • San Diego • San Francisco • Singapore • Sydney • Tokyo

Publishing Editor: *Judy Meyer*
Acquisition Editor: *Hilary Rowe*
Project Manager: *Justin Palmeiro*
Editorial Assistant: *Erin LaBonte-McKay*
Marketing Manager: *Kristin Banach*
Cover Design: *Cate Barr*
Full Service Provider: *Graphic World Publishing Services*
Composition: *Cepha Imaging Pvt., Ltd.*

Elsevier Academic Press
200 Wheeler Road, Burlington, MA 01803, USA
525 B Street, Suite 1900, San Diego, California 92101-4495, USA
84 Theobald's Road, London WC1X 8RR, UK

This book is printed on acid-free paper. 

Copyright © 2005, Elsevier Inc. All rights reserved.

No part of this publication may be reproduced or transmitted in any form or by any means, electronic or mechanical, including photocopy, recording, or any information storage and retrieval system, without permission in writing from the publisher.

Permissions may be sought directly from Elsevier's Science & Technology Rights Department in Oxford, UK: phone: (+44) 1865 843830, fax: (+44) 1865 853333, e-mail: permissions@elsevier.com.uk. You may also complete your request on-line via the Elsevier homepage (<http://elsevier.com>), by selecting "Customer Support" and then "Obtaining Permissions."

Library of Congress Cataloging-in-Publication Data

Application submitted

British Library Cataloguing in Publication Data

A catalogue record for this book is available from the British Library

ISBN: 0-12-333942-1

For all information on all Academic Press publications
visit our website at www.academicpressbooks.com

Printed in China

05 06 07 08 09 9 8 7 6 5 4 3 2 1

To

Molecular Geneticists/Clinical Pathologists

This Page Intentionally Left Blank

Contents

Authors and Coauthors of Volume 2	xi
Foreword	xv
Preface to Volume 2	xvii
Contents of Volume 1	xix
Prologue	xxiii
Selected Definitions	xxv

I Molecular Pathology 1

- 1.1 Laser Capture Microdissection-Microarray Technology: Global mRNA Amplification for Expression Profiling on Laser Capture Cells 3**
Kazuhiko Aoyagi and Hiroki Sasaki
- 1.2 Comparative Genomic Hybridization Analysis Using Metaphase or Microarray Slides 11**
Simon Hughes, Ben Beheshti, Paula Marrano, Gloria Lim, and Jeremy A. Squire
- 1.3 Microarray Immunoassay of Complex Specimens: Problems and Technologic Challenges 23**
Wlad Kusnezow, Timo Pulli, Yana V. Syagailo, and Jörg D. Hoheisel
- 1.4 Comparative Genomic Hybridization 37**
Isabel Zudaire

- 1.5 Microsatellite Instability in Cancer: Assessment by High Resolution Fluorescent Microsatellite Analysis 55**
Shinya Oda and Yoshihiko Maehara
- 1.6 The Role of Extreme Phenotype Selection in Cancer Research 65**
Jose Luis Perez-Gracia, Maria Gloria Ruiz-Ilundain, Ignacio Garcia-Ribas, and Eva Carrasco
- 1.7 Rolling Circle Amplification 73**
Vanessa King
- 1.8 Direct, *in situ* Assessment of Telomere Length Variation in Human Cancers and Preneoplastic Lesions 83**
Alan Meeker, Wesley R. Gage, Angelo De Marzo, and Anirban Maitra
- 1.9 Clinical Flow Cytometry of Solid Tumors 89**
Mathie P.G. Leers and Marius Nap
- 1.10 Suppression Subtractive Hybridization Technology 113**
Isik G. Yulug and Arzu Atalay

II Colorectal Carcinoma 127

- 2.1 **Colorectal Carcinoma: An Introduction** 129
M.A. Hayat
- 2.2 **Role of Immunohistochemical Expression of p53 in Colorectal Carcinoma** 139
Jin-Tung Liang and Yung-Ming Jeng
- 2.3 **Applying Tissue Microarray in Rectal Cancer: Immunostaining of Ki-67 and p53** 149
Mef Nilbert and Eva Fernebro
- 2.4 **Role of Immunohistochemical Expression of p21 in Rectal Carcinoma** 159
Nobuhiro Takiguchi, Nobuhito Sogawa, and Masaru Miyazaki
- 2.5 **Role of p107 Expression in Colorectal Carcinoma** 163
Tsutomu Masaki, Kazutaka Kurokohchi, Fei Wu, and Shigeki Kuriyama
- 2.6 **Expression of Gastric MUC5AC Mucin During Colon Carcinogenesis** 167
Jacques Bara, Marie-Elisabeth Forgue-Lafitte, and Marie-Pierre Buisine
- 2.7 **Role of Cyclooxygenase2 Expression in Colorectal Cancer** 183
Sven Petersen
- 2.8 **Role of Immunohistochemical Expression of Bcl-2 in Colorectal Carcinoma** 193
N.J. Agnantis, A.C. Goussia, E. Ioachim, and D. Stefanou
- 2.9 **Immunohistochemical Detection of CD97 Protein in Colorectal Carcinoma** 201
Gabriela Aust

