DIAGNOSTIC MOLECULAR PATHOLOGY

DIAGNOSTIC MOLECULAR PATHOLOGY

A Guide to Applied Molecular Testing

Edited by

WILLIAM B. COLEMAN, PHD

Department of Pathology and Laboratory Medicine, Program in Translational Medicine, UNC Lineberger Comprehensive Cancer Center, University of North Carolina School of Medicine, Chapel Hill, NC, United States

GREGORY J. TSONGALIS, PHD, HCLD, CC

Laboratory for Clinical Genomics and Advanced Technology (CGAT), Department of Pathology and Laboratory Medicine, Dartmouth-Hitchcock Medical Center and Norris Cotton Cancer Center, Lebanon, NH, United States; Geisel School of Medicine at Dartmouth, Hanover, NH, United States

AMSTERDAM • BOSTON • HEIDELBERG • LONDON
NEW YORK • OXFORD • PARIS • SAN DIEGO
SAN FRANCISCO • SINGAPORE • SYDNEY • TOKYO

Academic Press is an imprint of Elsevier

Academic Press is an imprint of Elsevier
125 London Wall, London EC2Y 5AS, United Kingdom
525 B Street, Suite 1800, San Diego, CA 92101-4495, United States
50 Hampshire Street, 5th Floor, Cambridge, MA 02139, United States
The Boulevard, Langford Lane, Kidlington, Oxford OX5 1GB, United Kingdom

Notices
Knowledge and best practice in this field are constantly changing. As new research and experience broaden our understanding, changes in research methods, professional practices, or medical treatment may become necessary.

Practitioners and researchers must always rely on their own experience and knowledge in evaluating and using any information, methods, compounds, or experiments described herein. In using such information or methods they should be mindful of their own safety and the safety of others, including parties for whom they have a professional responsibility.

To the fullest extent of the law, neither the Publisher nor the authors, contributors, or editors, assume any liability for any injury and/or damage to persons or property as a matter of products liability, negligence or otherwise, or from any use or operation of any methods, products, instructions, or ideas contained in the material herein.

British Library Cataloguing-in-Publication Data
A catalogue record for this book is available from the British Library

Library of Congress Cataloging-in-Publication Data
A catalog record for this book is available from the Library of Congress

ISBN: 978-0-12-800886-7

Publisher: Mica Haley
Acquisition Editor: Tari Broderick
Editorial Project Manager: Jeff Rosetti
Production Project Manager: Julia Haynes
Designer: Inês Cruz

Typeset by MPS Limited, Chennai, India

Endorsements

Diagnostic Molecular Pathology is destined to become an important cornerstone and go-to volume for pathologists, researchers, and clinicians—indeed anyone who wants to understand the approach and application of modern molecular techniques in disease detection and diagnosis. Drs. Coleman and Tsongalis—and their impressive stable of contributors—are to be congratulated for making the exciting and rapidly expanding science of molecular diagnosis accessible to the novice, while also providing the expert with important details on the nuances. Beyond just a compendium of useful information, it is a well-curated journey through basic concepts, infectious diseases, malignancy, hematopathology, and genetic diseases—and even includes access to a website with lab test videos and decision-making exercises.

Richard N. Mitchell, MD, PhD (Brigham and Women's Hospital and Harvard Medical School)

With much fanfare, molecular techniques have revolutionized clinical medicine, from diagnosis to personalized medical therapeutics. It is with this background that *Diagnostic Molecular Pathology* approaches the daunting task of summarizing the advances in the field from infectious diseases, heritable and acquired genetic diseases, hematological malignancies, to pharmacogenomics. In a comprehensive publication, the editors perform a yeoman's effort in covering this dynamic and exciting field.

Lawrence M. Silverman, PhD (University of Virginia School of Medicine)

Diagnostic Molecular Pathology provides a panoramic view of diagnostic molecular pathology testing, written by leaders in the field. It is a great reference and learning tool.

Dani S. Zander, MD (University of Cincinnati Medical Center)

Dedication

This textbook describes the emerging field of *diagnostic molecular pathology* and its application to various forms of human disease. Despite the relative youthfulness of this field, *diagnostic molecular pathology* has become critically important in the contemporary practice of personalized medicine and is built upon the collective knowledgebase that reflects our understanding of the pathology, pathogenesis, and pathophysiology of human disease. As such, the information contained in this textbook represents the culmination of innumerable small successes that emerged from the ceaseless pursuit of new knowledge by countless clinical and experimental pathologists working around the world on all aspects of human disease. Their ingenuity and hard work have dramatically advanced the field of molecular pathology over time, and particularly during the last 25 years. This book is a tribute to the dedication, diligence, and perseverance of individual scientists who contributed to the advancement of our understanding of the molecular basis of human disease, especially graduate students, laboratory technicians, and postdoctoral fellows, whose efforts are so frequently taken for granted, whose accomplishments are so often unrecognized, and whose contributions are so quickly forgotten.

