CANCER AND THE ENVIRONMENT

GENE-ENVIRONMENT INTERACTION

WORKSHOP SUMMARY

INSTITUTE OF MEDICINE

CANCER and the ENVIRONMENT

GENE-ENVIROMENT INTERACTION

Samuel Wilson, Lovell Jones, Christine Coussens, and Kathi Hanna, *Editors*

Roundtable on Environment Health Sciences, Research, and Medicine

Board on Health Sciences Policy

INSTITUTE OF MEDICINE

NATIONAL ACADEMY PRESS
Washington, D.C.

NATIONAL ACADEMY PRESS • 2101 Constitution Avenue, N.W. • Washington, DC 20418

Support for this project was provided by the National Institute of Environmental Health Sciences, National Institute of Health (Contract No. 282-99-0045, TO#5); National Center for Environmental Health and Agency for Toxic Substances and Disease Registry, Centers for Disease Control and Prevention (Contract No. 200-2000-00629, TO #7); National Institute for Occupational Safety and Health, Centers for Disease Control and Prevention, Centers for Disease Control and Prevention (Contract No. 0000166930); National Health and Environmental Effects Research Laboratory and National Center for Environmental Research, Environmental Protection Agency (Contract No. 282-99-0045, TO#5); American Chemistry Council (unnumbered grant); and Exxon-Mobil Corporation (unnumbered grant). This support does not constitute endorsement of the views expressed in the report.

International Standard Book Number 0-309-08475-X

Library of Congress Control Number 2002109770

Additional copies of this report are available for sale from the National Academy Press, 2101 Constitution Avenue, NW, Box 285, Washington, DC 20055. Call (800) 624-6242 or (202) 334-3313 (in the Washington metropolitan area), or visit the NAP's on-line bookstore at **www.nap.edu.**

The full text of the report is available on-line at **www.nap.edu**

For more information about the Institute of Medicine, visit the IOM home page at **www.iom.edu.**

Printed in the United States of America.

The serpent has been a symbol of long life, healing, and knowledge among almost all cultures and religions since the beginning of recorded history. The serpent adopted as a logotype by the Institute of Medicine is a relief carving from ancient Greece, now held by the Staatliche Museen in Berlin.

"Knowing is not enough; we must apply.
Willing is not enough; we must do."
—Goethe

INSTITUTE OF MEDICINE

Shaping the Future for Health

THE NATIONAL ACADEMIES

National Academy of Sciences
National Academy of Engineering
Institute of Medicine
National Research Council

The **National Academy of Sciences** is a private, nonprofit, self-perpetuating society of distinguished scholars engaged in scientific and engineering research, dedicated to the furtherance of science and technology and to their use for the general welfare. Upon the authority of the charter granted to it by the Congress in 1863, the Academy has a mandate that requires it to advise the federal government on scientific and technical matters. Dr. Bruce M. Alberts is president of the National Academy of Sciences.

The **National Academy of Engineering** was established in 1964, under the charter of the National Academy of Sciences, as a parallel organization of outstanding engineers. It is autonomous in its administration and in the selection of its members, sharing with the National Academy of Sciences the responsibility for advising the federal government. The National Academy of Engineering also sponsors engineering programs aimed at meeting national needs, encourages education and research, and recognizes the superior achievements of engineers. Dr. Wm. A. Wulf is president of the National Academy of Engineering.

The **Institute of Medicine** was established in 1970 by the National Academy of Sciences to secure the services of eminent members of appropriate professions in the examination of policy matters pertaining to the health of the public. The Institute acts under the responsibility given to the National Academy of Sciences by its congressional charter to be an adviser to the federal government and, upon its own initiative, to identify issues of medical care, research, and education. Dr. Harvey V. Fineberg is president of the Institute of Medicine.

The **National Research Council** was organized by the National Academy of Sciences in 1916 to associate the broad community of science and technology with the Academy's purposes of furthering knowledge and advising the federal government. Functioning in accordance with general policies determined by the Academy, the council has become the principal operating agency of both the National Academy of Sciences and the National Academy of Engineering in providing services to the government, the public, and the scientific and engineering communities. The council is administered jointly by both Academies and the Institute of Medicine. Dr. Bruce M. Alberts and Dr. Wm. A. Wulf are chairman and vice-chairman, respectively, of the National Research Council.

ROUNDTABLE ON ENVIRONMENTAL HEALTH SCIENCES, RESEARCH, AND MEDICINE (membership until March 31, 2002)

Paul Grant Rogers (Chair), Partner, Hogan & Hartson, Washington, DC
Lynn Goldman (Vice-Chair), Professor, Johns Hopkins University Bloomberg School of Public Health, Baltimore, MD
Pauline Abernathy, Program Officer, The Pew Charitable Trusts, Philadelphia, PA
M. Brownell Anderson, Associate Vice President for Medical Education, Association of American Medical Colleges, Washington, DC
Roger Bulger, President and CEO, Association of Academic Health Centers, Washington, DC
Mark Cullen, Professor of Medicine and Public Health, Yale Occupational and Environmental Medicine Program, Yale University School of Medicine, New Haven, CT
Ruth Etzel, Editor of the American Academy of Pediatrics *Handbook of Pediatric Environmental Health*, Adjunct Professor in the Department of Environmental and Occupational Health, George Washington University School of Public Health and Health Services, Washington, DC
Henry Falk, Assistant Administrator, Agency for Toxic Substance and Disease Registry, Atlanta, GA
Baruch Fischhoff, Professor of Social and Decision Sciences, Professor of Engineering and Public Policy, Department of Social and Decision Sciences, Carnegie Mellon University, Pittsburgh, PA
Howard Frumkin, Professor and Chair, Department of Environmental and Occupational Health, Emory University Rollins School of Public Health, Director of the Southeast Pediatric Environmental Health Specialty Unit, Atlanta, GA
Bernard D. Goldstein, Dean, University of Pittsburgh Graduate School of Public Health, Pittsburgh, PA
Robert Graham, Director, Center for Practice and Technology Assessment, Agency for Healthcare Research and Quality, Bethesda, MD
John T. Grupenhoff, President, Science and Health Communications Group, Inc., Bethesda, MD
Carol Henry, Vice President for Science and Research, American Chemistry Council, Arlington, VA
Richard J. Jackson, Director, National Center for Environmental Health, Centers for Disease Control and Prevention, Atlanta, GA
Lovell Jones, Professor, Gynecologic Oncology, University of Texas, Houston, TX
Patricia G. Kenworthy, Vice-President for Policy and Research and Senior Staff Attorney, National Environmental Trust, Washington, DC

Donald Mattison, Professor, Mailman School of Public Health, Columbia University, New York, NY
Roger McClellan, President Emeritus, Chemical Industry Institute of Toxicology, Albuquerque, NM
Sanford Miller, Senior Fellow and Adjunct Professor, Georgetown University, Washington, DC
Frank Mirer, Director, Health and Safety, International Union, United Auto Workers, Detroit, MI
Alan R. Nelson, Special Advisor to the CEO, American College of Physicians–American Society of Internal Medicine, Fairfax, VA
Kenneth Olden, Director, National Institute of Environmental Health Sciences, National Institutes of Health, Research Triangle Park, NC
Peter Preuss, Director, National Center for Environmental Research, U.S. Environmental Protection Agency, Washington, DC
Lawrence Reiter, Director, National Health and Environmental Effects Research Laboratory, U.S. Environmental Protection Agency, Research Triangle Park, NC
Kathleen Rest, Acting Director, National Institute of Occupational Safety and Health, Washington, DC
Samuel H. Wilson, Deputy Director, National Institute of Environmental Health Sciences, National Institutes of Health, Research Triangle Park, NC

IOM Health Sciences Policy Board Liaisons

Mark Cullen, Professor of Medicine and Public Health, Yale Occupational and Environmental Medicine Program, Yale University School of Medicine, New Haven, CT
Bernard D. Goldstein, Dean, University of Pittsburgh Graduate School of Public Health, Pittsburgh, PA

ROUNDTABLE ON ENVIRONMENTAL HEALTH SCIENCES, RESEARCH, AND MEDICINE
(Membership April 1, 2002 to Present)

Paul Grant Rogers (Chair), Partner, Hogan & Hartson, Washington, DC
Lynn Goldman (Vice-Chair), Professor, Johns Hopkins University Bloomberg School of Public Health, Baltimore, MD
Jacquelyne Agnew, Professor, Bloomberg School of Public Health, The Johns Hopkins University, Baltimore, MD
Jack Azar, Vice President, Environment, Health and Safety, Xerox Corporation, Webster, NY

Sophie Balk, Chairperson, Committee on Environmental Health, American Academy of Pediatrics, Bronx, NY

Roger Bulger, President and CEO, Association of Academic Health Centers, Washington, DC

Henry Falk, Assistant Administrator, Agency for Toxic Substance and Disease Registry, Atlanta, GA

Baruch Fischhoff, Professor of Social and Decision Sciences, Professor of Engineering and Public Policy,
Department of Social and Decision Sciences, Carnegie Mellon University, Pittsburgh, PA

John Froines, Professor and Director, Center for Occupational and Environmental Health,
Southern California Particle Center and Supersite, University of California, Los Angeles, CA

Howard Frumkin, Professor and Chair, Department of Environmental and Occupational Health, Emory University Rollins School of Public Health, Director of the Southeast Pediatric Environmental Health Specialty Unit, Atlanta, GA

Michael Gallo, Professor of Environmental and Community Medicine, University of Medicine and Dentistry, New Jersey–Robert Wood Johnson Medical School, Piscataway, NJ

Bernard D. Goldstein, Dean, University of Pittsburgh Graduate School of Public Health, Pittsburgh, PA

Robert Graham, Director, Center for Practice and Technology Assessment, Agency for Healthcare Research and Quality, Bethesda, MD

Charles Groat, Director, U.S. Geological Survey, Reston, VA

Myron Harrison, Senior Health Advisor, Exxon-Mobil, Inc., Irwing, TX

Carol Henry, Vice President for Science and Research, American Chemistry Council, Arlington, VA

Richard J. Jackson, Director, National Center for Environmental Health, Centers for Disease Control and Prevention, Atlanta, GA

Lovell Jones, Professor, Gynecologic Oncology, University of Texas, Houston, TX, University of Texas, M.D. Anderson Cancer Center, Houston, TX

Alexis Karolides, Senior Research Associate, Rocky Mountain Institute, Snowmass, CO

Donald Mattison, Professor, Mailman School of Public Health, Columbia University, NY

Michael McGinnis, Senior Vice President and Director of the Health Group, Robert Wood Johnson Foundation, Princeton, NJ

James Melius, Director, Division of Occupational Health and Environmental Epidemiology, New York State Department of Health, New York, NY

James Merchant, Professor and Dean, College of Public Health, Iowa University, Iowa City, IA

Sanford Miller, Senior Fellow, Center for Food and Nutrition Policy, Virginia Polytechnic Institute and State University, Alexandria, VA
Alan R. Nelson, Special Advisor to the CEO, American College of Physicians–American Society of Internal Medicine, Fairfax, VA
Kenneth Olden, Director, National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health, Research Triangle Park, NC
Peter Preuss, Director National Center for Environmental Research, U.S. Environmental Protection Agency, Washington, DC
Lawrence Reiter, Director, National Health and Environmental Effects Research Laboratory, U.S. Environmental Protection Agency, Research Triangle Park, NC
Kathleen Rest, Acting Director, National Institute of Occupational Safety, and Health, Centers for Disease Control and Prevention, Washington, D.C.
Samuel Wilson, Deputy Director, National Institute of Environmental Health Sciences, National Institutes of Health, Research Triangle Park, NC

IOM Health Sciences Policy Board Liaisons

Mark Cullen, Professor of Medicine and Public Health, Yale Occupational and Environmental Medicine Program, Yale University, School of Medicine, New Haven, CT
Bernard D. Goldstein, Dean of the University of Pittsburgh Graduate School of Public Health, Pittsburgh, PA

Study Staff

Christine Coussens, Study Director
Dalia Gilbert, Research Assistant
Jason Harris, Senior Project Assistant

Division Staff

Andrew Pope, Board Director
Alden Chang, Administrative Assistant
Carlos Gabriel, Financial Associate
Kathi Hanna, Consultant

INDEPENDENT REPORT REVIEWERS

This report has been reviewed in draft form by individuals chosen for their diverse perspectives and technical expertise, in accordance with procedures approved by the National Research Council's Report Review Committee. The purpose of this independent review is to provide candid and critical comments that will assist the institution in making its published report as sound as possible and to ensure that the report meets institutional standards for objectivity, evidence, and responsiveness to the study charge. The contents of the review comments and draft manuscript remain confidential to protect the integrity of the deliberative process. We wish to thank the following individuals for their participation in the review of this report:

Dr. Roger Giese, Director, Environmental Cancer Research Program, Northeastern University, Boston, MA
Dr. Theodore Krontiris, Deputy Director, Beckman Research Institute, Duarte, CA
Ms. Stacey Neloms, Director, Maryland Cancer Registry, Baltimore, MD

Although the reviewers listed above have provided many constructive comments and suggestions, they were not asked to endorse the final draft of the report before its release. The review of this report was overseen by **Melvin Worth**, Scholar-in-Residence, Institute of Medicine, who was responsible for making certain that an independent examination of this report was carried out in accordance with institutional procedures and that all review comments were carefully considered. Responsibility for the final content of this report rests entirely with the authoring committee and the institution.

Foreword

Kenneth I. Shine

One of the more interesting aspects of the announcement by Ian Wilmut that he and his colleagues in Scotland had successfully "cloned" a sheep born in 1996 was the enormous public interest and misunderstanding about cloning. The common misperception, which persists to this day, is that a cloned individual would be an exact duplicate of another.

In the process of trying to explain to the American public and, in fact, to some members of the scientific community what is involved in "cloning," it became critical to point out that the relationship between cloned animals is, in fact, less close than that between identical twins. In a cloned organism the nucleus comes from one animal and the cytoplasm, which contains mitochondrial genes, comes from another. Thus, identical twins are closer in biological and genetic identity than "clones" would be. Yet, none of us would expect identical twins to actually behave the same way; in fact, they are very different people.

Consideration of identical twins, and of clones, emphasizes the notion that even when two individuals have identical or nearly identical genes, the social, cultural, and physical environment in which they live has a profound impact on who they are and what they become. The long tradition of studying twins emphasizes the need for us to understand the manner by which biology and the environment influence the health and development of individuals and populations.

The Institute of Medicine (IOM) has issued a number of consensus studies about environmental health. Several years ago the IOM produced a report on environmental justice, that is, how risky environments tend to be found in communities of lower socioeconomic status, resulting in disparate rates of cancer and other chronic diseases. Other aspects of IOM's recent work have focused on the unequal burden of cancer, which dramatizes that there are both increased incidence and increased prevalence of cancer in certain groups of our population. The National Cancer Policy Board has addressed a number of issues in cancer care, including its quality and accessibility, and has demonstrated that there are substantial gaps between the average care and the best care for cancer.

IOM roundtables provide a venue in which individuals representing many different perspectives can come together to discuss important issues facing health and medicine today. Roundtables provide a forum for exploring the interfaces between the various aspects of science and the diverse characteristics of health care and public health. This workshop follows on a successful meeting convened by the Roundtable of Environmental Health Sciences, Research, and Medicine last year, *Rebuilding the Unity of Health and the Environment*, which emphasized the multiple interrelationships that exist among the social, natural, and built environments as they relate to human health. All of these efforts have highlighted the importance of understanding the wide array of elements that might influence the health of individuals and populations—where they live, what they eat, how they live, where they work, how they raise their children, and how they do their work.