- 2.10 **Roles of Immunohistochemical Expression of Cyclin A and Cyclin-Dependent Kinase 2 in Colorectal Tumors** 207
Jia-Qing Li and Katsumi Imaida
- 2.11 **Role of Mismatch Repair Proteins and Microsatellite Instability in Colon Carcinoma** 215
Maria Lucia Caruso
- 2.12 **Role of CD-61 (Beta-3 Integrin) Glycoprotein in Colon Carcinoma** 227
A. Moreno, C. Lucena, D. Llanes, and J.J. Garrido
- 2.13 **Immunohistochemical and *in situ* Hybridization Analysis of Lumican in Colorectal Carcinoma** 237
Toshiyuki Ishiwata
- 2.14 **Role of Immunohistochemical Expression and *in situ* Hybridization Expression of Endothelin in Colon Carcinoma** 245
Florence Pinet
- 2.15 **Role of Fibroblastic Stroma in Colon Carcinoma** 255
Nicolas Wernert
- 2.16 **Role of Immunohistochemical Expression of p53, Rb, and p16 Proteins in Anal Squamous Cell Carcinoma** 267
Hanlin L. Wang

III Prostate Carcinoma 277

- 3.1 **Prostate Carcinoma: An Introduction** 279
M.A. Hayat

- 3.2 Genetic Alterations in Prostate Cancer** 299
Kotaro Kasahara, Takahiro Taguchi, Ichiro Yamasaki, and Taro Shuin
- 3.3 Alterations of Genes and Their Expression in Prostate Carcinoma** 307
Pedro L. Fernández and Timothy M. Thomson
- 3.4 *In situ* Hybridization of Human Telomerase Reverse Transcriptase mRNA in Prostate Carcinoma** 321
Bernd Wullich, Jörn Kamradt, Volker Jung, and Thomas Fixemer
- 3.5 Detection of Genetic Abnormalities Using Comparative Genomic Hybridization in Prostate Cancer Cell Lines** 327
Lisa W. Chu and Jan C. Liang
- 3.6 Markers for the Development of Early Prostate Cancer** 335
Michael D. Slater, Christopher Lauer, Angus Gidley-Baird, and Julian A. Barden
- 3.7 The Role of MUC18 in Prostate Carcinoma** 347
Guang-Jer Wu
- 3.8 Role of Immunohistochemical Expression of PCNA and p53 in Prostate Carcinoma** 359
Francesco Cappello, Fabio Bucchieri, and Giovanni Zummo
- 3.9 Role of the *p14^{ARF}* and *p16^{INK4a}* Genes in Prostate Cancer** 369
Noboru Konishi
- 3.10 P504S/ α -Methylacyl CoA Racemase: A New Cancer Marker for the Detection of Prostate Carcinoma** 377
Zhong Jiang
- 3.11 Role of Somatostatin Receptors in Prostate Carcinoma** 387
Jens Hansson
- 3.12 Role of Immunohistochemical Expression of Retinoid X Receptors in Prostate Carcinoma** 399
María I. Arenas, Juan Alfaro, and Ricardo Paniagua
- 3.13 Role of Androgen Receptor Cofactors in Prostate Cancer** 409
Peng Lee and Zhengxin Wang
- 3.14 Role of Androgen Receptor Gene Amplification and Protein Expression in Hormone Refractory Prostate Carcinoma** 423
Joanne Edwards and John M.S. Bartlett
- 3.15 Role of Immunohistochemical Loss of Bin1/Amphiphysin2 in Prostatic Carcinoma** 431
James B. DuHadaway and George C. Prendergast
- 3.16 Role of Prostate-Specific Glandular Kallikrein 2 in Prostate Carcinoma** 439
Pirkko Vihko and Annakaisa Herrala
- 3.17 Expression and Gene Copy Number Alterations of HER-2/neu (ERBB2) Gene in Prostate Cancer** 449
Kimmo J. Savinainen and Tapio Visakorpi
- 3.18 Combined Detection of Low Level HER-2/neu Expression and Gene Amplification in Prostate Cancer by Immunofluorescence and Fluorescence *in situ* Hybridization** 457
Regina Gandour-Edwards and Janine LaSalle
- 3.19 Calpain Proteases in Prostate Carcinoma** 463
Alan Wells, Sourabh Kharait, Clayton Yates, and Latha Satish
- 3.20 Immunohistochemical Expression of Raf Kinase Inhibitor Protein in Prostate Carcinoma** 471
Zheng Fu, Lizhi Zhang, and Evan T. Keller
- Index 481

This Page Intentionally Left Blank

Authors and Coauthors of Volume 2

N.J. Agnantis (193)

Department of Pathology, Medical School, University of Ioannina, 45110 Ioannina, Greece

Juan Alfaro (399)

Department of Cell Biology and Genetics, University of Alcalá, 28871 Alcalá de Henares, Madrid, Spain

Kazuhiko Aoyagi (1)

Genetics Division, National Cancer Center Research Institute, 1-1, Tsukiji 5-chome, Chuo-ku, Tokyo 104-0045, Japan

María I. Arenas (399)