Diagnostic Molecular Pathology: A Guide to Applied Molecular Testing is dedicated to the memory of Dr. Kathleen Rao who passed away on March 24, 2016, following a brief battle with cancer. Dr. Rao earned a PhD in genetics from the University of North Carolina at Chapel Hill and was a member of the faculty in the UNC School of Medicine from 1984 until the time of her death. Dr. Rao was a Professor of Pediatrics, Genetics, and Pathology and Laboratory Medicine, and served as the Director of the Cytogenetics Laboratory for UNC Hospitals. Dr. Rao made numerous contributions to the field of cytogenetics, was a Founding Fellow of the American College of Medical Genetics and Genomics, and was the recipient of the 2016 Distinguished Cytogeneticist Award. She served on the International Standing Committee on Cytogenetic Nomenclature, the Children's Oncology Group Cytogenetics Committee, and the Cancer and Leukemia Group B Cytogenetics Review Committee. Dr. Rao was also well-recognized as an extraordinary medical educator at the University of North Carolina where she was a Founding member of the UNC School of Medicine's Academy of Educators. As Director of the Cytogenetics Laboratory Fellowship Training Program at UNC, Dr. Rao taught and mentored numerous students who now work in the field of cytogenetics throughout the United States. Dr. Rao was a dear friend and cherished colleague to many people at the University of North Carolina and across the country. We are proud to have known her and worked with her through the years. We are also extremely honored to have her as a contributor to this textbook (see chapter: Molecular Testing in Pediatric Cancers) and regret that we will not have another chance to work with her on a project like this one. This book is dictated to the example Dr. Rao provides all of us—as a distinguished educator, an accomplished molecular pathologist, and a genuinely good person.

We also dedicate *Diagnostic Molecular Pathology: A Guide to Applied Molecular Testing* to the many people that have played crucial roles in our successes. We thank our many scientific colleagues, past and present, for their camaraderie, collegiality, and support. We especially thank our scientific mentors for their example of dedication to research excellence. We are truly thankful for the positive working relationships and friendships that we have with our faculty colleagues, for the mentoring we received from our elders, and for the opportunity to mentor those that follow us. We also thank our undergraduate students, graduate students, and postdoctoral fellows for teaching us more than we might have taught them. We thank our parents for believing in higher education, for encouragement through the years, and for helping make dreams into reality. We thank our brothers and sisters, and extended families, for the many years of love, friendship, and tolerance. We thank our wives, Monty and Nancy, for their unqualified love, unselfish support of our endeavors, understanding of our work ethic, and

appreciation for what we do. Lastly, we give special thanks to our children, Tess, Sophie, Pete, and Zoe. Their achievements and successes as young adults are a greater source of pride for us than our own accomplishments. As when they were children, we thank them for providing an unwavering bright spot in our lives, for their unbridled enthusiasm and boundless energy, and for giving us a million reasons to take an occasional day off from work just to have fun.

William B. Coleman
Gregory J. Tsongalis

List of Contributors

Kimberly H. Allison, MD Department of Pathology, Stanford University School of Medicine, Stanford, CA, United States

Megan A. Allyse, PhD Department of Health Sciences Research, Mayo Clinic School of Medicine, Rochester, MN, United States

Rodney C. Arcenas, PhD, D(ABMM) Molecular Microbiology and Immunology, Memorial Healthcare System, Pathology Consultants of South Broward, Hollywood, FL, United States

Michael J. Bartel, MD Division of Gastroenterology & Hepatology, Mayo Clinic, Jacksonville, FL, United States

Amir Behdad, MD Division of Hematopathology, Northwestern University, Feinberg School of Medicine, Northwestern Memorial Hospital, Chicago, IL, United States

Katie M. Bennett, PhD, MB (ASCP)CM, NRCC-CC Texas Tech University Health Sciences Center, School of Health Professions, Molecular Pathology Program, Lubbock, TX, United States

Jonathan S. Berg, MD, PhD Department of Genetics, University of North Carolina School of Medicine, Chapel Hill, NC, United States

D. Hunter Best, PhD Department of Pathology, University of Utah School of Medicine, Salt Lake City, UT, United States; Molecular Genetics and Genomics, ARUP Laboratories, University of Utah School of Medicine, Salt Lake City, UT, United States

Bryan L. Betz, PhD Department of Pathology, University of Michigan, Ann Arbor, MI, United States

Jessica K. Booker, PhD Department of Pathology and Laboratory Medicine; Department of Genetics, University of North Carolina at Chapel Hill, Chapel Hill, NC, United States

Kristi S. Borowski, MD Departments of Medical Genetics and Obstetrics and Gynecology, Mayo Clinic School of Medicine, Rochester, MN, United States