One of our goals is to encourage health professionals—physicians, nurses, and others in their communities—to recognize that these are not issues limited to the public health department or to sanitary engineers. These are issues for all of us in the health professions. Enhanced communications between the professions and the community will be necessary to convey information about the interactions between who we are genetically and the environment in which we live. These concerns remain central to gaining insight into what can be done to prevent, diagnose, and treat cancer as well as numerous other diseases, and they stress the multidisciplinary nature of the challenges we have before us.

Preface

In the early 1970s, Congress was at a pivotal point in shaping the future of cancer research and policy for the United States. I remember vividly the atmosphere "on the Hill" as legislation was pending before both the House and the Senate on the funding of the National Cancer Institute (NCI) and its position within the National Institutes of Health (NIH). The American people, the Congress, and President Nixon were concerned about cancer; at the time, most people diagnosed with cancer didn't have much hope for the future. We were losing many of our best and brightest to this deadly disease and we needed to do something about it. The result in this country was to declare a "war on cancer."

During this time, I was the chair of the Subcommittee on Health and the Environment in the House. We were known as the "Disease of the Month Club" because of the volume of legislation we were passing. We wore this label as a badge of honor because we were committed to improving the health of the citizens of the United States through increasing the government's commitment to biomedical research. In 1971, the Senate passed legislation to make an independent agency of NCI, and President Nixon appeared ready to sign the legislation. Our committee understood the importance of this legislation, and we proceeded to hold three weeks of hearings on the bill. (Three weeks of hearings were all but unheard of, but they were necessary to ensure that members of the House were educated on the topic.) Many scientists, including several Nobel laureates, testified to the value of keeping the NCI as part of NIH. They pointed out examples of how advances in one field can impact advances in other fields. A number of prominent researchers questioned whether the exchange of information would be as great if the NCI became independent of NIH. We also had to think of our research commitment to other diseases and whether this would result in separate agencies for each disease. We, on the committee and members of the House, struggled with these issues, but decided at the end of our deliberations to keep NCI a part of NIH. We passed the National Cancer Act of 1971, and our fight against cancer began in earnest.

It has been 30 years since that landmark legislation, and we have made tremendous strides in "the war." No longer is a person diagnosed with cancer served a death sentence. Our understanding of the mechanisms underlying cancers has allowed us to start targeting treatment and separating out a group of diseases that we call "cancer." Molecular techniques and advances in cell biology gained from cancer research have spilled over into other areas of science such as neuroscience and physiology. Thirty years of research and clinical investigations have given us hope and promise that one day most cancers will be sucessfully treated by the wide variety of new modalities being developed.

The statistics speak for themselves. A report released last year by the NCI, the American Cancer Society, the North American Association of Central Cancer Registries, the Centers for Disease Control and Prevention, and the National Center for Health Statistics reported a general decrease of 0.8 percent per year in the incidence rate for all cancers combined from 1990 to 1997. The greatest decline in cancer incidence rates has been among men, who overall have higher rates of cancer than women. There is reason for hope. While breast cancer incidence rates showed little change in the 1990s, breast cancer death rates have declined about 2 percent per year since 1990 and have dropped sharply since 1995.

With the growing use of electronic media, information is readily available to more and more individuals. Although these media allow for rapid distribution of information, there is no assurance that the information provided is accurate and scientifically sound. Members of the public have many questions when they have cancer, and they don't understand why one person develops cancer and another person doesn't. They have questions about how their environment may have contributed to the development of their disease. The scientific community doesn't have all the answers, but we assembled a group of researchers to discuss some of these questions.

As part of its task, the Roundtable convenes workshops to inform the debate on issues related to environmental health. We continue to explore the impact that the environment has on our cities, our families, and our health. It shouldn't be surprising to anyone that the places in which we work, eat, sleep, and play can have a dramatic impact on our health. As I have said many times, "Environmental laws are more than regulations—they are health laws!"

In September 2000, the Roundtable decided to convene a workshop on *Cancer and the Environment: Gene–Environment Interactions* on May 16–17, 2001. During the planning, it became clear that a two-day meeting would start the process but would still leave many questions unanswered. What is clear, however, is that understanding the role of cancer and the environment is one of the greatest challenges that we face in this new century.

As you can read in later sections of this report, there are significant differences in populations and the development of cancer. We have a significant vulnerable population that includes children, minorities, women, and the poor. We

are making progress against breast cancer, *but* not for all segments of the population. We are making progress again lung cancer, *but* not for all ethnic minorities. Scientists and government officials struggle with questions concerning cancer clusters. Are they just a statistical aberration or are they the result of an environmental exposure? More research and multidisciplinary approaches will begin to tease apart these issues. We hope that we will be able to continue to decrease both the cancer incidence and the mortality rates for all populations.

On behalf of the Roundtable, I would like to thank a number of individuals. First are the co-chairs, Drs. Franklin Mirer and Alan Nelson, for their leadership on this planning group. I would also like to thank other members of the planning group, Dr. Ruth Etzel, Dr. Michael Gallo, Dr. Lovell Jones, Ms. Patricia Kenworthy, Dr. John Minna, Dr. Samuel Wilson, and Ms. Gerri Wolfle, who worked with the co-chairs and study staff to put this program together.

The Roundtable, itself, does not take a position on any issue. The comments and summary presented here were captured to promote greater discussion of environmental influences on the health of our citizens. This summary is a report to the Roundtable, and the views and opinions expressed in the summary are the views of the speakers and workshop participants, not the Roundtable. Announcements of upcoming activities and workshops can be found at the Roundtable's Web site: www.iom.edu/ehsrt.

Paul G. Rogers, J.D.
Chair

Contents

Summary

Cancer is the second leading cause of death in the United States and results in more than half a million deaths each year. In the 1990s, we began to see a downward trend in cancer death rates with an increased survival rate of cancer patients. Most of the increase in survival rates can be attributed to earlier detection through screening programs and advances in chemotherapy. However, what these positive trends mask is the fact that the incidence of some cancers is still on the rise.

Both environmental and genetic factors are known to be involved in the development of cancer. For example, environmental factors such as exposures to certain chemicals or to sunlight have long been linked to the development of some types of cancers. In planning this workshop, the Roundtable on Environmental Health Sciences, Research, and Medicine wanted to address the link between environmental factors and the development of cancer in the light of recent advances in genomics and, more specifically, in toxicogenomics and gene–environment interactions. Speakers were invited from many scientific discliplines including epidemiology, molecular biology, oncology, microbiology and immunology, nutrition science, and human genetics. The goals of the workshop were to facilitate discussion among these scientists; to assess genetic–environmental interactions across diverse populations, including the underserved, women, children, and minorities; and to review what is known about gene–environment interactions in site-specific cancers. The language presented in this summary should not be viewed as an endorsement by the Roundtable on Environmental Health Sciences, Research, and Medicine or the Institute of Medicine of what action is needed for the future, but rather as an effort to synthesize the various perspectives presented.

This workshop came at a logical time to begin asking complex questions about gene–environment interactions. As discussed by Samuel Wilson, National Institute of Environmental Health Sciences, only a small percentage of cancer is attributed to the powerful dominant single genes or the strongest toxicants. With

the recent completion of an initial draft sequence of the human genome and with the evolving and enhanced view of environmentmental health, we will be able to conduct more precise studies of environmental contributions to cancer. He further suggested that we will need a new toolbox to address cancer resulting from the common modifier genes and multiple environmental exposures. In the charge to the speakers and participants, he outlined a number of questions that would begin to address what research tools will be needed, how new scientific information can be applied in a timely manner to reduce the burden of cancer, and how this can be flexible enough to treat the individual.

Sam Donaldson, of ABC News, opened the meeting with a keynote address in which he described the media's tendency to seek out scientific outliers rather than the conventional wisdom. He urged the scientific community to be clear in its messages about the linkages between cancer and the environment and to emphasize areas of agreement.

THE ROLE OF THE ENVIRONMENT IN CANCER

In a scientific keynote address, Joseph Fraumeni, National Cancer Institute, highlighted the importance of environmental factors in human cancer. He pointed to a growing body of knowledge that dramatically illustrates the influence of such factors, for example, the observations of scrotal cancer among young chimney sweeps in London in 1775 and the international variation reported in cancer incidence. John Milner, Pennsylvania State University, using a broader perspective of environment—one that includes diet and lifestyle, described the evidence of dietary interactions and cancer. He discussed the findings that both essential and nonessential dietary nutrients can markedly influence several key biological events, including cell cycle regulation, processes involved in replication or transcription, immunocompetence, and factors involved in apoptosis, or programmed cell death. These observations suggest that specific foods or components may have the potential to markedly reduce cancer risk.

Analyses of the incidence of cancer in twin pairs and in families are traditional methods for answering questions about the relationships between cancer etiology, genes, and the environment. Kari Hemminki, Karolinska Institute, and Curtis Harris, National Cancer Institute, described recent progress in identifying and characterizing susceptibility genes in familial cancer. This work, taken together, has revolutionized our understanding of the critical genetic mechanisms in cancer etiology. Studies that combine genetic analysis with assessment of exposures and diet can explain why not everyone exposed to a particular cancer-causing chemical will develop cancer. Recent research has identified functional polymorphisms that influence an individual's cancer risk and has focused on gene products involved in the activation and detoxification of carcinogens and DNA repair. Gene polymorphisms that are important in apoptosis will increasingly be recognized as clues to individual susceptibility to cancer.

SPECIAL POPULATIONS

Despite recent good news about decreasing cancer mortality rates, not all population subgroups are sharing in this success story. Cancer rates are higher and accelerating in some racial and ethnic groups. The reasons for these disparities may include the environment, hormones, and genetics. Lovell Jones, M.D. Anderson Cancer Center, described how progress toward preventing, diagnosing, and treating cancer will be hampered by the nation's inability to deal effectively with the greater cancer burden borne by certain vulnerable populations. These populations are typically defined as groups at higher-than-average risk of death, disease, and disability, and include people with low incomes, low literacy rates, the elderly, those in rural communities, African Americans, Hispanics, American Indians and Alaska Natives, and other ethnic minorities.

Trying to assess how genes and the environment compound cancer risk in populations considered vulnerable because of their social or economic status requires new approaches, according to some workshop participants. Demographic studies of cancer must consider the diversity within affluent groups as well as within less economically affluent groups, said Armin Weinberg, Baylor College of Medicine. They also must consider immigration patterns and countries of origin because those factors play a primary role in predisposition to cancer.

María Hernández-Valero described how populations, such as migrant farmworkers and children, are particularly vulnerable to developing cancer following environmental exposures. Farmworkers, who include pregnant women and chil-

A number of questions concerning future research emerged from the workshop:

If we move toward large-scale clinical trial studies and large-scale population studies, how (and by whom) will the large repositories for DNA and tissues be created?

If large amounts of lifestyle, medical, and environmental exposure information are needed, do we have the resources necessary to collect and organize this information?

How will we solve policy issues related to access to databases, while maintaining protection of patient rights?

How do we encourage more "discovery-driven" research to define the molecular landscape of cancer? How can this be incorporated into an interdisciplinary research involving epidemiologists and molecular biologists?

With the greater emphasis on environmental surveillance and environmental disease registries, how do we ensure that all populations, including special populations, are involved?

dren, endure a high burden of exposure to pesticides and other agents that are known carcinogens. The extent of the burden is unknown because it is particularly difficult to study exposures and cancer clusters in migrant farmworkers due to their mobility and hesitancy to be the subjects of investigation, said Richard Jackson, Centers for Disease Control and Prevention.

Starting in infancy, children are vulnerable to environmental exposures. Greta Bunin, Children's Hospital of Philadelphia, and Leslie Robison, University of Minnesota, discussed trends in incidence and survivorship in childhood cancers and presented preliminary evidence about linkages between childhood cancer and diet. In addition to in utero exposures and, in some cases, preconceptual exposures of the parents, diet and other environmental influences may combine with genetic predispositions to form a strong link between these factors and the development of childhood cancers. The developing child may be particularly sensitive to exposures affecting specific organs, since the types of cancer found in children are disproportionately different from those found in adults. Some meeting participants suggested that longitudinal studies are needed to be able to identify risk factors.

SITE-SPECIFIC CANCERS

Presenters described some recent advances in understanding the linkages between genes and the environment in site-specific cancers, including breast, lung, colorectal, and prostate cancer. More refined studies have been focused on understanding how genetics may account for the differences among individuals in their responses to harmful exposures. For example, John Minna, University of Texas Southwestern Medical Center, and Margaret Spitz, M.D. Anderson Cancer Center, described how some genes signal the synthesis of enzymes in the lung. Ordinarily these enzymes destroy cancer-causing substances in tobacco smoke, but a gene variation might reduce these enzymes or their efficiency, and therefore make people more susceptible to lung cancer.

Understanding the role of environment in breast cancer is an area of ongoing research. Brian Henderson, University of Southern California; Mary Wolff, Mount Sinai School of Medicine; and Olufunmilayo Olopade, University of Chicago, discussed studies under way to address how complex genetic factors or hormonal milieu may alter environmental risk factors. These effects may be responsible for differences in breast cancer among racial or ethnic groups.

Presentations by Raymond DuBois, Vanderbilt University, and David Alberts, Arizona Cancer Center, highlighted what is known about environmental risk factors in the development of colorectal cancer. Understanding the twenty-fold variation in incidence rates in different geographic regions around the country may provide a clue to the etiology of colorectal cancer. In addition, studying the relationship of the environment and adenomatous polyps—the precursors to colorectal cancer—may help identify relatively asymptomatic individuals who

are at increased risk of cancer and would benefit most from diagnostic follow-up or intervention.

Development of multigenic models of cancer susceptibility will be an important future approach to predicting, preventing, and diagnosing cancers, said some participants. For example, prostate cancer is a common disease for which there are few well established risk factors. Pedigree analyses suggest a genetic component for some individuals; however, the majority of prostate cancer cases cannot be explained by a single-gene model, suggesting multigenic etiology. Moreover, the international and racial–ethnic variations in prostate cancer incidence, combined with the effects of migration on risk patterns, suggest that gene–environment interaction may be involved in determining prostate cancer risk. Donald Coffey, Johns Hopkins University, and Robert DiPaola, Cancer Institute of New Jersey, discussed the relative roles of diet, nutritional supplements, and hormones in risks for prostate cancer.

Finally, one area of research has focused on using the body's own immune response to combat the growth and development of cancer. Steven Rosenberg, National Cancer Institute, described how these so-called immunotherapies are in their initial stages but offer much hope for future vaccines and treatment approaches.

Several speakers emphasized the need for combining cancer registry data with other databases to identify new etiologic leads. For example, large epidemiologic consortia could be formed to pool data and publish results from several independent investigations to quickly determine whether a given result in one study is supported by other studies. Some participants also noted the need for a linked environmental surveillance system. Investigations of the cancer-related significance of hormonal, metabolic, genetic, and environmental factors could then be compared and contrasted. Additionally, some workshop participants suggested that we need better technologies and generally enhanced skills in the area of risk communication. The combination of these efforts could lead to better cancer prevention and control.

1

Charge to Participants and Workshop Objectives

REMARKS AND CHARGE TO PARTICIPANTS

Samuel H. Wilson

As I am sure you will all agree, we have accumulated a wealth of knowledge about cancer through many years of investment in fundamental and applied research. During this time, many promising drugs and treatments have been discovered, and survivorship and prevention are increasing. We have begun to unravel the mysteries of the molecular architecture of cancer, and this has revealed that cancer is not a singular disease. It is, rather, a closely linked group of molecular disorders, varying in etiology and mechanism, but with some common intersections. Additionally, we know that a person's susceptibility to cancer can be governed by the interaction of common modifier genes or "susceptibility genes" and environmental factors.