Department of Cell Biology and Genetics, University of Alcalá, 28871 Alcalá de Henares, Madrid, Spain

Arzu Atalay (113)

Bilkent University, Faculty of Science, Department of Molecular Biology and Genetics, 06800 Ankara, Turkey

Gabriela Aust (201)

University of Leipzig, Institute of Anatomy, Research Laboratories in the Department of Obstetrics and Gynecology, Ph.-Rosenthal-Str.55, 04103 Leipzig, Germany

Jacques Bara (167)

U-482 INSERM, Hôpital St-Antoine, 184, rue du Fbg St-Antoine, 75012 Paris, France

Julian A. Barden (335)

Institute for Biomedical Research, Department of Anatomy and Histology, the University of Sydney, Sydney NSW 2006, Australia

John M.S. Bartlett (423)

Endocrine Cancer Group, Glasgow Royal Infirmary, Glasgow, Scotland, G32 2ER

Ben Beheshti (11)

Ontario Cancer Institute, Princess Margaret Hospital, 610 University Avenue, Room 9-721, Toronto, Ontario, Canada M5G 2M9

Fabio Bucchieri (359)

Sezione di Anatomia Umana, Dipartimento di Medicina Sperimentale, Università degli Studi di Palermo, via del Vespro 129, 90127—Palermo—Italy

Marie-Pierre Buisine (167)

U-560 INSERM 1, Pl de Verdun, 59045 Lille, France

Francesco Cappello (359)

Sezione di Anatomia Umana, Dipartimento di Medicina Sperimentale, Università degli Studi di Palermo, via del Vespro 129, 90127—Palermo—Italy

Eva Carrasco (65)

Clinical Research Department, Eli Lilly & Co., Avenida de la Industria 30, 28108 Alcobendas, Madrid, Spain

Maria Lucia Caruso (65)

Department of Pathology, Scientific Institute for Digestive Diseases “S de Bellis,” Via della Resistenza, 70013 Castellana Grotte (BA), Italy

Lisa W. Chu (327)

Division of Life Sciences, Lawrence Berkeley National Laboratory, Berkeley, CA 94720

Angelo De Marzo (83)

Department of Pathology, Ross Research Building, John Hopkins University School of Medicine, 720 Rutland Avenue, Baltimore, MD 21205-2196

James B. DuHadaway (431)

Lankenau Institute for Medical Research, Department of Pathology, Anatomy, and Cell Biology, Thomas Jefferson University, 100 Lancaster Avenue, Wynnewood, PA 19096

Joanne Edwards (423)

Endocrine Cancer Group, Glasgow Royal Infirmary, Glasgow, Scotland, G32 2ER

Pedro L. Fernández (307)

Department of Anatomical Pathology, Hospital Clinic, Villarreal 170, Barcelona 08036, Spain