Thomas Bourlet, PharmD, PhD GIMAP EA3064, University of Lyon, Saint-Etienne, France; Laboratory of Infectious Agents and Hygiene, University Hospital of Saint-Etienne, Saint-Etienne, France

Pierre Brissot, MD, PhD National Center of Reference for Rare Genetic Iron Overload Diseases, Pontchaillou University Hospital, Rennes, France; Inserm-UMR 991, University of Rennes 1, Rennes, France

Noah A. Brown, MD Department of Pathology, University of Michigan, Ann Arbor, MI, United States

Marcin Bula, PhD The Wolfson Centre for Personalised Medicine, Institute of Translational Medicine, University of Liverpool, Liverpool, United Kingdom

Richard M. Caprioli, PhD Mass Spectrometry Research Center, and Department of Biochemistry, Vanderbilt University School of Medicine, Nashville, TN, United States

Subhankar Chakraborty, MD Division of Gastroenterology & Hepatology, Mayo Clinic, Rochester, MN, United States

William B. Coleman, PhD Department of Pathology and Laboratory Medicine, Program in Translational Medicine, UNC Lineberger Comprehensive Cancer Center, University of North Carolina School of Medicine, Chapel Hill, NC, United States

Kristy R. Crooks, PhD Department of Pathology, University of Colorado, Anschutz Medical Campus, Aurora, CO, United States

Jianli Dong, MD Department of Pathology, University of Texas Medical Branch, Galveston, TX, United States

Harry A. Drabkin, MD Department of Medicine, Division of Hematology/Oncology, Medical University of South Carolina, Charleston, SC, United States

Daniel L. Duncan, MD Department of Pathology and Laboratory Medicine, University of North Carolina School of Medicine, Chapel Hill, NC, United States

Jawed Fareed, PhD Department of Pathology, Loyola University Health System, Maywood, IL, United States

Andrea Ferreira-Gonzalez, PhD Division of Molecular Diagnostics, Department of Pathology, Virginia Commonwealth University, Richmond, VA, United States

Birgit H. Funke, PhD, FACMG Laboratory for Molecular Medicine, Partners Personalized Medicine, Boston, MA, United States; Department of Pathology, Harvard Medical School, Boston, MA, United States; Department of Pathology, Massachusetts General Hospital, Boston, MA, United States

Larissa V. Furtado, MD Department of Pathology, University of Chicago, Chicago, IL, United States

Giorgio Gallinella, MD, PhD Department of Pharmacy and Biotechnology, S. Orsola-Malpighi Hospital – Microbiology, University of Bologna, Bologna, Italy

Sonzalo Gonzalo, PharmD GIMAP EA3064, University of Lyon, Saint-Etienne, France; Laboratory of Infectious Agents and Hygiene, University Hospital of Saint-Etienne, Saint-Etienne, France

Florence Grattard, MD, PhD GIMAP EA3064, University of Lyon, Saint-Etienne, France; Laboratory of Infectious Agents and Hygiene, University Hospital of Saint-Etienne, Saint-Etienne, France

Danielle B. Gutierrez, PhD Mass Spectrometry Research Center, and Department of Biochemistry, Vanderbilt University School of Medicine, Nashville, TN, United States

Gloria T. Haskell, PhD Department of Genetics, Duke University, Durham, NC, United States

Amin A. Hedayat, MD Department of Pathology, Dartmouth-Hitchcock Medical Center, Lebanon, NH, United States

W. Edward Highsmith, Jr, PhD Departments of Laboratory Medicine and Pathology, and Medical Genetics, Mayo Clinic School of Medicine, Rochester, MN, United States

Susan J. Hsiao, MD, PhD Department of Pathology & Cell Biology, Columbia University Medical Center, New York, NY, United States

Omer Iqbal, MD Department of Pathology, Loyola University Health System, Maywood, IL, United States

Nahed Ismail, MD, PhD, D(ABMM), D(ABMLI) Department of Pathology, University of Pittsburgh, Pittsburgh, PA, United States

Anne-Marie Jouanolle, PharmD National Center of Reference for Rare Genetic Iron Overload Diseases, Laboratory of Molecular Genetics and Genomics, Pontchaillou University Hospital, Rennes, France

Sarah E. Kerr, MD Department of Laboratory Medicine and Pathology, College of Medicine, Mayo Clinic, Rochester, MN, United States

Olivier Loréal, MD, PhD National Center of Reference for Rare Genetic Iron Overload Diseases, Pontchaillou University Hospital, Rennes, France; Inserm-UMR 991, University of Rennes 1, Rennes, France

Heather M. McLaughlin, PhD Laboratory for Molecular Medicine, Partners Personalized Medicine, Department of Pathology, Harvard Medical School, Massachusetts General Hospital, Boston, MA, United States