It is clear that if we are to continue making progress in prevention and treatment of cancer, we will have to place additional focus on the interplay between modifier genes and environment. It is this interplay that holds the greatest promise in the fight to prevent and control cancer—a central theme of this workshop.

Workshops such as this assist members of the Institute of Medicine Roundtable on Environmental Health Sciences, Research, and Medicine in clarifying issues concerning environmental health. The presentations and discussions further define the topics that will be important for the Roundtable to discuss and consider in the future. When the Roundtable met in September of 2000, it became evident that this was an ideal time to hold a workshop on gene–environment interactions in cancer because of two recent advances in genetics and environmental studies. The first advance is the completion of an initial draft sequence of the human genome. We now are in the early stages of the postsequencing genomics era, and we are beginning to comprehend the genetic variations that

modify an individual's susceptibility to cancer. The second development is that we are working with an evolving, expanded, and enhanced view of environmental health and exposures that includes factors such as diet, lifestyle, metabolic alterations, socioeconomic status, and environmental pollutants. It is this expanded view of environmental health that will allow us to conduct more meaningful and precise studies of environmental contributions to cancer.

Although the research model of the rare dominant cancer gene, or the strong environmental toxicant, has served us well in the past in defining the molecular biology of cancer, it will not be sufficient in the future. Only a small percentage of cancer is attributable to powerful dominant single genes or the strongest toxicants. Instead, new science and a new scientific toolbox will be needed, and more research involving the common modifier genes and the multiple environmental factors must be considered.

Fortunately, the genomics era can provide us with many of these new tools. Cancer research in the future will require an integration of new molecular genetic measurements, environmental exposure measurements, and precisely defined population groups.

As the group planning this workshop explored the themes and questions surrounding gene–environment interactions, we identified a number of key unanswered questions. I list these as a challenge and a charge to our speakers, panelists, and participants to consider during the workshop:

- What are the approaches and the assay tools that will allow us to conduct the most precise molecular evaluations of cancer susceptibility?
- What are the approaches that will allow us to understand the lag time or the interval between the earliest stages of precancer and the eventual clinical end points of cancer?
- What are the research strategies that will allow us to measure the multiple stages during cancer development so that early interventions can be facilitated?
- How will we apply information on genetic and environmental factors to reduce the burden of cancer through education, prevention, and intervention?
- How can this be done in ways that are both sensitive to local community needs and flexible enough to allow individual approaches?

All of these questions are difficult and open ended. Even though we may not find all of the answers, this workshop will help us to frame approaches in the future and to find the answers more rapidly. We must rise to the challenge of understanding the complex equation of gene–environment interaction and apply this understanding to the prevention and treatment of cancer. This is a time of great promise for environmental cancer research as we are poised to use new technologies and multidisciplinary approaches to further unravel this devastating disease we call cancer.

STATEMENT OF WORKSHOP OBJECTIVES

Franklin Mirer

Cancer is still the second leading cause of death in the United States; more than half a million lives are claimed each year. For industrial workers, occupation-related cancer is responsible for 5 to 20 times the number of work-related deaths as traumatic injury. After decades of steady increases in cancer death rates, the 1990s saw the beginning of a downward trend. Fortunately, cancer survival rates have increased, in large part because of earlier detection, screening programs, and advances in chemotherapy and other treatments. These positive trends, however, are only part of the story, because the incidence of certain cancers is still on the rise.

Both environmental and genetic factors are involved in the development of cancer. In fact, one of the earliest observations of an environmental cause of cancer was reported by Bernardino Ramazzini in 1714. Today, we know that exposure to chemicals and sunlight, diet, lifestyle, economic status, and infections can contribute to the development of certain cancers. Yet not everyone exposed to a particular cancer-causing agent or chemical develops the disease. For example, 90 percent of heavy smokers do not get cancer. Complex interactions require future research on individual susceptibilities, examining how multiple modifier genes interact with the environment.

Since the 1960s we have been developing the field of occupational health along a particular paradigm that has three main questions. First, does a carcinogenic effect at a higher exposure exist at lower exposures? Second, does a carcinogenic effect observed in a laboratory animal study reliably predict a carcinogenic effect in people? Third, are there enough people exposed to identified carcinogens at high enough levels to account for a significant fraction of observed cancers?

It is important to study the interactions of all the factors that influence health. For example, if an investigator does not know anything about chemical exposures, then he or she might conclude that all of the variations of cancer rates in the populations are based on genetics or other host factors. Actually, if everyone in a particular population has the same exposure, all the variation will be due to host factors.

Second, if an investigator does not know anything about genetics, he or she will presume that all variation in cancer rates is due to exposure. Finally, if an investigator focuses on only one carcinogen, then he or she will overattribute the role of that agent in all cancers.

Over the past 40 years, we have reached a limited consensus on what environmental agents contribute to the development of cancer. As we use new information to evaluate the carcinogenic risks of chemicals, we must also look backward at how hard it was to draw the final conclusions about the largest single cause of preventable mortality—cigarette smoking.

As noted earlier, this workshop is being held at a time when new technologies and new intellectual perspectives are available to push research into new research frontiers. This workshop was designed to bring together clinicians, epidemiologists, researchers, technologists, public health practioners, policymakers, and other interested parties to discuss the recent opportunities in cancer research. We will discuss many of the opportunities for areas of future research and prevention. Finally, we will use the broader perspective of environments—one that encompasses the effects of the social, built (including occupational), and natural environment—to begin to understand why not everyone or every group that is exposed to an environmental stressor develops cancer.

2

Keynote Addresses

CANCER, THE ENVIRONMENT, AND THE MEDIA

Samuel Donaldson

I know a little bit about cancer and the environment, because I am a member of the cancer club—the melanoma branch—and since receiving this diagnosis, I have paid more attention to developments in cancer research than I did in the past. We have learned a lot about the effects of the environment in causing cancer, in some cases, far too late.

When I was in the army in the 1950s, I wangled an assignment to Frenchman Flat in Nevada. In 1958, along with some of my buddies, I crouched in a trench 3,000 yards from a tower where a nuclear device was exploded. The device turned out to be no larger than the ones we dropped on Hiroshima and Nagasaki, but witnessing this event made me a believer in the power of the nuclear bomb. The ground shook and the countryside—the desert—came in on us. We had our hands over our closed eyes in a 6-foot slit trench, yet I saw bright daylight. Then, when the implosion occurred and the countryside came back in on us, we jumped out of the trench. At this point, the fireball was just decreasing in luminance. It was mainly white. In a few moments, as we sat there watching, white rain began to fall on us. The winds had changed. They carted us quickly to water trucks, which were about 2 or 3 miles away, and we stripped and they hosed us down. The photo tags we wore showed we had been exposed only to background radiation.

> If we can teach young people early on these good habits, we have a better chance of facing down melanoma in the future.
>
> *Sam Donaldson*

I do not think that this incident caused my melanoma; rather, overexposure to the sun is the more likely culprit. We now know to wear sunscreens and

hats, and if we can teach young people early on these good habits, we have a better chance of facing down melanoma in the future.

The point of telling the story about the atomic explosion was that exploding the atmosphere was something that seemed necessary during the Cold War, but we now know that exposure to strontium-90 and other noxious radiation from atmospheric testing could eventually kill us all. In 1963, led by President John F. Kennedy, we signed the first test ban treaty. For many years, nearly no country has tested in the atmosphere and we are healthier because of it. What is important about this story is that there was such broad agreement in the scientific community about the health effects of such testing that it was impossible for policymakers to ignore the evidence.

Let me just tell you at this point what you already know about those of us in the news media. We look for the people who are the "odd steppers"—who march to different drummers. If you think about it, you really want us to do that. In many areas, it is the odd steppers—Copernicus, Galileo—who turn out to be right. If the news media ignores such people because conventional wisdom contradicts them, then a lot gets lost. For the media to do its job, we must seek out all opinions, even if they are unpopular and unconventional.

I recognize that in science this works against you. For example, in 1964 I was at a press conference when Dr. Luther Terry presented his first great Surgeon General's report on smoking. As a smoker and a reporter, I had to pay attention. So, I actually studied the graphs and looked at the charts and supporting documents and was almost instantly converted into a believer. Of course, it took me seven more years before I could finally kick the habit and not smoke another cigarette. However, during that period and long after, there were many other voices saying that the link between smoking and cancer had not yet been proven. Of course, many of these voices were coming from the tobacco industry's "smoking and health" divisions, even into the 1990s. Today, to a large extent, those voices have been discredited. Even so, the fact that they existed and came from platforms that seemed to give them standing, helped dilute the message that smoking can kill you.

I recently moderated a panel on global warming, during which young people were asking questions. One young lady asked this distinguished panel whether global warming was really occurring. She asked, "Is it true? Does everybody agree on this?" One of the scientists gave a very good answer, telling her that several years ago an international panel of scientists developed a consensus statement that the global temperature will rise anywhere from 3.5 to 9 degrees within 100 years if nothing is done about present carbon dioxide emissions and other causative factors. He also responded that the news media would always be able to find one scientist who will say, "No, it is not true." He lamented that the media would then report that some scientists say global warming is a fact and others say it is not, which will confuse the issue. He was quite right.

In another example, in the mid-1970s the House Select Committee on As-

sassinations assembled 20 top forensic scientists to look at the autopsy photographs from the Kennedy assassination. Nineteen of these scientists said, despite what the Zapruder film appears to show, that President Kennedy was killed by a shot from the rear. They postulated that the large flash seen on his forehead and the appearance of his neck jerking back on the film is the result of an exit wound, that is, representing the natural reflexes of the muscles when there is a trauma to the back of the head. One forensic scientist, said, "No, I think that could be an entry wound," forever leading the popular press and the film industry to beliefs about conspiracy and cover-up.

What I am saying to the scientific community is this: as it pursues, discusses, and reports its work, to the extent possible it must make clear the degree to which there is unanimity in the scientific community. That is, give the media a sense of how widespread the agreement is on a given issue. It is not our mission as reporters to be in on the science of science, but to report it. I am not going to work against the scientific community, but if I hear somebody with credibility that disagrees, I am going to call them and see what they think. I am not suggesting that scientific findings must be compromised, but rather that the magnitude of scientific differences should be explained. For example, in the case of my global warming story, one of the panelists later told me that he actually thinks the rise in temperature due to global warming over the next 100 years is likely to be closer to 9 degrees than 3.5 degrees. That helped me put the widely touted range of 3.5 to 9 into better perspective. It would also be useful if the general scientific community believes the outlier, or odd stepper, is really wrong, they should say it loud and clear, and avoid the tendency toward professional courtesy.

It has been six years since I had a melanoma tumor removed from my right groin. I know with every passing year that my odds improve, but the same cannot be said for all cancer patients. Each one is an individual. The graphs and charts showing cancer rates and survival give us a sense of the field, but any one point on the graph is an individual, whose risks and odds of survival might differ. All of us who have had cancer know that once it strikes, it is always stalking. It is always in the back of your mind, and we want to avoid its occurence in others as much as its recurrence in our own bodies.

I know with every passing year that my odds improve, but the same cannot be said for all cancer patients. Each one is an individual.

Sam Donaldson

Thus, when you are looking for the relationship between, for example, the hole in the ozone layer and cancer, I can't help but think how important this information is to the future incidence of melanoma. The scientific community seems unanimous in saying that chlorofluorocarbons help destroy the ozone, particularly in the southern hemisphere. If we don't do something about this, my

> If there is broad agreement about the need to act, then the scientific community must provide a solid front.
>
> *Sam Donaldson*

division of the melanoma cancer club will grow, rather than decrease. If there is broad agreement about the need to act, then the scientific community must provide a solid front. I appreciate that this is a challenge—that scientists are by their very nature skeptical. To the extent that the scientific community can reach broad agreement, we will all be in your debt.

GENES AND THE ENVIRONMENT IN CANCER ETIOLOGY

Joseph F. Fraumeni, Jr.

For some time, the epidemiologic evidence has suggested that the bulk of cancer in the population is related to environmental exposures, which are broadly defined here to include lifestyle factors, such as smoking, nutrition, and reproductive variables. Although genetic mechanisms are fundamental to the development and progression of all forms of cancer, the actual role of inherited susceptibility as an etiologic factor has been very difficult to assess.

The causes of cancer in the population can be assigned to one of four broad categories: (1) inherited susceptibility alone; (2) environment alone; (3) interactions between genes and the environment; or (4) a "spontaneous" category of tumors that may arise randomly from the play of chance (see Figure 2-1). It is in the category of gene–environment interactions that tremendous interest is build-

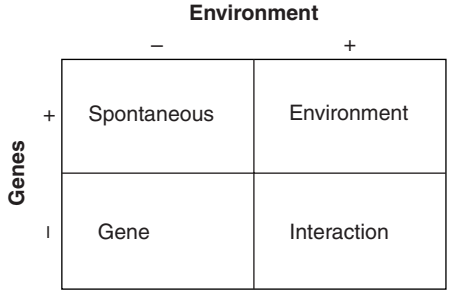

FIGURE 2-1 Categories of cancer causation in the general population. This figure illustrates the potential interaction of genes and the environment in the development of cancer. For example, the absence of genetic (-) and environment (-) influences reults in the development of a spontaneous tumor. SOURCE: Adapted from Knudson (1996). Reprinted with permission.

ing, particularly as advances in molecular biology and genome technology are incorporated into epidemiologic strategies.

The earliest recorded observation of an environmental risk factor occurred in Italy just over three centuries ago, when Bernardino Ramazzini reported an unusually high frequency of breast cancer in Catholic nuns, which can now be largely explained by reproductive factors and their effect on endogenous hormones. The next milestone was in 1775, when the British surgeon Percivall Pott (see Figure 2-2) discovered a cluster of scrotal cancer among young chimney

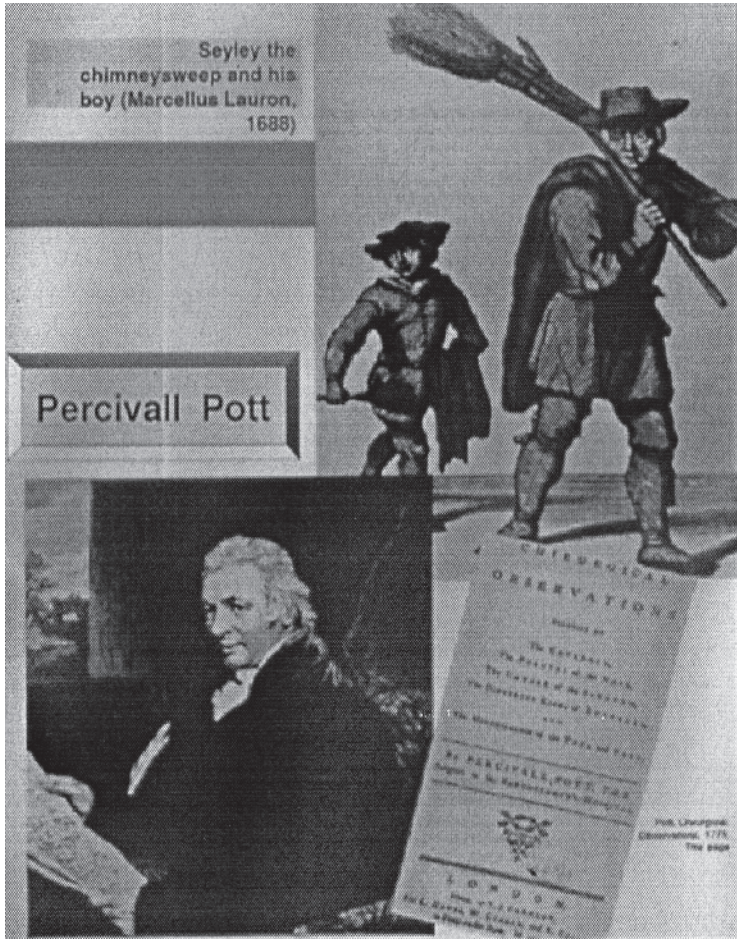

FIGURE 2-2 In 1775, Dr. Percivall Pott, a British surgeon, reported one of the earliest observations on environmental cancer. SOURCE: U.S. Government.

sweeps exposed to soot, which was subsequently found to contain mixtures of carcinogenic polycyclic hydrocarbons.