- Eva Fernebro (149)
Department of Oncology, Lund University Hospital,
221 85 Lund, Sweden
- Thomas Fixemer (321)
Clinic of Urology and Pediatric Urology, University of
the Saarland, 66421 Homburg/Saar, Germany
- Marie-Elisabeth Forgue-Lafitte (167)
U-482 INSERM, Hôpital St-Antoine, 184, rue du Fbg
St-Antoine, 75012 Paris, France
- Zheng Fu (471)
Department of Oncology, Mayo Clinic,
200 First Street SW, Rochester, MN 55905
- Wesley R. Gage (83)
Department of Pathology, Ross Research Building,
John Hopkins University School of Medicine,
720 Rutland Avenue, Baltimore, MD 21205-2196
- Regina Gandour-Edwards (457)
Department of Pathology, University of California
Davis Health System, 4400 V Street,
Sacramento, CA 95817
- Ignacio Garcia-Ribas (65)
Clinical Research Department, Eli Lilly & Co.,
Avenida de la Industria 30, 28108 Alcobendas,
Madrid, Spain
- J.J. Garrido (227)
Department of Genetics, Campus Rabanales,
Edificio C-5, Spanish Research Council, University of
Córdoba, 14071 Córdoba, Spain
- Angus Gidley-Baird (335)
Institute for Biomedical Research, Department of
Anatomy and Histology, University of Sydney,
Sydney NSW 2006, Australia
- A.C. Goussia (193)
Department of Pathology, Medical School, University of
Ioannina, 45110 Ioannina, Greece
- Jens Hansson (387)
Lund University, Department of Urology, Wallenberg
Laboratory, Building 46, Level 4, Malmö University
Hospital, S-205 02 Malmö, Sweden
- M.A. Hayat (129, 279)
Department of Biological Sciences, Kean University,
1000 Morris Avenue, Union, NJ 07083
- Annakaisa Herrala (439)
Biocenter Oulu and Research Center for Molecular
Endocrinology, P.O. Box 5000, FIN-90014,
University of Oulu, Finland
- Jörg D. Hoheisel (23)
Functional Genome Analysis, Deutsches
Krebsforschungszentrum, Im Neuenheimer Feld 580,
D-69120 Heidelberg, Germany
- Simon Hughes (11)
Ontario Cancer Institute, Princess Margaret Hospital,
610 University Avenue, Room 9-717, Toronto, Ontario,
Canada M5G 2M9
- Katsumi Imaida (207)
Onco-pathology, Kagawa Medical University, 1750-1
Ikenobe, Miki-cho, Kita-gun, Kagawa 761-0793, Japan
- E. Ioachim (193)
Department of Pathology, Medical School, University of
Ioannina, 45110 Ioannina, Greece
- Toshiyuki Ishiwata (237)
Department of Pathology, Nippon Medical School,
1-1-5 Sendagi, Bunkyo-ku, Tokyo 113-8602, Japan
- Yung-Ming Jeng (139)
Division of Colorectal Surgery, Department of Surgery,
National Taiwan University Hospital, No.7, Chung-Shan
South Road, Taipei, Taiwan
- Zhong Jiang (377)
Department of Pathology, University of Massachusetts
Medical School, 55 Lake Avenue North, Worcester,
MA 01655
- Volker Jung (321)
Clinic of Urology and Pediatric Urology, University of
the Saarland, 66421 Homburg/Saar, Germany
- Jörn Kamradt (321)
Clinic of Urology and Pediatric Urology, University of
the Saarland, 66421 Homburg/Saar, Germany
- Kotaro Kasahara (299)
Department of Urology, Kochi Medical School,
Nankoku, Kochi 783-8505, Japan
- Evan T. Keller (471)
Rm 5111 CCGC Building, 1500 E. Medical Center Dr.,
University of Michigan, Ann Arbor, MI 48109-0940
- Sourabh Kharait (463)
Department of Pathology, 711 Scaife, University of
Pittsburgh, Pittsburgh, PA 15261
- Vanessa King (73)
Chemistry and Immunohistochemistry, Dade Behring,
Inc., Box 707, Glasgow Business Community,
P.O. Box 6101, Newark, DE 19714
- Noboru Konishi (369)
Department of Pathology, Nara Medical University,
840 Shijo-Cho, Kashihara, Nara 634-8521, Japan
- Shigeki Kuriyama (163)
Third Department of Internal Medicine,
Kagawa Medical University, 1750-1 Miki-cho,
Kita-gun, Kagawa 761-0793, Japan
- Kazutaka Kurokohchi (163)
Third Department of Internal Medicine,
Kagawa Medical University, 1750-1 Miki-cho,
Kita-gun, Kagawa 761-0793, Japan

Wlad Kusnezow (23)

Functional Genome Analysis, Deutsches
Krebsforschungszentrum, Im Neuenheimer Feld 580,
D-69120 Heidelberg, Germany

Janine LaSalle (457)

Department of Medical Microbiology and Immunology,
University of California Davis, One Shields Avenue,
Davis, CA 95616

Christopher Lauer (335)

Missenden Medical Centre, Camperdown,
NSW 2050, Australia

Peng Lee (409)

Department of Cancer Biology-Box 173,
The University of Texas, M.D. Anderson Cancer
Center, 1515 Holcombe Boulevard,
Houston, TX 77030-4009

Mathie P.G. Leers (89)

Atrium Medical Center Heerlen, Department of Clinical
Chemistry & Hematology, P.O. Box 4446,
6401 CX Heerlen, The Netherlands

Jia-Qing Li (207)

Onco-pathology, Kagawa Medical University,
1750-1 Ikenobe, Miki-cho, Kita-gun,
Kagawa 761-0793, Japan

Jan C. Liang (327)

Division of Life Sciences, Lawrence Berkeley National
Laboratory, Berkeley, CA 94720

Jin-Tung Liang (139)

Division of Colorectal Surgery, Department of
Surgery, National Taiwan University Hospital, No. 7,
Chung-Shan South Road, Taipei, Taiwan

Gloria Lim (11)

Ontario Cancer Institute, Princess Margaret Hospital,
610 University Avenue, Room 9-721, Toronto,
Ontario, Canada M5G 2M9

D. Llanes (227)

Department of Genetics, Campus Rabanales,
Edificio C-5, Spanish Research Council, University of
Córdoba, 14071 Córdoba, Spain

C. Lucena (227)

Department of Genetics, Campus Rabanales,
Edificio C-5, Spanish Research Council, University of
Córdoba, 14071 Córdoba, Spain

Yoshihiko Maehara (55)

Department of Surgery and Science, Graduate School
of Medical Sciences, Kyushu University,
Fukuoka 812-8582, Japan

Anirban Maitra (83)

Department of Pathology, Ross Research Building,
Johns Hopkins University School of Medicine,
720 Rutland Avenue, Baltimore, MD 21205-2196

Paula Marrano (11)

Ontario Cancer Institute, Princess Margaret Hospital,
610 University Avenue, Room 9-721, Toronto, Ontario,
Canada M5G 2M9

Tsutomu Masaki (163)

Third Department of Internal Medicine, Kagawa
Medical University, 1750-1 Miki-cho, Kita-gun,
Kagawa 761-0793, Japan

Alan Meeker (83)

Department of Pathology, Ross Research Building,
Johns Hopkins University School of Medicine,
720 Rutland Avenue, Baltimore, MD 21205-2196