Meriam Memmi, PhD GIMAP EA3064, University of Lyon, Saint-Etienne, France; Laboratory of Infectious Agents and Hygiene, University Hospital of Saint-Etienne, Saint-Etienne, France

Tomasz I. Michalak, MD, PhD, FAASLD, FCAHS Molecular Virology and Hepatology Research Group, Division of BioMedical Science, Faculty of Medicine, Health Sciences Centre, Memorial University, St. John's, Newfoundland, Canada

Melissa B. Miller, PhD Department of Pathology and Laboratory Medicine, UNC School of Medicine, Chapel Hill, NC, United States

Patricia M. Mulrooney-Cousins, PhD Molecular Virology and Hepatology Research Group, Division of BioMedical Science, Faculty of Medicine, Health Sciences Centre, Memorial University, St. John's, Newfoundland, Canada

Yuri E. Nikiforov, MD, PhD Division of Molecular & Genomic Pathology, Department of Pathology, University of Pittsburgh School of Medicine, Pittsburgh, PA, United States

Jeremy L. Norris, PhD Mass Spectrometry Research Center, and Department of Biochemistry, Vanderbilt University School of Medicine, Nashville, TN, United States

Nirali M. Patel, MD Department of Pathology and Laboratory Medicine, University of North Carolina School of Medicine, Chapel Hill, NC, United States

Peter L. Perrotta, MD Department of Pathology, West Virginia University, Morgantown, WV, United States

Benjamin A. Pinsky, MD, PhD Department of Medicine, Division of Infectious Diseases and Geographic Medicine, Department of Pathology, Stanford University School of Medicine, Stanford, CA, United States

Munir Pirmohamed, MBChB (Hons), PhD, FRCP, FRCP(E) The Wolfson Centre for Personalised Medicine, Institute of Translational Medicine, University of Liverpool, Liverpool, United Kingdom

Rongpong Plongla, MD, MSc Division of Infectious Diseases, Chulalongkorn University and King Chulalongkorn Memorial Hospital, Bangkok, Thailand; Department of Pathology and Laboratory Medicine, UNC School of Medicine, Chapel Hill, NC, United States

Bruno Pozzetto, MD, PhD GIMAP EA3064, University of Lyon, Saint-Etienne, France; Laboratory of Infectious Agents and Hygiene, University Hospital of Saint-Etienne, Saint-Etienne, France

Victoria M. Pratt, PhD, FACMG Pharmacogenomics Laboratory, Department of Medical and Molecular Genetics, Indiana University School of Medicine, Indianapolis, IN, United States

Gary W. Procop, MD, MS Section of Clinical Microbiology, Department of Laboratory Medicine, Cleveland Clinic, Cleveland, OH, United States

Massimo Raimondo, MD Division of Gastroenterology & Hepatology, Mayo Clinic, Jacksonville, FL, United States

***Kathleen W. Rao, PhD** Departments of Pediatrics, Pathology and Laboratory Medicine, and Genetics, University of North Carolina School of Medicine, Chapel Hill, NC, United States; Cytogenetics Laboratory, McLendon Clinical Laboratories, UNC Hospitals, Chapel Hill, NC, United States

Stuart A. Scott, PhD, FACMG Department of Genetics and Genomic Sciences, Icahn School of Medicine at Mount Sinai, New York, NY, United States

Chanjuan Shi, MD, PhD Department of Pathology, Microbiology, and Immunology, Vanderbilt University Medical Center, Nashville, TN, United States

Carolyn J. Shiau, MD, FRCPC Department of Pathology, University Health Network, Toronto, ON, Canada

Yue Si, PhD Department of Pathology, University of Utah School of Medicine, Salt Lake City, UT, United States

Steven C. Smith, MD, PhD Department of Pathology, Virginia Commonwealth University School of Medicine, Richmond, VA, United States

Matthew B. Smolkin, MD Department of Pathology, West Virginia University, Morgantown, WV, United States

Kathleen A. Stellrecht, PhD Department of Pathology and Laboratory Medicine, Albany Medical College; Albany Medical Center Hospital, Albany, NY, United States

Susanna K. Tan, MD Department of Medicine, Division of Infectious Diseases and Geographic Medicine, Stanford University School of Medicine, Stanford, CA, United States

Jessica S. Thomas, MD, PhD, MPH Department of Pathology, Microbiology, and Immunology, Vanderbilt University Medical Center, Nashville, TN, United States

Scott A. Tomlins, MD, PhD Department of Pathology, Michigan Center for Translational Pathology, Department of Urology, Comprehensive Cancer Center, University of Michigan Medical School, Ann Arbor, MI, United States

Dimitri G. Trembath, MD, PhD Division of Neuropathology, Department of Pathology and Laboratory Medicine, The University of North Carolina at Chapel Hill, Chapel Hill, NC, United States