Over the years, alert clinicians continued to provide an early clue for a series of epidemiologic studies of environmental cancer, particularly by identifying case clusters of tumors that were uncommon in the general population and appeared related to a particular exposure (see Table 2-1).

The overall importance of the environment to cancer etiology can be roughly gauged by the international variation in statistics, gathered from the periodic volumes entitled *Cancer Incidence in Five Continents*, published by the International Agency for Research on Cancer. The differences between geographic areas with the highest and the lowest rates have ranged between 50- and 150-fold for melanoma and for cancers of the nasopharynx, prostate, and liver, to about 5-fold for leukemia (Table 2-2). Some of the geographic and ethnic variation can be related to diagnostic and reporting practices, as well as genetic factors for certain tumors such as melanoma, which tends to occur in fair-skinned populations. Nonetheless, the available evidence for most tumors suggests that environmental factors may be driving the patterns: when cancer rates from the lowest-risk countries were substracted from U.S. rates, it was estimated that perhaps 80 percent of all cancers in the United States are related in some way to environmental factors and are, thus, potentially avoidable.

More persuasive evidence for environmental factors comes from studies of migrant populations in which the risk of various cancers tends to shift away from the country of origin toward that of the new country. In a case-control study of breast cancer among Asian American women, the risk varied about sixfold according to migration history. The risk was lowest in migrants from rural parts of Asia who had lived less than a decade in the United States, and highest in those born in the United States along with at least three grandparents. Thus, for breast

TABLE 2-1 Some Examples of Cancer and Environmental Exposure

Cancer	Exposure
Scrotal cancer	Chimney sweeps
Liver angiosarcoma	Vinyl chloride
Acute leukemia	Benzene
Nasal adenocarcinoma	Hardwood dust
Bone sarcoma	Radium
Multifocal skin cancer	Arsenic
Mesothelioma	Asbestos
Vaginal cancer	DES
Kaposi sarcoma	AIDS/HIV

NOTE: DES = diethylstilbestrol.
SOURCE: U.S. Government.

TABLE 2-2 International Variation in Cancer Incidence

Type of Cancer	H/L	Highest Rates	Lowest Rates
Melanoma	155	Australia	Japan
Nasopharynx	100	Hong Kong	U.K.
Prostate	70	U.S. (blacks)	China
Liver	50	China	Canada
Cervix uteri	28	Brazil	Israel
Stomach	22	Japan	Kuwait
Lung	19	U.S. (blacks)	India
Colon	19	U.S. (whites)	India
Bladder	16	Switzerland	India
Pancreas	11	U.S. (blacks)	India
Ovary	8	N.Z. (Maori)	Kuwait
Breast	7	Hawaii (Hawaiian)	Israel (non-Jews)
Leukemia	5	Canada	India

SOURCE: Parkin et al. (1992). Reprinted with permission.

cancer, it takes a few generations of acculturation for the rates in migrants to approach the rates in the general U.S. population. Studies so far suggest that this gradient in incidence is related partly to changing reproductive factors, a more westernized diet, and an increase in height and body mass, as well as weight gain during adult life, but there are probably other factors that have not yet been detected.

Further evidence for environmental factors appears in the temporal variation in incidence or mortality for certain cancers, although again the trends may be influenced by improvements in detection and reporting. Based on recent statistics from the National Cancer Institute's (NCI's) Surveillance, Epidemiology, and End Results (SEER) program,[1] a number of cancers are continuing to show substantial average annual increases in incidence, including lung cancer among women due to cigarette smoking; melanoma due largely to sunlight exposure; pleural mesothelioma due to asbestos exposure; non-Hodgkin's lymphoma in part due to the AIDS epidemic, but largely unexplained; hepatocellular carcinoma due to hepatitis C virus infection; renal adenocarcinoma, particularly in African Americans, possibly related to increases in obesity and the prevalence of hypertension; and esophageal cancer.

When we divided esophageal cancer by cell type, again using data from the SEER program, there was a remarkable increase in one cell type, esophageal

[1]The SEER program collects and publishes cancer incidence and survival data from 11 population-based cancer registries and 3 supplemental registries covering approximately 14 percent of the U.S. population (see http://seer.cancer.gov for more information).

adenocarcinoma. This tumor was once uncommon but is now rising more rapidly than any other cancer, by about 8–9 percent per year. In a case-control study of esophageal adenocarcinoma, we have found substantial excess risks associated with gastroesophageal reflux and obesity. Gastroesophageal reflux is also increasing in incidence and may lead to an intestinal-type metaplasia called Barrett's esophagus, a precursor to adenocarcinoma.

It is noteworthy that esophageal adenocarcinoma is much more common in Caucasian men than in African American men, whereas squamous cell carcinoma is six times more common in African American men than in Caucasian men. The vast majority of squamous tumors of the esophagus are strongly related to drinking habits and, to a lesser extent, smoking habits and a low intake of fruits and vegetables. In our case-control study of this tumor, we found that these risk factors interact with one another and contribute to the higher rates in the African American population. We also found a strong effect of low socioeconomic status, which persists after adjusting for other risk factors, contributes to the ethnic differential in risk, and probably reflects a correlated exposure (perhaps a virus or dietary component) that has so far eluded detection.

Although the geographic variation of cancer within the United States is far less pronounced than the international patterns, we thought that mapping cancer mortality on a small-area scale (i.e., county level) might uncover spatial clusters of the more common tumors. A strategy was developed, with a stepwise progression of studies ranging from detailed mapping and correlation studies that generated etiologic hypotheses, to field studies in high-rate areas that tested hypotheses. We have recently published an updated atlas of cancer mortality among the Caucasian and African American populations, with the maps and other displays available on an interactive NCI Web site. The computer-generated maps in the new atlas are shown at the level of both the county and the state economic areas (combinations of counties with similar cultural and socioeconomic characteristics) for two time periods, 1950–1969 and 1970–1994.

For lung cancer in men, the earlier maps showed elevated rates in urban counties in the Northeast, but the highest rates by far were displayed in a string of counties along the Southeast Atlantic Coast and along the Gulf Coast. In the more recent maps, through 1994, there was a remarkable shift, with the elevated rates moving from the urban Northeast and coastal areas to broad stretches across the Southeast, a pattern that tracks the regional changes that have occurred in smoking habits. The earlier maps for lung cancer prompted a series of case-controlled studies in the high-rate coastal counties, which drew attention to the unexpected scale and impact of asbestos exposures in shipyards, particularly those operating during World War II, and to synergistic interactions between asbestos and cigarette smoking.

Another unexpected finding from the earlier maps was the clustering of elevated rates for oral cancer among women in the rural South. This pattern prompted a case-control study in North Carolina that implicated the long-stand-

ing practice of snuff dipping. The risks reached as high as fiftyfold for cancers of the gum and buccal mucosa, the tissues in direct contact with the smokeless tobacco product. The study promptly led to congressional hearings and regulatory actions aimed at controlling the advertising and labeling of smokeless tobacco, educational campaigns aimed at young people, and laboratory studies that implicated tobacco-specific nitrosamines as the likely carcinogen.

Since our first atlas was published, cancer mapping on a small-area scale has become standard practice in virtually every nation with vital statistics. Most remarkable have been the mortality patterns in China, where there is enormous geographic variation that has provided special opportunities to study environmental cancer. For example, the map for lung cancer among women revealed very high rates in the northeast, where case-control studies have implicated several indoor air pollutants as pulmonary carcinogens (such as cooking oil fumes from wok cooking, polycyclic hydrocarbons from coal-heating stoves, and indoor radon in poorly ventilated houses).

In some high-risk areas in China, intervention studies are under way to clarify etiologic factors and hopefully develop preventive measures for certain cancers. In an endemic area for squamous esophageal cancer associated with poor nutritional status, the effects of vitamin and mineral supplements are being evaluated, both in very high risk individuals who have esophageal dysplasia and in the general population. Similar studies are under way in a high-incidence area for gastric cancer, where it is possible to monitor through endoscopy the progression of precancerous lesions (ranging from atrophic gastritis to intestinal metaplasia to dysplasia), which are present in virtually the entire adult population. Since *Helicobacter pylori* infection is a major risk factor for this cancer, its eradication by antibiotic therapy is one of the treatment arms in our study.

The epidemiologic study of risk factors in the U.S. population is far from complete, particularly when exposure assessment is difficult—such as with diet and environmental pollution—and when relative risks are small. As expected, tobacco smoking is the main culprit, accounting for at least 30 percent of all cancer deaths. Alcohol interacts with tobacco to cause tumors of the upper aerodigestive system and liver, and also appears to be involved in breast cancer and possibly in colon and pancreas cancer.

It is clear that diet- and nutrition-related variables, including obesity and physical inactivity, are very important risk factors, but there are still many questions about their overall impact on cancer, as well as the specific causative and protective elements in the diet, not to mention the role played by hormonal, metabolic, and other alterations affected by nutritional status. Infectious agents appear to contribute less to the cancer burden in the United States than in developing countries, where cancers of the liver (hepatitis B), cervix (human papilloma virus), and stomach (*H. pylori*) are very common, but further studies are needed on the possible role of viruses and bacteria in other tumors.

In addition, the impact of chemicals from occupational exposure, environ-

mental pollution, and pharmaceutical agents still has to be clarified, as does ionizing radiation to some extent, including indoor radon. Ultraviolet radiation, of course, is the major cause of skin cancers, but these tumors are usually not reflected in mortality data except for melanoma.

The greatest uncertainty at present is genetic or inherited susceptibility, but there is growing evidence that its overall impact on cancer risk is likely to be greater than previously estimated from the demographic patterns of cancer, the low levels of familial risk for common tumors, the low frequency of hereditary cancer syndromes, or the calculations based on twin studies that have sharply partitioned genetic from environmental factors. In addition, as we learn more about the role of genetic susceptibility and its pathways, we are likely to gain further insights into the cancer risks associated with common exposures, including dietary components, endogenous hormones, and environmental hazards, that are acted upon by functional variants of candidate genes.

A great deal of progress has been made in linking hereditary cancer syndromes to highly penetrant genes, mainly through family-based studies. As shown in Table 2-3, most of the cloned genes have turned out to be tumor suppressors, while others are proto-oncogenes (such as the RET gene in multiple endocrine neoplasia type II) or mismatch repair genes that are associated with familial nonpolyposis colon cancer. In general, once a gene is mapped to a particular locus, cloning of the gene and characterizing its function soon follow.

As we learn more about the hereditary syndromes, it appears that many consist of constellations of tumors that appear to share genetic pathways. This can be seen with germline mutations of BRCA-1 and 2, which are associated with breast cancer, ovarian cancer, and other tumors as well, and with the mis-

TABLE 2-3 Familial Syndromes and Cloned Tumor Suppressor Genes

	Gene	Locus	Date
Retinoblastoma	RB1	13q14	1986
Wilms' tumor	WT1	11p13	1990
Li–Fraumeni syndrome	P53	17p13	1990
Familial adenomatous polyposis	APC	5q21	1991
Neurofibromatosis 1	NF1	17q11	1990
Neurofibromatosis 2	NF2	22q11	1993
Von Hippel–Lindau syndrome	VHL	3p25	1993
Familial melanoma 1	P16	9p21	1994
Tuberous sclerosis 2	TSC2	16p13	1993
Familial breast cancer 1	BRCA1	17q21	1994
Familial breast cancer 2	BRCA2	13q12	1995
Basal cell nevus syndrome	PTC	9q22	1996

SOURCE: Adapted from Knudson (1996). Reprinted with permission.

match repair genes that predispose to cancer of the colon, endometrium, and other sites.

The most striking example of this phenomenon is a familial constellation of cancers among children and young adults that features extremely high risks for soft tissue and bone sarcomas, and breast cancer, along with excesses of brain tumors, acute leukemia, and adrenocortical neoplasms (Li–Fraumeni syndrome). Our recent experience with these families suggests that there are excesses of other cancers arising at an early age, but at lower levels of relative risk. As in other hereditary syndromes, the affected individuals often develop multiple primary cancers, including a predisposition to sarcomas associated with radiotherapy, indicating gene–environment interaction. The variety of tumors in this syndrome has helped to dispel an earlier notion that inherited susceptibility to cancer is site-specific or tissue-specific, and is thus in line with molecular studies suggesting that fundamental biological mechanisms controlling susceptibility and cell proliferation are shared by several, if not all, forms of cancer. In the case of Li–Fraumeni syndrome, the search for an underlying mechanism was unrewarding until the molecular technology was available to uncover germline mutations of p53 in most families. This finding was of special interest, since alterations of this tumor suppressor gene are somatically acquired in more than 50 percent of all cancers in the population.

It is important to note that even for highly penetrant cancer genes such as p53 or the retinoblastoma gene RB-1, tumor expression may be affected by other modifying genes or by environmental exposures, such as radiotherapy and smoking. In our cohort study of hereditary retinoblastoma, we found that half the cases developed second cancers by age 50, particularly sarcomas, melanomas, and brain tumors. The cumulative risks were 58 percent in the irradiated group and 26 percent in those who received no radiotherapy, suggesting an interaction between the RB-1 gene and radiation. An extended follow-up of this group has revealed a significant excess of lung cancer that is limited to smokers, again consistent with gene–environment interaction. In contrast, there has been no excess risk of second cancers among the cases with nonhereditary retinoblastoma.

Although the single-gene mutations associated with hereditary cancer are highly penetrant and tend to carry high relative and absolute risks, they are relatively rare and appear to account for a small percentage of cancers overall. On the other hand, the polymorphic susceptibility genes or genetic variants are generally associated with low penetrance and low relative and absolute risks, but they are very common in the population (more than 1 percent) and may be involved in a high proportion of cancers through biologic interactions with environmental or endogenous exposures.

It is clear that family studies and linkage analyses have been highly successful in identifying major genes and helping to revolutionize our understanding of carcinogenic mechanisms, and they can still provide insights into interactions

with other genes and with epigenetic and environmental factors that modulate risk. However, it now appears that population studies of a case-control or cohort design are needed to clarify and quantify the risks associated with common susceptibility genes and their interactions with exposures.

The first associations linking cancer to these genetic variants involved the so-called metabolic genes, including the cytochrome P-450 activating genes such as CYPIA1 and CYP2E1, and the conjugating or detoxifying genes such as GSTM1 and NAT2. By knowing the substrate and the metabolic pathway of the gene, one may learn about mechanisms and the exogenous or endogenous carcinogens that are otherwise difficult to identify. For example, a metabolite of alcohol—acetaldehyde—has been implicated by the heightened risk of alcohol-related oral cancer associated with a rapid-acting genotype of alcohol dehydrogenase-3, which accelerates the metabolism of alcohol to acetaldehyde. In Japan, a gene variant of aldehyde dehydrogenase, which blocks the metabolism of acetaldehyde to acetate, has been linked to oral and esophageal cancer risk. These two observations point to acetaldehyde as the likely carcinogen in alcohol-related cancer.

In addition, a protective effect of dietary folate on colon cancer risk is supported by the relation to a methylenetetrahydrofolate reductase gene, while various estrogen- and androgen-metabolizing genes are under active study for breast and prostate cancer.