Masaru Miyazaki (159)

Department of Gastroenterological Surgery,
Chiba Cancer Center Hospital, 666-2 Nitonacho,
Chuo-ku, Chiba 260-0801, Japan

A. Moreno (227)

Department of Genetics, Campus Rabanales,
Edificio C-5, Spanish Research Council, University of
Córdoba, 14071 Córdoba, Spain

Marius Nap (89)

Atrium Medical Center Heerlen, Department of Clinical
Chemistry & Hematology, P.O. Box 4446,
6401 CX Heerlen, The Netherlands

Mef Nilbert (149)

Department of Oncology, Lund University of Hospital,
221 85 Lund, Sweden

Shinya Oda (55)

Department of Pathology, Institute for Clinical
Research, National Kyushu Cancer Center,
Notame 3-1-1, Fukuoka 811-1395, Japan

Ricardo Paniagua (399)

Department of Cell Biology and Genetics,
University of Alcalá, 28871 Alcalá de Henares,
Madrid, Spain

Jose Luis Perez-Gracia (65)

Medical Oncology Department, Clinica Universitaria de
Navarra, Avenida Pio XII, 36, 31008, Pamplona, Spain

Sven Petersen (183)

Department of General and Abdominal Surgery,
General Hospital Dresden-Friedrichstadt,
Teaching Hospital Technical University Dresden,
Dresden, Germany

Florence Pinet (245)

INSERM Unit 508, Institut Pasteur de Lille, 1 rue du
professeur Calmette, 59019 Lille cedex, France

George C. Prendergast (431)

Lankenau Institute for Medical Research, Department of
Pathology, Anatomy, and Cell Biology,
Thomas Jefferson University, 100 Lancaster Avenue,
Wynnewood, PA 19096

- Timo Pulli (23)
Functional Genome Analysis, Deutsches
Krebsforschungszentrum, Im Neuenheimer Feld 580,
D-69120 Heidelberg, Germany
- Maria Gloria Ruiz-Ilundain (65)
Medical Oncology Department, Clinica Universitaria de
Navarra, Avenida Pio XII, 36, 31008, Pamplona, Spain
- Hiroki Sasaki (1)
Genetics Division, National Cancer Center Research
Institute, 1-1, Tsukiji 5-chome, Chuo-ku,
Tokyo 104-0045, Japan
- Latha Satish (463)
Department of Pathology, 711 Scaife, University of
Pittsburgh, Pittsburgh, PA 15261
- Kimmo J. Savinainen (449)
Laboratory of Cancer Genetics, Institute of Medical
Technology, University of Tampere and Tampere
Hospital, Tampere, Finland
- Taro Shuin (299)
Department of Urology, Kochi Medical School,
Nankoku, Kochi 783-8505, Japan
- Michael D. Slater (335)
Institute for Biomedical Research, Department of
Anatomy and Histology, University of Sydney,
Sydney NSW 2006, Australia
- Nobuhito Sogawa (159)
Department of Gastroenterological Surgery,
Chiba Cancer Center Hospital, 666-2 Nitonacho,
Chuo-ku, Chiba 260-0801, Japan
- Jeremy A. Squire (11)
Ontario Cancer Institute, Princess Margaret Hospital,
610 University Avenue, Room 9-721, Toronto, Ontario,
Canada M5G 2M9
- D. Stefanou (193)
Department of Pathology, Medical School, University of
Ioannina, 45110 Ioannina, Greece
- Yana V. Syagailo (23)
Functional Genome Analysis, Deutsches
Krebsforschungszentrum, Im Neuenheimer Feld 580,
D-69120 Heidelberg, Germany
- Takahiro Taguchi (159)
Department of Anatomy, Kochi Medical School,
Nankoku, Kochi 783-8505, Japan
- Nobuhiro Takiguchi (159)
Department of Gastroenterological Surgery,
Chiba Cancer Center Hospital, 666-2 Nitonacho,
Chuo-ku, Chiba 260-0801, Japan
- Timothy M. Thomson (307)
Department of Anatomical Pathology, Hospital Clinic,
Villarroel 170, Barcelona 08036, Spain
- Pirkko Vihko (439)
Biocenter Oulu and Research Center for Molecular
Endocrinology, P.O. Box 5000, FIN-90014, University
of Oulu, Finland
- Tapio Visakorpi (449)
Institute of Medical Technology, University of Tampere,
Biokatu 6, FIN-33520, Tampere, Finland
- Hanlin L. Wang (267)
Department of Pathology & Immunology, Division of
Anatomic Pathology, Campus Box 8118, Washington
University School of Medicine, 660 South Euclid
Avenue, St. Louis, MO 63110-1093
- Zhengxin Wang (409)
Department of Cancer Biology, Box 173, The
University of Texas, M.D. Anderson Cancer Center,
1515 Holcombe Boulevard, Houston, TX 77030-4009
- Alan Wells (463)
Department of Pathology, 713 Scaife, University of
Pittsburgh, Pittsburgh, PA 15261
- Nicolas Wernert (255)
Institute of Pathology, University of Bonn, Sigmund-
Freud-Str. 25, P.O. Box 2120, 53011 Bonn, Germany
- Fei Wu (163)
Third Department of Internal Medicine, Kagawa
Medical University, 1750-1 Miki-cho, Kita-gun,
Kagawa 761-0793, Japan
- Guang-Jer Wu (347)
Department of Microbiology and Immunology and the
Winship Cancer Institute, Emory University School of
Medicine, 1510 Clifton Rd., NE, Atlanta, GA 30322
- Bernd Wullich (321)
Clinic of Urology and Pediatric Urology, University of
the Saarland, 66421 Homburg/Saar, Germany
- Ichiro Yamasaki (299)
Department of Urology, Kochi Medical School,
Nankoku, Kochi 783-8505, Japan
- Clayton Yates (463)
Department of Pathology, 711 Scaife, University of
Pittsburgh, Pittsburgh, PA 15261
- Isik G. Yulug (113)
Bilkent University, Faculty of Science, Department of
Molecular Biology and Genetics, 06800 Ankara, Turkey
- Lizhi Zhang (471)
Rm 5111 CCGC Building, 1500 E. Medical Center Dr.,
University of Michigan, Ann Arbor, MI 48109-0940
- Isabel Zudaire (37)
Department of Genetics, University of Navarra,
C/Irunlarrea s/n, 31008 Pamplona, Spain
- Giovanni Zummo (359)
Sezione di Anatomia Umana, Dipartimento di
Medicina Sperimentale, Università degli Studi di
Palermo, via del Vespro 129, 90127—Palermo—Italy