Ming-Sound Tsao, MD, FRCPC Department of Pathology, University Health Network, Toronto, ON, Canada

Gregory J. Tsongalis, PhD, HCLD, CC Laboratory for Clinical Genomics and Advanced Technology (CGAT), Department of Pathology and Laboratory Medicine, Dartmouth-Hitchcock Medical Center and Norris Cotton Cancer Center, Geisel School of Medicine at Dartmouth, Hanover, NH, United States

Richard M. Turner, MB, BChir, MA, MRCP The Wolfson Centre for Personalised Medicine, Institute of Translational Medicine, University of Liverpool, Liverpool, United Kingdom

Scott A. Turner, MD, PhD Laboratory for Clinical Genomics and Advanced Technology (CGAT), Department of Pathology and Laboratory Medicine, Dartmouth-Hitchcock Medical Center and Norris Cotton Cancer Center, Geisel School of Medicine at Dartmouth, Hanover, NH, United States

Aaron M. Udager, MD, PhD Department of Pathology, University of Michigan Medical School, Ann Arbor, MI, United States

Paul Verhoeven, MD, PhD GIMAP EA3064, University of Lyon, Saint-Etienne, France; Laboratory of Infectious Agents and Hygiene, University Hospital of Saint-Etienne, Saint-Etienne, France

David H. Walker, MD Department of Pathology, University of Texas Medical Branch, Galveston, TX, United States

Myra J. Wick, MD Departments of Medical Genetics and Obstetrics and Gynecology, Mayo Clinic School of Medicine, Rochester, MN, United States

Kathryn Willoughby, MD Department of Medicine, Division of Hematology/Oncology, Medical University of South Carolina, Charleston, SC, United States

Shaofeng Yan, MD, PhD Department of Pathology, Dartmouth-Hitchcock Medical Center, Lebanon, NH, United States

Belinda Yen-Lieberman, MS, PhD Section of Clinical Microbiology, Department of Laboratory Medicine, Cleveland Clinic, Cleveland, OH, United States

Preface

Pathology is the scientific study of the nature of disease and its causes, processes, development, and consequences. The field of pathology emerged from the application of the scientific method to the study of human disease. Thus, pathology as a discipline represents the complimentary intersection of medicine and basic science. Early pathologists were typically practicing physicians who described the various diseases that they treated and made observations related to factors that contributed to the development of these diseases. The description of disease evolved over time from gross observation to structural and ultrastructural inspection of diseased tissues based upon light and electron microscopy. As hospital-based and community-based registries of disease were developed, the ability of investigators to identify factors that cause disease and assign risk to specific types of exposures expanded to increase our knowledge of the epidemiology of disease. While descriptive pathology can be dated to the earliest written histories of medicine and the modern practice of diagnostic pathology dates back perhaps 200 years, the elucidation of mechanisms of disease and linkage of disease pathogenesis to specific causative factors occurred more recently from studies in experimental pathology. The field of experimental pathology embodies the conceptual foundation of early pathology—the application of the scientific method to the study of disease—and applies modern investigational tools of cell and molecular biology to advanced animal model systems and studies of human subjects. Whereas the molecular era of biological science began over 50 years ago, recent advances in our knowledge of molecular mechanisms of disease have propelled the field of molecular pathology. These advances were facilitated by significant improvements and new developments associated with the techniques and methodologies available to pose questions related to the molecular biology of normal and diseased states affecting cells, tissues, and organisms. Today, molecular pathology encompasses the investigation of the molecular mechanisms of disease and interfaces with translational medicine where new basic science discoveries form the basis for the development of new therapeutic approaches and targeted therapies for the new strategies for prevention, and treatment of disease. Diagnostic molecular pathology is a new field that is focused on exploitation of molecular features and mechanisms of disease for the development of practical molecular diagnostic tools for disease detection, diagnosis, and prognostication. Diagnostic molecular pathology is essential for the realization of true personalized medicine. As this field continues to expand and mature, new molecular tests will emerge that will have utility in the sensitive and specific detection, diagnosis, and prognostication of human disease. Over time, the molecular technologies required will become increasingly economically practical and accessible to all patients whether treated in academic medical centers or community hospitals.