More recently, research on genetic variants has broadened to include other functional classes of cancer susceptibility genes that have more distal or downstream effects. These include polymorphisms affecting DNA repair and processing, cell cycle control, immune function, inflammation, growth factors, apoptosis, and angiogenesis.

Further study of interactions between relatively common alleles and exposures will rely on case-control studies, which may be population or hospital based, but are often embedded within prospective studies. These studies will require sufficent sample sizes for statistical power to evaluate gene–gene and gene–environment interactions, especially when the effects are multiplicative. It also is important to ensure careful epidemiologic design with appropriate control groups, as well as highly accurate genotyping and exposure assessment, which may include acquired genetic biomarkers such as macromolecular adducts and mutational "fingerprints" of exposure. Any misclassification of the gene or the exposure will greatly decrease the power of the study and increase the sample size needed to show an effect.

The challenges to population studies are formidable but they can be met. Large sample sizes are possible through collaborative multicenter studies. The development of simple, noninvasive approaches to collecting genomic DNA, such as the mouthwash rinse to collect buccal cells, should decrease the costs of sample collection and improve participation rates. The studies will require not only sound epidemiology, but also specimen processing and repository facilities,

and close collaboration between epidemiologists, molecular biologists, genomicists, and bioinformaticians.

Despite efforts to ensure high-quality and cost-effective experience in genotype data, the sheer volume of information and the complexity of interactions will generate a prodigious number of multiple comparisons and false-positive findings—more so when it becomes possible to conduct whole-genome scans and subclassify tumors on a molecular basis, not to mention new technologies and developments in proteomics and other areas. To avoid publication bias and assist in interpretation, it would seem important to report all the tests that are conducted in a particular study and to make available all negative as well as positive results through comprehensive databases. A major problem at present is the blizzard of positive associations that are being reported on almost a daily basis and are followed in due course by a flurry of contradictory observations by other investigators. Whenever possible, it would seem important to assemble a coalition of research groups to coordinate approaches and conduct parallel case-control studies that can provide quick replication of positive or negative findings, using independent data sets prior to publication.

Other potential difficulties with population-based designs include linkage disequilibrium, in which the candidate gene may be a marker allele rather than a disease allele itself, but this problem can be minimized by further progress in sequencing the genome and understanding the biologic relevance of the allele. The potential for population stratification, also known as ethnic confounding, can be handled in the main by appropriate epidemiologic techniques.

In conclusion, the available epidemiologic evidence indicates that while environmental exposures drive the demographic patterns for most cancers, there are growing indications that gene variants may have a sizable impact on cancer development by modifying the effects of exogenous or endogenous risk factors and by helping to uncover low levels of relative risk from common exposures in genetically susceptible subgroups. The identification of susceptibility or modifier genes should also help identify potential carcinogens and protective factors acted upon by the gene products and should provide insights into mechanisms and interactions that will multiply the opportunities for preventive intervention.

> The big challenge for epidemiology now is to develop strategies to ensure that the advances in human genomics are incorporated appropriately into population studies, as well as family-based, and hybrid studies.
>
> *Joseph Fraumeni*

The National Institutes of Health has moved in various ways to seize the opportunities in this important area, such as the initiatives of the environmental genome project coordinated by the National Institute of Environmental Health Sciences. Another blueprint is sketched out in the NCI *Bypass Budget Proposal*

for Fiscal Year 2002, which summarizes the extraordinary opportunities for investment in cancer research. In a chapter entitled "Genes and the Environment," NCI outlines a series of objectives and plans that combine epidemiologic and molecular approaches in ways that may enlarge our understanding of cancer etiology and inform new clinical and public health approaches aimed at preventing and controlling cancer. The big challenge for epidemiology now is to develop strategies to ensure that the advances in human genomics are incorporated appropriately into population studies as well as family-based and hybrid studies (consisting of population and family-based components) and that these studies have the power and sensitivity to dissect the environmental and genetic influences on cancer risk.

3

The Links Between Environmental Factors, Genetics, and the Development of Cancer

The past decade has witnessed important advances in the understanding of factors that influence cancer risk. Several environmental factors continue to surface as potentially instrumental in explaining the wide global variation in the incidence and biological behavior of various tumors. For example, discoveries that both essential and nonessential dietary nutrients can markedly influence several key biological events—including cell cycle regulation, processes involved in replication or transcription, immunocompetence, and factors involved with apoptosis, or programmed cell death—have strengthened convictions that specific foods or components may markedly influence cancer risk.

Analyses of the incidence of cancer in twin pairs and in families are traditional methods for answering questions about the relationships between cancer etiology, genes, and the environment. Sorting out the relative roles of each in the initiation and progression of cancer can lead to clearer elucidation of how shared environmental influences can disparately affect the health of individual members of a community, that is, why some people exposed to a specific agent develop cancer when others do not.

Finally, although environmental, occupational, and recreational exposures to carcinogens contribute to cancer risk in humans, variation in incidence and progression of cancers among individuals can be attributed to interindividual variation in genetic makeup. Recent research has identified functional polymorphisms that influence an individual's cancer risk and has focused on gene products involved in activation and detoxification of carcinogens and DNA repair. Gene polymorphisms that are important in apoptosis will increasingly be recognized as clues to individual susceptibility to cancer, explaining why individuals with shared environmental exposures do not always share cancer morbidity and mortality.

DIET AS A MODIFIER OF CANCER RISK

There are unprecedented opportunities for using the food supply to achieve genetic potential, that is, to optimize our performance and reduce the risks of diseases, said John Milner, Department of Nutrition, Pennsylvania State University. Although 80 percent of cancers are related to environmental factors, the influence of diet in the development of cancer is somewhat uncertain. However, the general consensus is that approximately 35 to 40 percent of cancers relate to dietary habits, although the range might be quite large.

The influence of diet in the development of cancer is somewhat uncertain. However, the general consensus is that approximately 35 to 40 percent of cancers relate to dietary habits

John Milner

Even though science has come a long way in understanding what factors are important in controlling cancer risks or modifying health in general, we still do not really know who is going to benefit, and under what circumstances, said Milner. In fact, we do not yet know if there are some people who would be placed at risk because of exaggerated intakes of certain types of foods or food components. The whole issue of the role of diet in health is exceedingly complex when trying to assess the relative roles of individual foods as they relate to overall cancer risk. There are some areas of agreement, however, said Milner. More than 80 percent of the studies that have been published reveal a reduction in cancer risk with an increase in fruit and vegetable consumption. However, there is considerable variability among populations, suggesting that a person's genetics may be important in determining the response. He added that we need to have a better understanding of how genes are involved in the cancer process and how individual nutrients can modify these genes and ultimately influence the probability of developing cancer.

Some of the strongest evidence linking diet and cancer comes from the epidemiological observation that increased vegetable and fruit consumption is associated with a reduction in the risk for cancers of the mouth and pharynx, esophagus, lung, stomach, colon, and rectum. Likewise considerable evidence points to a host of essential and nonessential nutrients as modifiers of cancer risk at a variety of sites. Milner noted that part of this variation in cancer risk may arise from variation in the intake of one or more essential nutrients supplied by either plant or animal food sources. Vegetables derived from various parts of plants including roots (e.g., carrots, parsnips), leaves (e.g., spinach, lettuce), flowers (e.g., artichoke, broccoli), stalks (e.g., celery, rhubarb), and seeds (e.g., corn, peas), as well as a host of fruits, provide thousands of chemically diverse phytonutrients that may contribute to these observations. Some of these phytonutrients—including flavonoids, carotenoids, organosulfides, and

isothiocyanates—have been the focus of recent research to determine both their effects on risk and their mechanisms of action.

Despite the clear linkages that have been found between the risk of developing some types of cancers and dietary patterns, inconsistencies have been detected, which might reflect the multifactorial and complex nature of cancer, the specificity that individual dietary constituents have in modifying specific genetic pathways, and the temporal relationship between dietary intervention and phenotypic changes in tumor incidence or behavior. The chemical and biological diversity of dietary components in combination with a range of molecular targets makes pinpointing the importance of diet in various cancers a challenge, emphasized Milner. It is likely that this challenge will be augmented by advances in cell biology and epidemiology. For instance, when limonene (found in citrus fruits) is added to tumor cells it has been found to enhance several genes while suppressing others. Since several of the identified genes are involved in the pathways leading to apoptosis, it is possible that agents such as limonene could play a role in the cell signaling involved in programmed cell death. Similarly, studies with a variety of other nutrients, including selenium, isothiocyanates, and allyl sulfide, have been reported to modify at least 20 different gene products associated with cancer prevention.

In addition, knockout and transgenic animals can provide important clues about the specific site of action of dietary components. The use of these genomic technologies to evaluate the effects of nutrients offers exciting opportunities for determining which cellular change is most important in bringing about a change in the incidence or behavior of a tumor.

Preclinical evidence suggests that diverse dietary constituents including selenium, allyl sulfur, genistein, and resveratrol can influence the same genetic pathways associated with tumor cell proliferation and apoptosis. Such common effects raise concerns about potential interactive and cumulative effects among nutrients, said Milner. In addition, compounds such as diallyl disulfide, which is found in crushed garlic, can actually suppress the growth rate of cells, and indole-3-carbinol, found in cabbage, can shift estradiol metabolism, which can affect tumor formation. The only problem, said Milner, is that we may have to consume about three-quarters of a pound of cabbage a day and several cloves of garlic to bring about a response. We know of a few examples where isolated food components and intact foods do not bring about the same biological response. Thus, a reductionist

> A reductionist approach to diet and cancer prevention may produce oversimplifications and confusion. We clearly need to know what the mechanisms are that account for specific bioactive food components but must also recognize that we eat whole foods.
>
> *John Milner*

approach to diet and cancer prevention may produce oversimplifications and confusion. We clearly need to know what the mechanisms are that account for specific bioactive food components but must also recognize that we eat whole foods.

Astonishing strides have been made in understanding how molecules and genetic pathways differ in precancerous and malignant cells and from their normal counterparts. Capitalizing on the differences in cellular signatures that are characterized by active and inactive genes and cellular products could assist in determining who should and should not benefit from intervention strategies. Clearly, added Milner, such information will help clarify the reason for discrepancies among preclinical, epidemiological, and intervention studies.

At least part of the variation in response to dietary components can probably be explained by the consumer's genetic profile. It is now becoming apparent that the prevalence of polymorphisms is variable among studied populations, and these differences could influence the response to diet. Evidence exists that genetic polymorphisms may modulate cancer risk through their influence on folate metabolism. For example, epidemiologic studies have reported that the relationship between dietary folate and colorectal cancer risk is influenced by polymorphism in methylenetetrahydrofolate reductase activity. Variation in the response to folate metabolism is not unique since other studies suggest that variation in receptors for vitamin D may also be linked to cancer risk. Considerably more information is needed about how genetic polymorphisms influence the response to dietary components and ultimately cancer risk, added Milner.

Unquestionably, cancer is intertwined with environmental factors including diet. Strategies to prevent cancer through modification of either diet or specific dietary patterns will probably not be uniformly effective for all individuals, said Milner. He stressed that a better understanding of gene–nutrient interactions will be needed to determine those who might benefit most from dietary intervention and those who might be placed at risk. For example, there are data suggesting that some women who consume large amounts of fruits and vegetables may be at increased risk of giving birth to children with infantile leukemia. These women appear to have a reduced ability to remove some of the flavonoids from their system, which thus accumulate and become toxic to the developing fetus. Although in most cases there likely will be benefits from increased consumption of fruits and vegetables during pregnancy, in a small subset of the population an opposite response may occur. Future research in nutrition and cancer prevention must give top priority to studies that seek to understand the basic molecular and genetic mechanisms by which nutrients influence the various steps in carcinogenesis. "By understanding the importance of the genetic profile, we can identify who is going to benefit and who is not going to benefit from dietary intervention," concluded Milner.

GENETIC EPIDEMIOLOGY AS A TOOL FOR STUDYING GENE–ENVIRONMENT INTERACTIONS

Mounting evidence supports the concept that cancer is generally a polygenic multifactorial disease, which makes environment an important modifier in the risk of cancer, stated Kari Hemminki, Karolinska Institute. It is estimated that only 1 percent of cancers are caused by "cancer syndromes" and up to 5 percent result from highly penetrant single-gene mutations; thus, the majority are polygenic. Studies with various animal and in vitro models, initiation and promotion models, adenoma carcinoma models, and immortalized human cells provide evidence that polygenic mechanisms are important in cancer, at least in experimental systems.

Almost all of the known cancer syndromes are monogenic and conform to a two-stage model of development; that is, they require inactivation of two copies of a tumor suppressor gene in order to initiate. These syndromes tend to be dominant Mendelian conditions, which can be assessed in family studies covering two or more generations. However, such studies provide no data on recessive Mendelian conditions and have a limited resolving power in polygenic conditions. Consequently, apart from highly penetrant single-gene mutations, the risks posed by low-penetrance single-gene mutations, polygenes, and recessive genes are poorly understood.

Hemminki described a study of data obtained from 44,000 same-sex twin pairs to assess cancer risks for co-twins of twins with cancer. There were almost 10,000 pairs in which one of the members had cancer. The analysis of environmental and inherited contributions was based on correlations between monozygotic twins who share the genome completely, that is, 100 percent concordance in their genomes. A similar concordance was carried out with dizygotic twins, the difference being the assumption that only 50 percent of the genes are common. The assumption is that the environment is affecting monozygotic and dizygotic twins similarly. Some of these different effects will then be 100 percent, or

Twin studies as tools for understanding genes, the environment, and cancer

Genetic: if monozygotic twins are more similar for a given trait than dizygotic twins

Shared Environment (e.g., diet and childhood experiences): if there is twin similarity not accounted for by genetic effects

Nonshared Environment: anything that is not hereditary and not shared between relatives, that is, sporadic causes of cancer

1. The nonshared random environmental effect was the largest factor for all cancers, accounting for 58 to 82 percent of the total variation (Table 3-1) (Lichtenstein et al., 2000). Statistically significant heritability estimates were detected for cancers of the colorectum (35 percent), breast (27 percent), and prostate (42 percent). The estimates for shared environmental effects ranged from 0 to 20 percent, but none were statistically significant.

A Swedish family cancer database, containing 10 million people, is the largest population-based data set ever used for studies on familial cancer, said Hemminki. The data are used to develop estimates for the environmental and inherited components in cancer, using the genetic relationships among family members to calculate the effects of genotype, shared environment, and nonshared environment. The database has been used in modeling cancer causation and has revealed that environmental causes explained most of the total variation for all neoplasms except thyroid cancer, for which heritable causes were largest. There also appears to be a subgroup of cancer patients who develop a second cancer to which there is a strong genetic predisposition, that often cannot be predicted by a family history. This phenomenon is typical of polygenic disease.

Hemminki reported that the twin and family data quantified nonshared environmental effects as ranging from 40 to 90 percent for different cancers. It is of interest to note that this effect was large for some cancers of identified environmental causes, such as lung and cervical cancers. In contrast, shared environment—common family experiences and habits—accounted for 0 to 30 percent of cancer etiology. For all cancer, the genetic effect was estimated to be 26 percent; however, there is evidence supporting heritability for all cancers.

TABLE 3-1 Heritable and Environmental Effects from Twin Studies

	Proportion of Variance Attributed to		
Cancer	Heritable Effects	Shared Environmental Effects	Nonshared Environmental Effects
Stomach	0.28	0.10	0.62[a]
Colorectum	0.35[a]	0.05	0.60
Lung	0.26	0.12	0.62[a]
Breast	0.27[a]	0.06	0.67[a]
Cervix uteri	0	0.20	0.80[a]
Ovary	0.22	0	0.78[a]
Prostate	0.42	0	0.58
Bladder	0.31	0	0.69
Leukemia	0.21	0.12	0.66[a]

[a]95% does not include 0.0.