Foreword

According to mortality data from the National Center for Health Statistics, approximately 1,334,100 new cases of cancer will have been diagnosed, and 556,500 people will have died from cancer in the United States by the end of 2003. Though the number of cancer-related deaths has been on the decline since 1992, the incidence has increased over the same period. This increase is largely due to the implementation of improved screening techniques that have in turn been made possible by advances in immunochemical diagnostic testing. As immunochemical techniques such as *in situ* hybridization (ISH) and immunohistochemistry (IHC) continue to be refined, their use in improving patient care through research and improved methods of diagnosis is becoming ever more valuable.

In situ hybridization is a well-established approach for identifying the organization and physical position of a specific nucleic acid within the cellular environment, by means of hybridizing a complementary nucleotide probe to the sequence of interest. The use of deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) as probes to assay biologic material has been in use for approximately 30 years. However, recently, advances in ISH have seen a replacement of radioactive detection by more adaptable colorimetric and fluorescent (FISH) methods for the interrogation of nuclei, metaphase chromosomes, DNA fibers, patient tissue, and, most recently, deriving information from patient samples using DNA microarrays. Technologic advances, including array comparative genomic hybridization, spectral karyotyping, and multicolor banding, have provided a refinement in the study of genome organization and chromosomal rearrangements. In addition, ISH using RNA has allowed for a determination of the expression pattern and the abundance of specific transcripts on a cell-to-cell basis. Advances in DNA and RNA ISH have migrated from the research setting and are becoming routine tests in the clinical setting permitting examination of the steps involved in tumorigenesis, which would not have been possible by the use of classical cytogenetic analysis.

Since the introduction of monoclonal antibodies, immunohistochemistry has developed into a vital tool,

which is now extensively used in many research laboratories and for clinical diagnosis. Immunohistochemistry is a collective term for a variety of methods, which can be used to identify cellular or tissue components by means of antigen-antibody interactions. Immunostaining techniques date back to the pioneering work by Albert Coons in the early 1940s, using fluorescein-labeled antibodies. Since then, developments in the techniques have permitted visualization of antigen-antibody interactions by conjugation of the antibody to additional fluorophores, enzyme, or radioactive elements. As there are a wide variety of tissue types, antigen availabilities, antigen-antibody affinities, antibody types, and detection methods, it is essential to select antibodies almost on a case-to-case basis. The consideration of these factors has led to the identification of several key antibodies that have great utility in the study and diagnosis of tumors.

The scientific advances in the field of immunochemistry have necessitated rapid developments in microscopy, image capture, and analytical software in order to objectively quantify results. These cutting-edge experimental systems have already produced many significant differences between cancers that might not have been distinguished by conventional means.

The focus of these volumes is the use of ISH and IHC to study the molecular events occurring at the DNA, RNA, and protein levels during development and progression of human carcinomas. Continued investment of time and expertise by researchers worldwide has contributed significantly to a greater understanding of the disease processes. As the technical requirements for many immunochemical techniques is quite demanding and as the methodology itself poses many pitfalls, the step-by-step methods provided in these volumes will serve as an excellent guide for both clinical and basic researchers studying human malignancies.

Simon Hughes
Ontario Cancer Institute
Princess Margaret Hospital
Toronto, Canada

This Page Intentionally Left Blank

Preface to Volume 2

The primary objectives of this volume remain the same as those of volume 1—that is, discussion of immunohistochemical and *in situ* hybridization (ISH), including fluorescence *in situ* hybridization (FISH) and chromogenic *in situ* hybridization (CISH) procedures as they are used in the field of pathology, especially cancer diagnosis. The practical importance of the antigen retrieval protocols in immunohistochemistry was realized in 1991, and since then they have been used routinely in pathology laboratories. Many chapters in this volume contain the details of these protocols. However, detection of certain antigens even in formalin-fixed tissues can be accomplished without using antigen retrieval methods.