With the remarkable pace of scientific discovery in the field of *diagnostic molecular pathology*, basic scientists, clinical scientists, and physicians have a need for a source of information on the current state-of-the-art of our understanding of the molecular basis of human disease and how we harness the molecular features of disease for practical molecular testing. More importantly, the complete and effective training of today's graduate students, medical students, postdoctoral fellows, and others, for careers related to the investigation and treatment of human disease requires textbooks that have been designed to reflect our current knowledge of the molecular mechanisms of disease pathogenesis, as well as emerging concepts related to translational medicine. In this volume on *Diagnostic Molecular Pathology: A Guide to Applied Molecular Testing* we have assembled a group of experts to discuss the molecular basis and mechanisms of major human diseases and disease processes, presented in the context of traditional pathology, and how these molecular features of disease can be effectively harnessed to develop practical molecular tests for disease detection, diagnosis, and prognostication. This volume is intended to serve as a multiuse textbook that would be appropriate as a classroom teaching tool for medical students, biomedical graduate students, allied health students, and others (such as advanced undergraduates). Further, this textbook will be valuable for pathology residents and other postdoctoral

fellows who desire to advance their understanding of molecular mechanisms of disease and practical applications related to these mechanisms, beyond what they learned in medical/graduate school. In addition, this textbook is useful as a reference book for practicing basic scientists and physician scientists who perform disease-related basic science and translational research, who require a ready information resource on the molecular basis of various human diseases and disease states and the molecular tests that are used during patient workup in a modern hospital laboratory. To be sure, our understanding of the many causes and molecular mechanisms that govern the development of human diseases is far from complete, and molecular testing has not yet become available for all human diseases. Nevertheless, the amount of information related to the practical exploitation of molecular mechanisms of human disease has increased tremendously in recent years and areas of thematic and conceptual consensus have emerged. We hope that *Diagnostic Molecular Pathology: A Guide to Applied Molecular Testing* will accomplish its purpose of providing students, researchers, and practitioners with in-depth coverage of the molecular basis of major human diseases and associated molecular testing so as to stimulate new research aimed at furthering our understanding of these molecular mechanisms of human disease and practice of molecular medicine through the development of new and novel molecular technologies and tests.

William B. Coleman
Gregory J. Tsongalis

CHAPTER

1

Basic Concepts in Molecular Pathology—Introduction to Molecular Testing in Human Disease

W.B. Coleman[1] *and G.J. Tsongalis*[2]

[1]Department of Pathology and Laboratory Medicine, Program in Translational Medicine, UNC Lineberger Comprehensive Cancer Center, University of North Carolina School of Medicine, Chapel Hill, NC, United States
[2]Laboratory for Clinical Genomics and Advanced Technology (CGAT), Department of Pathology and Laboratory Medicine, Dartmouth-Hitchcock Medical Center and Norris Cotton Cancer Center, Geisel School of Medicine at Dartmouth, Hanover, NH, United States

INTRODUCTION

Human diseases reflect a spectrum of pathologies and mechanisms of disease pathogenesis. The general categories of disease affecting humans include (1) hereditary diseases, (2) infectious diseases, (3) inflammatory diseases, and (4) neoplastic diseases. Pathologic conditions representing each of these general categories have been described for every tissue in the body. Despite the grouping of diseases by the common features of the general disease type, the pathogenesis of all of the various diseases is unique, and in some cases multiple distinct mechanisms can give rise to a similar pathology (disease manifestation). Disease causation may be related to intrinsic factors or extrinsic factors, but many/most diseases are multifactorial, involving a combination of intrinsic (genetic) and extrinsic factors (exposures). It is now well recognized that most major diseases are ultimately the result of aberrant gene expression, and that susceptibility to disease is significantly influenced by patterns of gene expression in target cells or tissues for a particular type of pathology. It follows that gene mutations and other genetic alterations are important in the pathogenesis of many human diseases. Hence, molecular diagnostic testing for genetic alterations may (1) facilitate disease detection, (2) aid in disease classification (diagnosis), (3) predict disease outcomes (prognostication), and/or (4) guide therapy (Fig. 1.1). Likewise, nongenetic alterations affecting the expression of key genes (termed epimutations) may also contribute to the genesis of disease at many tissue sites. Molecular testing focused on epigenetic alterations in human disease is emerging and in development. Like genetic alterations, epigenetic changes may significantly impact on certain disease characteristics that confer diagnostic value. Epigenetic alterations can lead to gene silencing events (which are mechanistically equivalent to inactivating mutations or gene deletions) and may contribute to gene expression signatures that have predictive value with respect to clinical features of disease.

In this chapter we describe basic concepts in molecular pathology and molecular diagnostic testing for human disease. This is intended to be an introductory review of the field, rather than a comprehensive review of the field. Hence, when needed, examples are drawn preferentially from the cancer literature. Interested readers will find comparable literature in numerous other biomedical fields.