SOURCE: Lichtrnstein et al. (2000). Reprinted with permission.

The data presented by Hemminki on twins, families, and second cancers provide additional support to the multistage theory of carcinogenesis. If most cancers are indeed polygenic, this should be adequately considered in study designs for gene mapping approaches. Linkage analysis in families of multiple affected individuals is not sufficient to identify cancer-related genes, said Hemminki. Instead, what are needed are large case-control studies with stringent clinical criteria so that the different types of cancer can be distinguished and there is a large enough sample size to enable even the rare homozygotes to be scored, emphasized Hemminki. In addition, it will be important to study people with multiple cancers or second cancers, because they can provide a good indication of whether polygenic effects are operating.

MOLECULAR CARCINOGENESIS, MOLECULAR EPIDEMIOLOGY, AND HUMAN RISK ASSESSMENT

The macroenvironment—our lifestyle, the air we breathe, the food we eat, the chemicals we are exposed to, as well as viruses, radiation, and physical agents we come in contact with—that combines with the microenvironment of our cells to either prevent or enhance carcinogenesis was described by Curtis Harris, National Cancer Institute (see Figure 3-1; Wang et al., 1997).

In addition, a great deal of interindividual variation in our genetic makeup plays a role in the incidence and variability of cancer. Much of the work on identifying functional polymorphisms that influence an individual's cancer risk has focused on gene products involved in the activation and detoxification of carcinogens and, more recently, on DNA repair.

The idea that genomic instability might play a role in cancer is also an old one. Aneuploidy was recognized in the nineteenth century and was postulated to play a role in some cancers. More recently, tripolar spindles of DNA have been associated with the overexpression of an oncoprotein from the hepatitis B virus, which could explain how the virus contributes to hepatocellular carcinoma.

In the 1970s and 1980s a set of genes, called tumor suppressor genes, was elucidated, one of which was called p53. These genes are so named because they prevent cancer by recognizing defective cell programming. The p53 gene recognizes the signal created by a precancerous condition and responds by killing the cell by a process called programmed cell death, or apoptosis. It has subsequently been shown that p53 mutations are common in diverse types of human cancer, where they are involved in genomic instability. The gene is involved in some pathways of apoptosis and cell cycle control, and among its many functions, it is a transcription factor. It suppresses some genes and upregulates others. It is at the crossroads of multiple cellular stress response pathways, DNA damage of varying kinds, hypoxia, and oncogene activation. (Figure 3-2).

In 1979, the p53 tumor suppressor gene was identified. It has been the subject of intense research in the past 20 years and is involved in many cellular

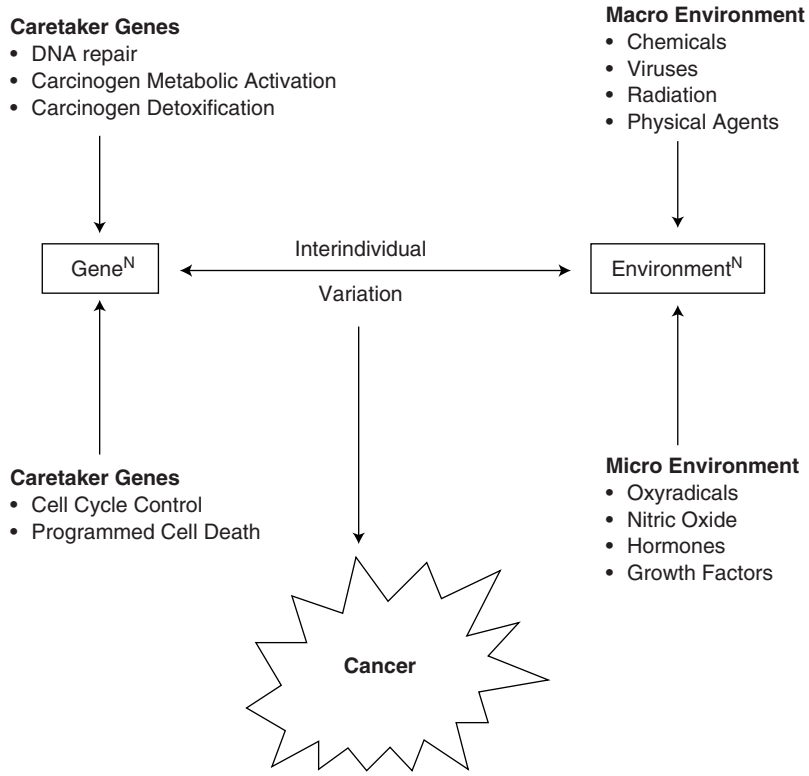

FIGURE 3-1 Multiple genes ($Gene^N$) can interaction with a number of environments ($Environment^N$) in the development of cancer. SOURCE: Harris (1997). Reprinted with permission.

responses including differentiation, DNA repair, inhibition of angiogenesis, and apoptosis—programmed cell death.

It is suggested that p53 plays a role in the development of cancer through mutation at various sites. Knockout mice missing p53 can develop normally, but they are highly tumor prone. Molecular studies estimate that approximately half of all human cancers, including some forms of skin, lung, and liver cancers, carry p53 mutations. Interestingly, the mutational sites in radon-associated lung cancer differ from lung cancer caused by tobacco smoking alone. These differences may have implications for cancer diagnosis and treatment in the future.

Cancer formation is a multistage process involving the activation of proto-oncogenes and the inactivation of tumor suppressor genes. Harris explained how carcinogens could affect any of these stages through genetic and epigenetic mechanisms.

Examining the mutational spectra of cancer-related genes (e.g., p53, BRCA1, and $p16^{INK4}$) may provide a molecular link between etiological agents and human cancer. For example, mutations in the evolutionarily conserved codons of the p53 tumor suppressor gene are common in diverse types of human cancer, and the p53 mutational spectra differ among cancers of the colon, lung, esophagus, breast, liver, brain, reticuloendothelial tissues, and hemopoietic tissues. Analysis of these mutations can provide clues to the mutagenic mechanisms and the function of specific regions of p53. Genetic polymorphisms are likely to play a role in the risk of lung cancer in smokers, ex-smokers, and individuals exposed to secondhand smoke. For example, women with a GST-null (glutathione *S*-transferase) genotype have about a twofold increased risk of developing lung cancer, and if they are exposed to high levels of environmental tobacco smoke, the risks are five- or sixfold higher. The hypothesis that women are more susceptible than men to tobacco-smoke-induced lung cancer is another controversial area deserving study, said Harris. Studies show more carcinogen-induced DNA damage in lungs of women than men who smoke, possibly due to a relative decrease in DNA repair capacity in women versus men.

Much of the work in the field right now is investigating the mechanisms that lead to the activation of p53, largely through the kinase pathways. Studies are concentrating on the type and location of mutations found in the p53 gene in a variety of cancers. For example, in liver tumors from persons living in geographic areas where aflatoxin B_1 and hepatitis B virus are cancer risk factors,

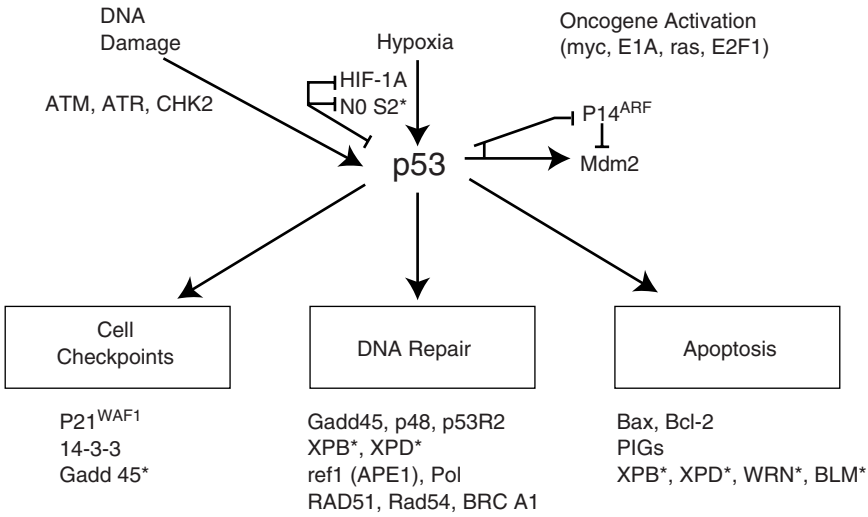

FIGURE 3-2 The p53 gene: at the crossroads of the cellular response. SOURCE: Harris (2000). Reprinted with permission.

most p53 mutations are at the third nucleotide pair of codon 249. A dose-dependent relationship between dietary aflatoxin B_1 intake and codon 249^{ser} p53 mutations is observed in hepatocellular carcinoma. Exposure of human liver cells to aflatoxin B_1 in vitro produces specific p53 mutants; the mutation load in vivo is positively correlated with dietary aflatoxin B_1 exposure. These results demonstrate that the expression of a specific mutant p53 protein provides a specific growth or survival advantage to liver cells.

Other associations between the p53 mutational spectra and exposures to carcinogens have been observed. For example, the induction of skin cancer by ultraviolet light is accompanied by specific p53 mutations. In another example, the p53 mutational spectrum in radon-associated lung cancer from uranium miners differs from that in lung cancer caused by tobacco smoking alone, noted Harris. These genetic changes in the tumor suppressor genes have implications for cancer diagnosis, prognosis, and therapy, according to Harris.

The association of a suspected carcinogenic exposure and cancer risk in populations can be studied with classic epidemiologic techniques. However, these techniques are not applicable to the assessment of risk in individuals, stressed Harris. A goal of molecular epidemiology is to integrate molecular biology, in vitro and in vivo laboratory models, biochemistry, and epidemiology to infer individual cancer risk. Carcinogen–macromolecular adduct levels, somatic cell mutations, and DNA adducts can be measured to determine the biologically effective dose of a carcinogen. Molecular epidemiology also explores host cancer susceptibilities, such as carcinogen metabolic activation, DNA repair, endogenous mutation rates, and inheritance of mutated tumor suppressor genes.

Substantial interindividual variation for each of these biologic end points has been shown, highlighting the need to assess cancer risk on an individual basis. As Ernst Mayr (1982) wrote in *The Growth of Biological Thought,* "In biology one rarely deals with classes of identical entities, but nearly always studies of populations consisting of unique individuals." There is a wide variation from one individual to another in the ability to metabolically activate and damage DNA. Given the pace of the past decade, said Harris, it is feasible that future advances will allow molecular epidemiologists to develop a cancer risk profile for an individual that includes assessment of a number of exposure and host factors. Cancer-related genes, such as p53, provide a useful molecular link between environmental agents and cancer itself. This will help focus preventive strategies and strengthen quantitative risk assessments.

SUMMARY

Cancer is the second leading cause of mortality in the United States today, resulting in more than half a million deaths each year. Although recent data show a downward trend in mortality rates due to cancer—mostly as a result of early detection and improved therapies—the incidence of some cancers is in-

creasing. Important advances have increased our understanding of the factors that influence cancer risk, explaining in part why some cancer rates are increasing while others are decreasing, and guiding us closer to the means to reverse adverse trends.

A growing body of knowledge dramatically illustrates the influence of the environment, genes, and their interactions in the international variation reported in cancer incidence. A variety of linkages clearly exist between environmental exposures, diet, lifestyle factors, and cancer. Genetic factors also are known to be involved in the predisposition to and development of some cancers. Recent progress in identifying and characterizing highly penetrant susceptibility genes in familial cancer has revolutionized our understanding of the critical genetic mechanisms in cancer etiology. Studies that combine genetic analysis with assessment of exposures and diet can explain why not everyone exposed to a particular cancer-causing chemical will develop cancer. Genetic research also is shedding light on why some cancer patients respond to therapy and others do not.

The interactions of multiple modifier genes with various environmental factors—that is, gene–environment interactions—explain why cancer rates vary across populations, among exposed groups, and even within families. The research community is now studying cancer with an expanded and enhanced view of environmental health and exposures that include factors such as diet, lifestyle, metabolic alterations, socioeconomic status, and various environmental exposures.

4

Gene–Environment Interaction in Special Populations

One of the challenges in cancer research and prevention is to ensure that the benefits in cancer prevention and treatment are available to individuals from all areas of the United States regardless of gender, socioeconomic status, age, ethnic origin, or migration. The workshop planning group, recognizing the need to look at research in diverse communities, devoted a session of the agenda to discuss advances and research in these areas. One challenge according to Armin Weinberg, Baylor College of Medicine, is the need to continue to describe and understand what researchers call special populations, priority populations, and vulnerable populations. "It is important to describe these populations, but not to label them" said Weinberg. As we continue to go forward in our research, we will most likely find that within communities and groups, there are subgroups. These subgroups will have issues and special circumstances that will have to be addressed.

HEALTH DISPARITIES

According to Lovell Jones, M.D. Anderson Cancer Center, "When we approach efforts to deal with the lack of real progress in addressing health disparities, we tend to fall back on what we did before. It may be under a different name or it may be packaged in a different box, but ultimately it is the same strategy. In other words, if you always do what you have always done, we will always get what we already got." He added that more data are needed to document the reality of health disparities, to characterize them accurately, and to determine their causes. Disparities are not necessarily racially or ethnically linked; they can be associated with lack of insurance, access to medical care, age, employment status, and migration patterns. Nonethe-

> We need new approaches to effectively deal with health disparities.
>
> *Lovell Jones*

less, minorities, the poor, the medically underserved, and children suffer disproportionately from these burdens in terms of health disparities.

In some racial and ethnic groups, cancer rates are higher and accelerating, according to Jones (see Newell, 1988). The reasons for these disparities may include the environment, hormones, and genetics but also involve socioeconomic status. "Although racial classifications are a social construct, these classifications continue to have an impact on the health of this nation," said Jones, adding that "health is probably the best indicator of the failure of this nation to address the issue of skin color and social class and the future well-being of this nation." Because we do not apply what we know about prevention and treatment equally to all parts of society, we are not achieving the health gains that are currently possible.

Because we do not apply what we know about prevention and treatment equally to all parts of society, we are not achieving the health gains that are currently possible.

Lovell Jones

Higher income permits increased access to medical care and enables people to afford better housing, live in better neighborhoods, and have opportunities to promote their health behaviors. Higher incomes also tend to help people participate in clinical research studies, said Weinberg; thus, disparities in access to health care can affect enrollment in research studies. Demographic studies of cancer must consider the diversity within affluent groups as well as within less economically affluent groups.

INFLUENCE OF MIGRATION

Researchers have to consider immigration patterns and countries of origin because these factors play a primary role in predisposition to cancer. Individuals from many geographic locations have different diets, exposures, and degrees of acculturations but are commonly grouped together. For example, the "Hispanic" group in the United States consists of individuals who have migrated from Mexico, South America, Cuba, the U.S. territory of Puerto Rico, as well as those born in the United States. Further, even though the vast majority (64–65 percent) of all Hispanics in the United States are Mexican Americans, there may be differences among this group. As seen in Figure 4-1, three distinct Mexican American populations have migrated to the United States. Although they are all grouped as Mexican Americans, they will have some differences in diet and exposure. What this suggests is that the outcomes of a study of Hispanics in Texas may differ from those of Hispanics in California.

As we address issues in cancer, "we need to remember that one size does not fit all," said Jones. Within special populations, vulnerable groups, or ethnic minorities, we have to remember that not all members are the same polymorphically and that children are not small adults.