Immunohistochemistry, ISH, FISH, and CISH of two major carcinomas (colorectal and prostate) are presented. The biomarkers of two other major carcinomas (lung and breast) were explained in Volume 1, and others will be discussed in the forthcoming Volume 3. The procedures are explained in maximum details in a step-by-step fashion so that the readers can use them without additional references. Materials required to carry out the procedures are also included. These procedures are also useful in clinical laboratories.

Another objective of this volume is the discussion of the role of molecular pathology (molecular genetics, molecular medicine, molecular morphology) to understand and achieve correct diagnosis and therapy in neoplastic diseases. Molecular pathology/genetics has the advantage of assessing genes directly. Knowledge of the genetic basis of disease will, in turn, allow more specific targeting of the cause, rather than the symptoms only, of the disease. The time is overdue to apply our knowledge of molecular genetics in conjunction with immunohistochemistry and histology to diagnostic, therapeutic, and prognostic decisions.

Genetic information will improve the prognosis used to monitor both the efficacy of treatment and the disease recurrence. Molecular markers, largely from tumors but also from germline, have great potential for diagnosis, for directing treatment, and as indicators of

the outcome. In other words, these markers are of considerable importance to clinical practitioners. For this and other reasons the role of gene mutations in cancer is emphasized because the characteristics of the tumor depend on the mutations that lead to their emergence. For example, down-regulation of tumor suppressor genes BRCA1/2 and their proteins is a well-known test for breast cancer susceptibility, resulting in poor prognosis. Indeed, methods of molecular testing of tumors are finally well established and are discussed in this and other volumes of this series of handbooks. Widespread molecular testing is the future for clinical practice.

Unfortunately, clinical practice has lagged behind the current knowledge of research in molecular genetics. Both technicians and pathologists need to be aware of the importance of molecular pathology testing. Somatic mutations are rarely performed, although some histopathology and cytogenetics laboratories have done limited testing such as chromosomal rearrangements in lymphoma. Molecular testing should be regarded as a means of complementing, rather than replacing, established methods such as immunohistochemistry and FISH.

There are several reasons for the limited use of molecular genetics in clinical practice. One reason is the high cost of establishing facilities for molecular techniques; another is our comparatively meager understanding of the nature of many diseases, including cancer. Although equipment for molecular testing is available, some investment is needed. Another reason is the dearth of clinician–scientist training programs, resulting in limited clinician–scientists. Also, an inequity in pay exists between those working in clinical practice versus research faculty. Accordingly, the differential in pay may be a disincentive for choosing a full-time career in medical research. The length of time (8 years as an average) to receive the M.D./Ph.D. is probably also a barrier in the development of new clinician–scientists. Many clinician–scientist trainees are married, or are in stable relationships, and personal time for family life and children is increasingly important.

Narrowing the gap in income between clinical practitioners and full-time medical researchers would provide a positive incentive for this profession.

Pathologists are well advised to adapt to modern therapeutic shifts (i.e., morphologic interpretation needs to be combined with molecular diagnostic modalities). The latter protocols can provide a second level of testing that is particularly useful for the analysis of neoplasms for which histologic and immunophenotypic data are inconclusive. Therapies already are beginning to progress more and more toward specific molecular targets. Examples are deoxyribonucleic acid (DNA) microarrays, differential display of gene expression, serial analysis of gene expression, comparative genomic hybridization, rolling circle amplification, reverse transcription polymerase chain reaction, FISH, Southern Blot hybridization, and specific cloned probes; most of these methods were discussed in Volume 1 and are also discussed in this volume. Flow cytometry technology is also presented. We already are down a path that has the potential to alter oncology clinical practice. My hope, through this series of volumes, is to expedite the translation of molecular genetics into clinical practice.

I am indebted to the authors of the chapters for their promptness and appreciate their dedication and hard work in sharing their expertise with the readers. In most cases the protocols presented were either introduced or refined by the authors and routinely used in their clinical pathology laboratories. The methods presented here offer much more detailed information than is available in scientific journals. Because of its relatively recent emergence from the research

laboratory, many molecular pathology protocols are still found in scientific journals only and have not appeared in a book. Each chapter provides unique individual practical knowledge based on the expertise of the author. As with all clinical laboratory testing, the results obtained should be interpreted in conjunction with other established and proven laboratory data and clinical findings.

This volume has been developed through the efforts of 97 authors, representing 15 countries. The high quality of each manuscript made my work as the editor an easy one. The authors were gracious and prompt. This volume is intended for use in research and clinical laboratories by medical technicians and pathologists, especially in the field of oncology. This volume will also be of interest and help to medical students.