MUTATIONS AND EPIMUTATIONS

Mutation refers to changes in the genome that are characterized by alteration in the nucleotide sequence

DOI: http://dx.doi.org/10.1016/B978-0-12-800886-7.00001-7

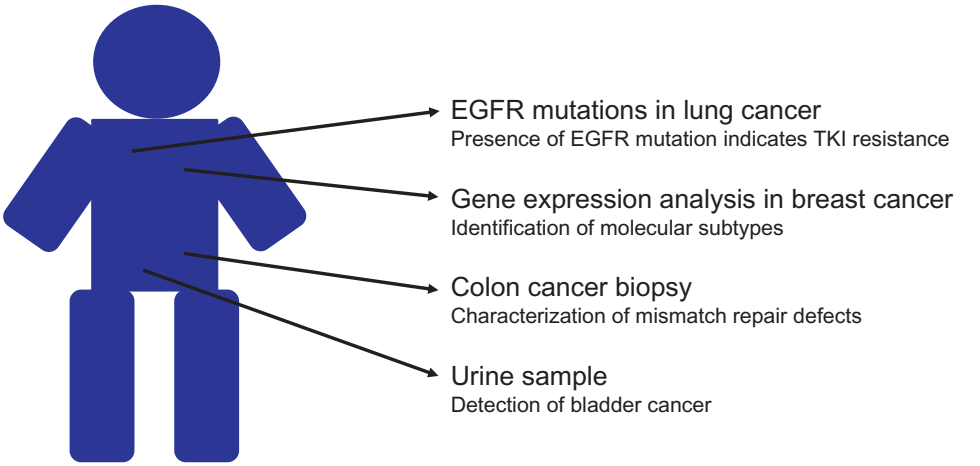

FIGURE 1.1 **Utilization of DNA biomarkers in disease detection, diagnosis, classification, and guided treatment.** This schematic provides examples from human cancer where DNA biomarkers obtained from non-invasive or invasive sources are used in the clinical workup of patients.

of a specific gene and/or other alterations at the level of the primary structure of DNA. Point mutations, insertions, deletions, and chromosomal abnormalities are all classified as mutations. In contrast, epimutation refers to alterations in the genome that do not involve changes in the primary sequence of the DNA. Aberrant DNA hypermethylation or hypomethylation and/or abnormal histone modifications resulting in alterations in chromatin structure are considered epimutations. Despite the differences between mutation and epimutation, the consequences of these molecular processes on the normal expression/function of critical genes/proteins may be the same—alteration of normal gene expression and/or normal protein function. These alterations may reflect (1) loss or reduction of normal levels of gene expression with consequent loss of protein function, (2) loss of function due to loss of protein or synthesis of defective protein, (3) increased levels of gene expression with consequent overexpression of protein, or (4) gain-of-function mutation with consequent altered protein function. Whereas many human diseases can be attributed to genetic alteration or epimutation affecting a single gene (or a few genes), the actual molecular consequence of these changes can be very dramatic, resulting in major alterations in gene expression patterns secondary to the primary genetic or epigenetic gene defect.

Genetic Alterations

Disease-related genetic alterations can be categorized into two major groups: nucleotide sequence abnormalities and chromosomal abnormalities. Examples of both of these forms of molecular lesion have been characterized in familial and acquired diseases affecting various human tissues.

Nucleotide sequence alterations include changes in individual genes involving single nucleotide changes (missense and nonsense), and small insertions or deletions (some of which result in frameshift mutations). Single nucleotide alterations that involve a change in the normal coding sequence of the gene (point mutations) can give rise to an alteration in the amino acid sequence of the encoded protein. Missense mutations alter the translation of the affected codon, while nonsense mutations alter codons that encode amino acids to produce stop codons. This results in premature termination of translation and the synthesis of a truncated protein product. Small deletions and insertions are typically classified as frameshift mutations because deletion or insertion of a single nucleotide (for instance) will alter the reading frame of the gene on the 3′-side of the affected site. This alteration can result in the synthesis of a protein that bears very little resemblance to the normal gene product or production of an abnormal/truncated protein due to the presence of a stop codon in the altered reading frame. In addition, deletion or insertion of one or more groups of three nucleotides will not alter the reading frame of the gene, but will alter the resulting polypeptide product, which will exhibit either loss of specific amino acids or the presence of additional amino acids within its primary structure.

Chromosomal alterations include the gain or loss of one or more chromosomes (aneuploidy), chromosomal rearrangements resulting from DNA strand breakage (translocations, inversions, and other rearrangements), and gain or loss of portions of chromosomes (amplification, large-scale deletion). The direct result of chromosomal translocation is the movement of a segment of DNA from its natural location into a new location within the genome, which can result in altered expression of the genes that are contained within the translocated region. If the chromosomal breakpoints utilized in a translocation are located within structural genes, then hybrid (chimeric) genes can be generated. The major consequence of a chromosomal deletion (involving a whole chromosome or a large chromosomal region) is the loss of specific genes that are

localized to the deleted chromosomal segment, resulting in changes in the copy number of the affected genes. Likewise, gain of chromosome number or amplification of chromosomal regions results in an increase in the copy numbers of genes found in these chromosomal locations.