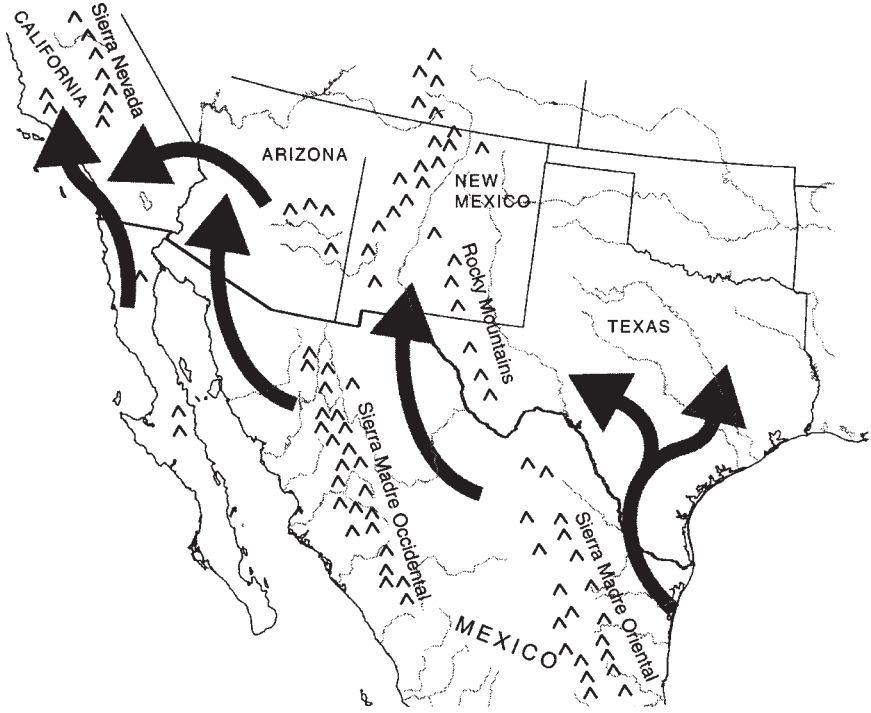

FIGURE 4-1 Geographical influence on migration. Migration patterns from Mexico show three distinct Mexican American populations that have migrated to the United States. SOURCE: L. Jones unpublished. Reprinted with permission.

CANCER DISPARITIES IN APPALACHIA

The National Cancer Institute (NCI) has stated that it considers rural residents to constitute a vulnerable population because rural Americans tend to be older, poorer, less educated, and more likely to be uninsured than their urban counterparts. In addition, rural communities have higher rates of chronic illness and disability and report poorer overall health status than urban communities. Residents in rural areas generally have less contact and fewer visits with physicians and lower levels of preventive care. In addition to factors related to rural health status and practices, there are systemic factors related to rural life in general—for example, lack of public transportation and lower levels of other community services—that may also contribute to less-than-optimal cancer control.

All of these factors are evident in the largely rural and predominantly white population of Appalachia, particularly in the Central Highlands, said Gilbert Friedell of the Markey Cancer Control Program in Lexington, Kentucky. Age-

TABLE 4-1 Age-Adjusted Cancer Incidence Rates (per 100,000 population), 1995–1997

	Kentucky			
Site	Overall	White	Black	Appalachia[a]
Lung	85.03	84.35	97.18	98.25
Invasive cervix	10.99	10.82	13.76	13.34

[a]Big Sandy, Kentucky River, and Cumberland Area Development Districts.

SOURCE: Friedell et al. (1999). Reprinted with permission (Kentucky Cancer Registry).

adjusted cancer mortality rates for Appalachia are higher than those in the rest of the United States. Lung cancer is a leading cause of male cancer deaths in central Appalachia—with the highest incidence in Appalachian Kentucky, the geographic area with the highest rate of cigarette smoking in the state.

The incidence of invasive cervical cancer and lung cancer in eastern Kentucky is higher than the incidence of these cancers in the overall Kentucky population (see Table 4-1). It is, however, quite similar to the incidence of lung cancer and cervical cancer in the predominantly urban, African American population of Kentucky. Poverty is a common characteristic of these two groups, said Friedell. Some of the counties in Appalachian Kentucky, for example, are among the poorest in the country and have the lowest levels of literacy. The use of race and ethnicity as surrogates for poverty has obscured the fact that the problems related to cancer in the poor white population are comparable in many ways to those seen in recognizable minority populations, added Friedell.

The use of race and ethnicity as surrogates for poverty has obscured the fact that the problems related to cancer in the poor white population are comparable in many ways to those seen in recognizable minority populations.

Gilbert Friedell

Individuals living in poverty often do not receive quality health care, including cancer prevention, diagnosis, treatment, and appropriate follow-up care because services are not available, accessible, or utilized. In addition, certain behavioral risk factors, such as tobacco use, poor nutrition, obesity, and underutilization of cancer screening examinations, are more prevalent in impoverished populations. Friedell pointed out that "the social environment in which poor people live prevents the development of healthy behaviors." However, until cancer surveillance incorporates socioeconomic status, the relationship between poverty and cancer in population groups will be difficult to sort out, said Friedell.

Some barriers to increased participation in cancer control programs exist at all socioeconomic levels, for example, lack of information about cancer and about the availability and the benefit of cancer screening. Other barriers, such as feelings of isolation and low literacy, are more prevalent in low-income, medically underserved populations such as those in Appalachia, said Friedell. For example, the average Kentucky high school completion rate was 65 percent, while the rate in Appalachian Kentucky counties was 55 percent. This barrier needs to be recognized by physicians and other health care personnel.

The NCI, recognizing the cancer control problems in Appalachia, has funded the Appalachia Leadership Initiative on Cancer for seven years as well as individual research projects in the region. Much of this effort has been aimed at the community level. Lessons drawn from this program indicate that enhancing cancer control efforts at the community level is possible, but it is labor intensive and requires commitment at the state and national levels and recognition that ongoing support will be necessary, concluded Friedell.

MIGRANT FARMWORKERS' CHILDREN AND PESTICIDES: A HIGH-RISK POPULATION

There are approximately 3 million to 6 million migrant and/or seasonal farmworkers (MSFs) in the United States. Approximately 85 to 90 percent are from ethnic and racial minorities, including Hispanics, African Americans, and Caribbean islanders. However, Hispanics of Mexican descent constitute the majority of this population, with children and adolescents comprising 20 to 25 percent of the total population. MSF children are chronically exposed to pesticides because of their parents' occupation. This exposure is of great concern given the vulnerability of children as determined by their body size and continuous development, which can increase the carcinogenic effects of these chemicals, according to María A. Hernández-Valero, M.D. Anderson Cancer Center.

There are several pathways by which these children can be exposed to agrochemicals at a very early age, including application drift; overspray; carry-home exposures from parents; exposure in utero; breast-feeding; going or working in the fields with their parents; and the foods they eat. Being chronically exposed to pesticides, some of which are known endocrine disrupters, may place MSF children who may also be genetically susceptible to these chemical substances at a higher risk of developing ill-health effects, including cancer. Studies of nonmigrant and agricultural workers also suggest that exposure to pesticides is associated with an increased risk of fetal death, miscarriages, developmental defects, and central nervous system disorders.

Yet cancer research among these workers and their families is almost nonexistent, which is attributed to the perceived difficulty in conducting epidemiologic studies among this underrepresented population.

Because organochlorine pesticides remain in the environment for many years

and MSF children are constantly exposed through many pathways, there is the need to monitor this high-risk population, said Hernández-Valero, and to consider options for reducing its pesticide exposures. Hernández-Valero recommends that these children be included in prospective cohort studies that are going on nationwide and that their exposure to pesticides be monitored to determine if chronic exposure at an early age will place them at a higher risk of developing deleterious health outcomes, including cancer, during childhood and later in life.

CHEMICALS AND CANCER CLUSTERS

"Cancer clusters are the bane of the existence of state and local health officials," said Richard Jackson, Centers for Disease Control and Prevention, "yet there are real opportunities embedded in cancer clusters to meet public health needs, and they can actually end up with good outcomes if you use good communication skills, good science, good medicine, and bring good policy to all of this."

> In the public's mind, clusters are environmental until proven otherwise.
>
> *Richard Jackson*

Jackson described his experience with pesticide use in Kern County, California, where agricultural chemicals have caused broad environmental contamination of wells with dibromochloro-propane, a known carcinogen that also causes sterility in males. Fifteen years ago, said Jackson, there was no way to deal with the public's concerns about these chemicals. There were no cancer or birth defects registries in the Central Valley of California. In addition, there was no obligatory reporting of pesticide use unless it was Category One (extremely toxic), and there was no record-keeping on these chemicals. Thus, it was very difficult to do investigations in this area. When the data gaps on these chemicals were filled, it turned out that an important percentage of them contained reproductive toxins and teratogens, as well as chemicals that cause skin toxicity. Many of these "grandfathered" chemicals were not tested before being put in the field, and as a result, unusual illnesses occurred. Some children with birth defects that resembled those caused by thalidomide were born to women who had been exposed in the fields. Migrant workers are particularly vulnerable, said Jackson, because in many cases they are undocumented workers, are poorly educated, and do not speak English. They are loathe to complain and unwilling to cooperate with investigations for fear of deportation. This highlights the need for public health investigators to learn how to communicate with communities and anticipate their concerns. "Thinking that you can deal with clusters and community problems without an on-site community person is a mistake," said Jackson. "Disease clusters are socially inexorable. You have to pay attention to them. You have to respond to people's concerns. Clusters are extremely power-

ful socially and they are extremely powerful politically." In the public's mind, added Jackson, clusters are environmental until proven otherwise.

As a result of an aggressive public health response and effective health advisory committees, there are now cancer and birth defects registries in the Central Valley of California. In addition, there is full record-keeping of all pesticide use in California in all toxicity categories. The challenge, said Jackson, is to actually document people's precise level of exposures so as to more accurately calibrate risks. This has to be considered in view of genetic variations in response to exposures. Dealing with cancer clusters with open dialogue and aggressive diligence can convert them from dreaded inevitabilities into genuine public health opportunities.

CANCER IN CHILDREN

Observations during the past several decades have identified a modest but consistent increase in the incidence of childhood cancers. Secular trends have varied with specific diagnostic categories, but the most consistent increases have been seen in acute leukemia and in tumors of the central nervous system, said Leslie Robison, University of Minnesota. An ecologic association has been noted between increases in brain and central nervous system (CNS) tumors and increased utilization of imaging techniques, suggesting that earlier detection may account for some of the observed increases.

Childhood cancer represents a relatively rare disease entity. In the general population, cancers in children under the age of 15 years are less than 2 percent of all the cancer burden in the United States. This is a small but important proportion, not only from what it can tell us scientifically but, more importantly, in terms of the number of years of potential life that are at stake, said Robison. In the United States, approximately 8,000 individuals under age 15 are diagnosed with cancer each year. The cumulative probability of a child developing cancer is approximately 1 in 630 before the age of 15 and 1 in 300 before the age of 20.

Distinct age-specific patterns of incidence occur among specific diagnostic classifications within the pediatric and adolescent age groups. There is a peak in incidence of acute lymphoblastic leukemia between the ages of 3 and 6 years; neuroblastoma, retinoblastoma, and Wilms' tumor aggregate in children less than 5 years old; lymphoma incidence rises with increasing age. Overall, males have a higher rate of malignancies than females, which is attributable primarily to a higher incidence of lymphomas and acute lymphoblastic leukemia among males. In the 15–20-year age group, females have a higher incidence of cancer than males.

> It is estimated that approximately 1 in every 900 individuals between the ages of 15 and 45 is now a survivor of childhood or adolescent cancer.
>
> *Leslie Robison*

The survival rate for childhood and adolescent cancer has increased dramatically during the past three decades. Currently, more than 70 percent of individuals diagnosed with cancer before age 15 will survive five or more years from diagnosis, with the majority being cured of their original malignancy. With these improvements in treatment and survival, it is estimated that approximately 1 in every 900 individuals between the ages of 15 and 45 is now a survivor of childhood or adolescent cancer. These survivors are, however, at increased risk for long-term complications of their initial cancer and subsequent therapy. Late sequelae of childhood cancer can include an increased risk of second and subsequent malignancies, as well as serious organ dysfunction and psychosocial effects. As more patients survive and the length of follow-up grows, patterns of second and subsequent malignancies are being identified in survivors, including increased rates of breast cancer, thyroid malignancies, CNS tumors, and leukemia.

Robison is interested in research on the long-term outcomes of these cancer survivors. Typically, studies of long-term outcomes and risk of second malignancies focus on the modalities used in the successful treatment of a patient. "Most patients receive multi-modality treatments, and we need to look at the interaction of these treatments as well as genetic effects," said Robison. The potential interaction between these treatments and the underlying genetics is of key importance and a high priority for study. Robison and others are piloting the creation of a national registry of children with cancer to identify environmental and other causes of cancer.

CHILDHOOD CANCER AND DIET

Diet can be considered part of the environment from several perspectives. All foods—including fish, fowl, meat, grains, vegetables, and fruits—may contain traces of contaminants such as pesticides used in food production and pollutants such as heavy metals and polychlorinated biphenyls. In addition, the nutrient composition of foods can vary with how and where the food is produced. Additives may be part of processing food.

Fewer than 20 studies have focused on diet in relation to childhood cancer, perhaps because scientists have thought it unlikely to play a role. Adult cancers that are most strongly linked with diet rarely occur in children and have latency periods of several decades. Limited research on this topic, however, suggests that diet may indeed affect risk, at least of some childhood cancers, said Greta Bunin, Children's Hospital of Philadelphia.

The childhood cancer studied the most in relation to diet is brain cancer, said Bunin. According to one hypothesis, children with greater exposure to *N*-nitroso compounds (NOCs) and their precursors are more likely to develop a brain tumor compared to other children. In many species of animals, NOCs are highly potent carcinogens, inducing nervous system tumors. For a few NOCs,

the risk is multiplied when the exposure occurs in utero. The fetus, of course, is growing rapidly and, for that reason, is likely to be more susceptible to some carcinogens. It is also possible that a mother's or father's diet before the child's conception could play a role, presumably by changes in the DNA of the sperm or the egg that would then lead to increased risk.

Human exposure to NOCs is widespread; these compounds have been detected in many common exposures and products, including cigarette smoke, automobile interiors, and cosmetics. In addition to being exposed to NOCs, humans are also exposed to precursors that combine to form NOCs in the gut and elsewhere in the body. In fact, most human exposure is thought to occur via synthesis in the body from precursors. Some substances, such as vitamins C and E, inhibit the formation of NOCs from precursors and protect animals from developing NOC-induced tumors.

Diet is a major source of NOCs, NOC precursors, and NOC inhibitors. Meats cured with nitrite, such as hot dogs and luncheon meat, contain NOCs and NOC precursors. Fruit, vegetables, and vitamin supplements contain NOC inhibitors. The NOC hypothesis predicts that a mother's frequent eating of cured meats and infrequent eating of fruits and vegetables during pregnancy would increase the risk of brain tumors in her children. Studies done to date generally support the hypothesis that frequent eating of cured meats during pregnancy increases the risk of such tumors and provide limited support for a protective effect of fruits and vegetables. However, individuals who eat a lot of cured meats might also have a diet high in fat, and that high-fat diet—rather than cured meats—could be responsible for the increased risk. Similarly, eaters of cured meats may have diets low in folate, which could increase the risk.

The most common childhood cancer, leukemia, has not been well studied in relation to diet, said Bunin. Although NOCs have not been linked to leukemia in animals, because of their potency as carcinogens in general and the ability of some of them to act transplacentally, a few studies have looked at foods with NOCs in relation to leukemia.