I appreciate the cooperation extended to me by Hilary Rowe, a valued, competent publishing editor. As the sponsoring editor, her understanding of the importance of this project in the field of human carcinomas helped me to embark on this uniquely difficult and complex endeavor and bring it to fruition. I am grateful to Dr. Frank Esposito and Dr. Dawood Farahi for their recognition of my teaching and scholarly contributions, and for their help. I acknowledge the hard, efficient work of Denise DeLancey, the production editor. I greatly appreciate receiving indispensable, expert help from Eliza McGovern in the preparation of the manuscript.

M.A. Hayat
February 2004

Contents of Volume 1

Selected Definitions

Classification Scheme of Human Cancers

Lung and Breast Carcinomas

I Introduction

Comparison of Immunohistochemistry, *in situ* Hybridization, Fluorescence *in situ* Hybridization, and Chromogenic *in situ* Hybridization

Comparison of Chromogenic *in situ* Hybridization, Fluorescence *in situ* Hybridization, and Immunohistochemistry

Target and Signal Amplification to Increase the Sensitivity of *in situ* Hybridization

II Molecular Pathology

Polymerase Chain Reaction Technology

DNA Microarrays Technology

Tissue Microarrays and Their Modifications in High-Throughput Analysis of Clinical Specimens

Gene Expression Profiling Using Laser Microdissection in Cancer Tissues

Differential Display of Gene Expression in Human Carcinomas

Serial Analysis of Gene Expression in Human Diseases

III Lung Carcinoma

Lung Carcinoma: An Introduction

Histopathologic Classification and Phenotype of Lung Tumors

Immunohistochemistry and *in situ* Hybridization of Mucin in Lung Carcinoma

Immunohistochemical Expression of MDM2 in Lung Cancer

Immunohistochemical Expression of E2F1 and p14ARF in Lung Carcinoma

Role of Immunohistochemical Expression of Beta Catenin in Lung Carcinoma

Role of Immunohistochemical Expression of Laminin-5 in Lung Carcinoma

Role of Immunohistochemical Expression of Caveolin-1 in Lung Carcinoma

Role of Thyroid Transcription Factor-1 in Pulmonary Adenocarcinoma

Role of Global Methylation of DNA in Lung Carcinoma

Immunohistochemical and Molecular Pathology of Angiogenesis in Primary Lung Adenocarcinoma

Immunohistochemistry of Human Leukocyte Antigen Expression in Lung Carcinoma

In situ Hybridization and Immunohistochemistry of Telomerase in Lung Carcinoma

Use of Fluorescence *in situ* Hybridization in Detection of Lung Cancer Cells

Role of Immunohistochemical Expression of *BCL-2* Gene in Lung Carcinoma

IV Breast Carcinoma

Breast Carcinoma: An Introduction

HER-2 (C-ERB-B-2) Oncoprotein

HER-2/neu Gene Amplification and Protein Overexpression in Breast Carcinoma: Immunohistochemistry and Fluorescence *in situ* Hybridization

HER-2/neu Amplification Detected by Fluorescence *in situ* Hybridization in Cytologic Samples from Breast Cancer

Detection of HER-2 Oncogene with Chromogenic *in situ* Hybridization in Breast Carcinoma

Immunohistochemical Evaluation of Sentinel Lymph Nodes in Patients with Breast Carcinoma

CD10 Expression in Normal Breast and Breast Cancer Tissues

Role of Immunohistochemical Expression of AKT Protein in Breast Carcinoma

Expression of Extracellular Matrix Proteins in Breast Cancer

Immunohistochemistry of Adhesion Molecule CEACAM 1 Expression in Breast Carcinoma

Role of Cadherins in Breast Cancer

Role of Immunohistochemical Expression of Erythropoietin and Erythropoietin Receptor in Breast Carcinoma

Loss of *BRCA1* Gene Expression in Breast Carcinoma

Role of Immunohistochemical Expression of *BRCA1* in Breast Carcinoma

Fluorescence *in situ* Hybridization of *BRCA1* Gene in Breast Carcinoma

Immunohistochemistry of C-MYC Expression in Breast Cancer

Immunohistochemical Localization of Neuropilin-1 in Human Breast Carcinoma: A Possible Molecular Marker for Diagnosis

Role of Epidermal Growth Factor Receptor in Breast Carcinoma

Alterations of the Cell-Cycle-Regulating Proteins in Invasive Breast Cancer: Correlation with Proliferation, Apoptosis, and Clinical Outcome

Role of Immunohistochemical Expression of Estrogen Receptor in Breast Carcinoma

Immunofluorescence and Immunohistochemical Localization of Progesterone Receptors in Breast Carcinoma

Immunohistochemical Expression of Cytosolic Thymidine Kinase in Patients with Breast Carcinoma

Immunohistochemical Detection of Melanoma Antigen E (MAGE) Expression in Breast Carcinoma

Male Breast Carcinoma: Role of Immunohistochemical Expression of

Receptors in Male Breast Carcinoma

Detection of Glycoconjugates in Breast Cancer Cell Lines: Confocal Fluorescence Microscopy

Role of *ETV6-NTRK3* Gene Fusion in Breast Carcinoma

Immunohistochemistry of CA6 Antigen Expression in Breast Carcinoma

Immunocytochemistry of Effusions

Immunohistochemistry of Needle Cytopunctures of Breast Carcinomas