Epigenetic Alterations

In contemporary terms, epigenetics refers to modifications of the genome that are heritable during cell division, but do not involve a change in the DNA sequence. Therefore epigenetics describes heritable changes in gene expression that are not simply attributable to nucleotide sequence variation. It is now recognized that epigenetic regulation of gene expression reflects contributions from both DNA methylation as well as complex modifications of histone proteins and chromatin structure. Nonetheless, DNA methylation plays a central role in nongenomic inheritance and in the preservation of epigenetic states, and remains the most accessible epigenomic feature due to its inherent stability. Thus DNA methylation represents a target of fundamental importance in the characterization of the epigenome and for defining the role of epigenetics in disease pathogenesis.

SOURCES OF NUCLEIC ACIDS FOR MOLECULAR TESTING

Molecular diagnostic testing is now firmly engrained in the clinical testing menu of most/all hospital clinical laboratories. To conduct molecular testing in the workup of patients with known or suspected disease, sources of nucleic acids (primarily DNA) for use as biomarkers in molecular diagnostic assays are required. There are a large number of potential sources for patient-derived DNA (Fig. 1.2). These sources can be divided based upon the difficulty in sampling and/or the discomfort to the patient during sampling as (1) invasive sources of DNA biomarkers or (2) noninvasive sources of DNA biomarkers (Fig. 1.2). Tremendous research effort is now focused on utilization of noninvasive sources of biomarkers in the detection, diagnosis, prognostication, and classification of human disease. Noninvasive sources of DNA cause minimal to no discomfort to the patient. Collection of a urine sample represents a procedure with no discomfort, while collection of peripheral blood is a procedure with minimal discomfort to the patient. In contrast, invasive sources of DNA, while valuable for molecular testing, can require surgical procedures to obtain (such as in the case of a tissue biopsy) and may involve considerable discomfort to the patient (which is the case for Pap smears and spinal taps). In all cases DNA from diseased tissue or cells is the desired product. This DNA may be derived from cells collected in one of these procedures or through isolation of cell-free DNA (in some cases). No matter the source of biomarkers or the procedures used to collect the sample, it is critical that the intended use of the sample is kept in mind to ensure that samples are collected, stored, and processed in a manner that will not compromise the DNA. Numerous commercial sources provide kits for preparation of nucleic acids from various bodily fluids and tissue samples. Furthermore, this process has been automated in many cases through the use of commercial instrumentation.

CLASSIFICATION OF DISEASE

The classification of disease has historically been based upon (1) site of the pathological lesion (organ or

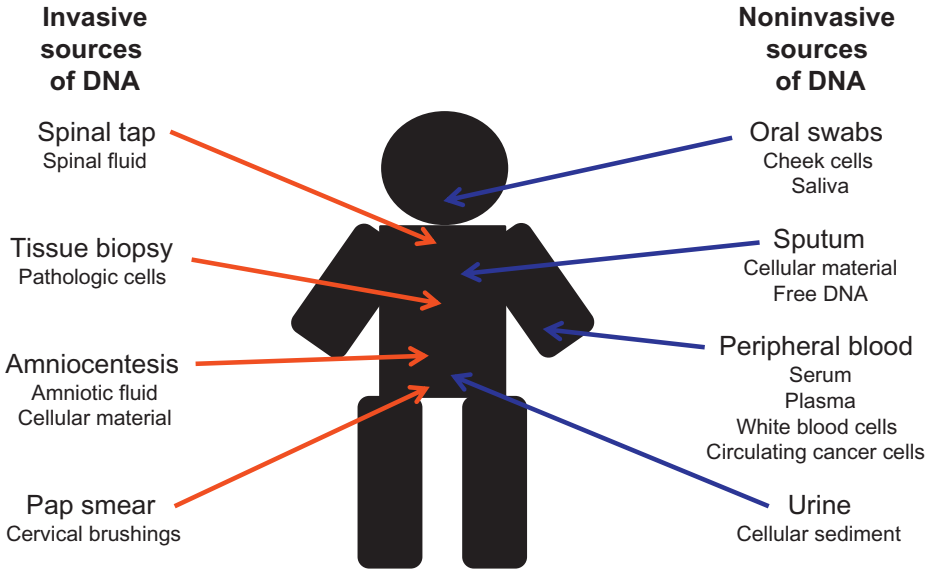

FIGURE 1.2 **Sources of DNA biomarkers for molecular testing.** Sources of DNA biomarkers for molecular testing can be grouped according to the relative difficulty in sampling and/or the relative discomfort to the patient during sample collection as (1) invasive sources, and (2) noninvasive sources. Among the invasive sources of DNA biomarkers, tissue biopsy may be used throughout the body to collect tissue for molecular testing (or routine pathologic examination). Some tissue biopsies can be obtained through simple surgical procedures (such as a skin biopsy), while others require a more extensive surgical procedure (bronchoscopy-based lung biopsy). In some cases the source of molecular biomarkers reflects an infectious agent (bacterium or virus) rather than host cells or cellular material.