A hypothesis has been proposed regarding the development of leukemia in the first year of life. In a majority of infant leukemias, the leukemic cells have abnormalities in band q23 of chromosome 11. Leukemias that occur after cancer treatment with epipodophyllotoxins, a class of chemotherapeutic agents, also have 11q23 abnormalities. These chemotherapy drugs inhibit an enzyme called topoisomerase II (Topo II) and increase the risk of leukemias with 11q23 abnormalities. If epipodophyllotoxins inhibit Topo II and increase the risk of leukemias with 11q23 abnormalities, perhaps other inhibitors of this enzyme also increase the risk of the same leukemias. Other inhibitors of Topo II exist in nature and include certain flavonoids and medications.

Some investigators have postulated that maternal exposure to Topo II inhibitors during pregnancy increases the risk of leukemias with 11q23 abnormalities in infants. In a preliminary study, said Bunin, no association between foods

containing these inhibitors and infant leukemia overall was observed. However, when the two subgroups of infant leukemia—acute lymphocytic leukemia and acute myeloid leukemia—were analyzed separately, strong and significant associations were seen for the myeloid leukemia but not for the lymphocytic leukemia.

Research linking diet and childhood cancers has been limited to brain tumors and leukemia. For most other childhood cancers, no studies have investigated the role of diet, said Bunin; thus, additional research on other childhood cancers may detect new risk factors.

SUMMARY

Years of research have resulted in a number of advances in the prevention and treatment of cancer, leading to an increase in survivorship across many cancers. While these results are promising, some researchers and community leaders have questioned whether the results are universal for all areas of our populations. Special populations such as migrant farmworkers, children, immigrants, and ethnic groups will have to be included in future research to ensure that they are also able to benefit from the new advances in research. Further understanding of environmental exposures in various subgroups through longitudinal studies will be necessary to more carefully identify risk in these groups, according to some participants.

5

Gene–Environment Interaction in Site-Specific Cancers

Through many years of fundamental research, we have begun to have a better understanding of cancer. Research underlying the basic phenomenon has been hampered by the fact that "cancer" is not a singular disease, but rather a closely linked group of molecular disorders. These disorders vary in their etiology and mechanisms but have some common intersections. By understanding the differences and similarities in cancers from various body sites (e.g., breast, prostate), we can continue to make advances in cancer research.

The role of the environment has been actively investigated in many site-specific cancers during the last century. Through the use of epidemiology, the advent of molecular biology, and advances in computer technologies, investigators are now able to answer more sophisticated questions than would have been possible 30 years ago. Large cohort studies are now able to be conducted to answer questions on a population level and also to probe research within a subgroup of individuals. This chapter covers some of the recent advances in understanding the relationships between genes and the environment in site-specific cancers, including breast, lung, colorectal, and prostate cancer, as described by various presenters.

BREAST CANCER

It is estimated that 184,200 women in the United States will be diagnosed with breast cancer this year. This is reflected in the high incidence rates observed for many racial–ethnic groups, both internationally and nationally. In the past 15–20 years, researchers have shed light on the etiology and risk factors involved in breast cancer, including reproductive hormones, genetic factors, and environmental factors. By exploring these complex interactions, researchers may be able to develop additional strategies to reduce the incident rate of breast cancer.

Reproductive Hormones

> A very important and reliable predictor of breast cancer risk is the amount of estradiol in the blood of both pre- and postmenopausal women
>
> *Brian Henderson*

During the 1990s, a series of landmark prospective epidemiologic studies were published showing that a very important and reliable predictor of breast cancer risk is the amount of circulating estradiol in the blood of both pre- and postmenopausal women. In fact, this is the best single predictor of risk, said Brian Henderson, University of Southern California. How this risk factor accounts for increased risk of breast cancer is a subject of intense research.

Henderson described a long-standing focus on the role of sex steroids in the etiology of breast cancer, especially related to stimulation of the breast by estrogen and, more recently, progestin. This focus has been driven by the premise that estrogen and progestin are the primary determinants of cell proliferation in breast epithelium and that cell proliferation is a prerequisite for many of the genetic changes necessary for cell transformation to a malignant phenotype. The strong and consistent association between a woman's menstrual history and breast cancer risk implicates lifetime exposure to sex steroid hormones as a major factor in the causation of breast cancer.

Recent epidemiologic studies have implicated estrogens more directly, by showing that circulating levels of the biologically most potent estrogen, estradiol (E_2), are significantly higher in breast cancer patients compared to normal controls. Moreover, said Henderson, plasma estrogen levels differ by racial–ethnic group and these differences appear to contribute to racial–ethnic variation in breast cancer rates. In addition, exogenous exposure to these steroids, as combined estrogen and progestin replacement therapy, also substantially increases the risk of breast cancer.

In addition to the level of circulating hormones, the role of hormone replacement therapy and the age at menarche and fecundity also have a relationship to breast cancer. For example, in the late nineteenth century in Europe and the United States, there was a pattern of a late age at menarche, a short period of fecundity, and an early onset of menopause. By the early twentieth century, the age of menarche had decreased and the length of fecundity increased, not only in the West but elsewhere in the world, including post-World War II Asia. This has coincided with a dramatic increase in breast cancer rates over the last 50 years. The use of multiethnic cohort studies will begin to address the complex interplay between genetic and environmental risk factors according to Henderson.

To further understand these risk factors, Henderson and others have turned to genetics. The process of breast cancer is driven by ovarian steroid hormones, mainly estradiol and progesterone, and these result from a biosynthetic pathway that involves a series of enzymes encoded by genes (see Figure 5-1). It is in these

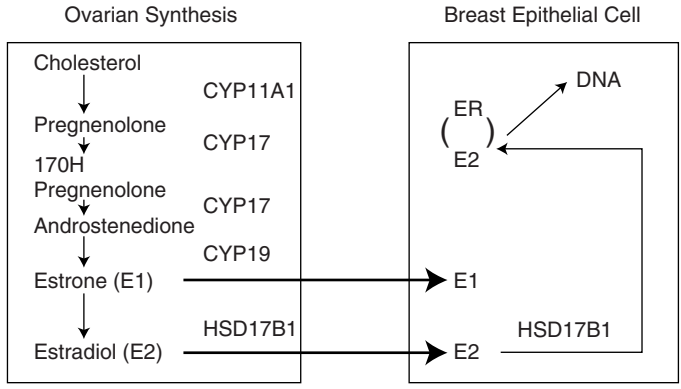

FIGURE 5-1 Estrogen biosynthesis pathway. SOURCE: B. Henderson (2001). Reprinted with permission.

genes that scientists have been looking for genetic variation that might explain differences in the amount of circulating estradiol, which can vary substantially from one woman to the next. This sort of variation is certainly consistent with the multigene model, said Henderson, so investigators have tried to express the risk of breast cancer—most easily represented by cumulative exposure to endogenous estrogen and progesterone—in terms of multiple susceptibility genes, contributing to build a multigenic model that separates women into high- and low-risk groups. By coupling epidemiologic research with other genetic studies, we will begin to build models of breast cancer susceptibility.

Breast Cancer Genetics

Breast and ovarian cancer appear to be coinherited in families. Breast cancer is thought to be caused by spontaneous mutations in somatic cells or by germline inheritance of mutations in breast cancer susceptibility genes. Approximately 30 percent of women who present with a diagnosis of breast cancer have at least one relative who has had cancer.

One such gene, the BRCA1 gene, was identified in 1990 by Mary-Claire King and her group and then cloned in 1994. BRCA2 was identified and cloned in 1995. Germline mutations in the BRCA1 or BRCA2 susceptibility genes result in breast cancers characterized by young age of onset, bilaterality, association with ovarian cancer and other tumor types, vertical transmission, and distinct tumor phenotypes. Many investigators have tried, so far unsuccessfully, to explain the functions of BRCA1 and BRCA2 and why they might be relevant to breast cancer. They probably function to maintain genomic stability and serve as tumor suppressor genes. However, there are multiple roles that these genes play

in the cell specifically (e.g., scaffold protein involved in oxidative DNA repair), and it is not clear why they are so highly penetrant for breast and ovarian cancer, said Olufunmilayo Olopade, of the University of Chicago.

Testing for the presence of BRCA1 and 2 mutations is now offered to women who have a family history of breast and ovarian cancer and are over the age of 18. Specific mutations have been identified in Caucasian and African American families, and some families have unique mutations, seen only in their family lineage. In some cases, the significance of mutations is hard to discern, so testing is not always clinically useful, said Olopade. What is known is that in families with BRCA1 mutations, the average age of cancer onset tends to be very young. With BRCA2, the mean age of onset is a little older than with BRCA1, and in one study by Olopade, there was a significant proportion of postmenopausal breast cancer that could be attributed to germline mutations in BRCA2.

Not everyone who inherits a mutation in the BRCA genes develops cancer. There are, in fact, other genetic and environmental factors that affect penetrance, and some of these factors may be modifying genes that are inherited by different populations at different rates. For example, hormonal reproductive factors and response to DNA damage affect risks, and some studies actually indicate that smoking reduces breast cancer risk in BRCA carriers. This does not suggest that these women should smoke, said Olopade, but it does suggest that there are issues of carcinogen metabolism and other enzyme activity that have to be considered.

Olopade noted that there is a clear distinction between BRCA1 and BRCA2 tumors. BRCA2 tumors tend to be estrogen receptor (ER) positive, whereas BRCA1 tumors are mostly ER negative and highly proliferative. This may have implications for prevention, said Olopade, because antiestrogen therapy with tamoxifen can reduce the incidence of ER-positive breast cancer. "If you can get a 50 percent reduction in breast cancer risk through hormonal ablation or through tamoxifen use, then you can imagine how you might be able to prevent some of these BRCA2 tumors," said Olopade. A lot of work needs to be done in terms of understanding how environmental factors influence the development of cancer and then how these individuals respond to different therapies, she concluded. These examples illustrate the clinical value of addressing the genetic basis of cancer and the importance of understanding genetic mechanisms in developing methods of cancer prevention, early detection, and targeted therapies.

Other genetic factors have been examined in addition to BRCA1 and 2. For example, several years ago, Kari Hemminki described a variant of the gene for cytochrome P-450, a key enzyme in the initial stages of sex steroid biosynthesis. He suggested that the C allele upregulated the gene and, therefore, would lead to more estrogen production. Subsequent studies showed that this allele appears to be associated with an increased risk of breast cancer, particularly with more regional or distant metastatic breast cancer than disease limited to the breast. This led to a hypothesis linking a genetic basis for greater lifetime estradiol

secretion with a higher risk of breast cancer. Henderson noted that more research is needed that looks at other parts of genetic pathways, for example, growth factor genes, hormone receptors, and other similar enzymes along the same pathways of biosynthesis or metabolism.

Environmental Factors

There has long been an assertion by the general population that environmental factors play a role in the generation of breast cancer. This has been fueled in part by the observation that established risk factors for breast cancer do not fully explain breast cancer risk. During the workshop, this was one of the more controversial topics discussed by researchers. According to Mary Wolff of the Mt. Sinai School of Medicine, researchers have been investigating issues surrounding lifestyle (including diet), obesity, and adverse exposures in an attempt to identify suceptible factors in initiating breast cancer. Unlike the senario for lung cancer where there is strong agreement between researchers that smoking is a risk factor for developing cancer, the role of environmental factors in breast cancer is not as clear.

Is there a role for the environment in breast cancer? The research is incomplete at this time according to many researchers. Investigations into pesticides such as DDT and DDE have not shown a consistent association with breast cancer (Snedeker, 2001). Diet and obesity may play a role in breast cancer development since they both contribute to changes in circulating hormone levels and age at menarche—all known risk factors for breast cancer. Further, one sees a change in incidence rates as ethnic groups shift to a more Western lifestyle (e.g., decrease in age of menarche, use of hormone replacement therapy)—for example, the increase in the incident rate of cancer in Asian women born in the United States compared to those living in Asia. Understanding the complex interactions of lifestyle, diet, established risk factors, and genetics will continue to be an important area of research.

LUNG CANCER

Molecular and Environmental Bases of Lung Cancer

There are approximately 160,000 new cases of lung cancer every year in the United States, and at least 80 percent of those people will die within five years of diagnosis. This results in more deaths from lung cancer in both men and women than from any other specific cancer. There are about 47 million current smokers and 44 million former smokers in the United States. Both groups are at increased risk of developing lung cancer, although current smokers have the higher risk.

Less than 20 percent of long-term smokers develop lung cancer by age 75, and we must develop ways to identify these people at an early stage of cancer

development to improve the cure rate, said John Minna. One possibility may be by genetic epidemiology. Genetically determined factors that abrogate the effects of environmental carcinogens may explain differences in susceptibility, said Margaret Spitz, of the M.D. Anderson Cancer Center. The challenge in risk assessment is to account for this interindividual variation in susceptibility to carcinogens. Evidence of familial aggregation of lung cancer provides indirect support for the role of a genetic predisposition to lung cancer. These studies of patterns of inheritance suggest that a small proportion of lung cancer is due to "lung cancer genes" that are probably of low frequency but high penetrance.

> Less than 20 percent of long-term smokers develop lung cancer by age 75.
>
> *John Minna*

Lung cancer risk from smoking is dependent on the dose of tobacco carcinogens, which is modulated by genetic polymorphisms in the enzymes responsible for carcinogen activation and detoxification, as well as by the efficiency of the host cells in monitoring and repairing DNA damage due to tobacco carcinogens. Individuals with susceptible genotypes (or adverse phenotypes) tend to develop lung cancer at earlier ages and with lower levels of tobacco exposure. On the other hand, the genetic component of risk tends to be lower at high-dose levels, when environmental influences overpower any genetic resistance.

By studying polymorphisms in DNA repair genes, Spitz and others are trying to establish genotype–phenotype correlations in the context of environmental insults. For example, by correlating polymorphisms in a DNA repair gene with the functional DNA repair assay, one can determine if markers in surrogate tissues reflect molecular events in the target lung tissue. Genotype–phenotype and diet–gene interactions are also being actively studied.

The gene that controls glutathione *S*-transferase activity, which is a protective detoxifying mechanism, is being studied intensively. The genotypes for these protective genes differ among various ethnic and racial groups. In general "null genotypes," those with little to no activity, have been shown to be associated with increased risk, but results have varied. To explain the inconsistencies in the results, Spitz and others have tried to estimate the significance of other unmeasured or unidentified covariates. They examined factors in the diet, specifically isothiocyanates, which are nonnutritive compounds found in the Brassica family of vegetables such as brussels sprouts, broccoli, and cabbage. These compounds are known to be very effective inhibitors of tumor formation in animal model systems, and many case-control and cohort studies have consistently shown an association between consumption of greater amounts of these vegetables and protection against the development of lung cancer.

Other studies have used "reporter genes" to measure the extent of DNA repair in cells transfected with carcinogen-damaged plasmids. "We know that DNA repair capacity declines with advancing age, however, the youngest cases

seem to have the poorest DNA repair capacity," said Spitz. Women have significantly poorer repair capacity than men, and the longest-term smokers have the best DNA repair capacity, perhaps as an adaptation to long-term smoking.

Many of the markers are relevant not only for risk assessment but also for predicting response to chemo- and radiation therapy. Thus, individuals on chemotherapy regimens who have good DNA repair capacity actually do worse because they are more likely to remove the therapeutic agent that causes damage to cancer cells.

> We know that DNA repair capacity declines with advancing age, however, the youngest cases seem to have the poorest DNA repair capacity.
>
> *Margaret Spitz*

The true dimensions of gene–environment interactions probably depend on multiple susceptibility factors, concluded Spitz. In the near future, micro-array technology will enable the performance of large-scale, low-cost genotyping. The resulting ethical, educational, social, and informatics considerations will be challenging. However, the ability to identify smokers with the highest risks of developing cancer will have major preventive implications for intensive screening and smoking cessation interventions and for chemoprevention trials.

Molecular Pathogenesis of Lung Cancer

John Minna of the University of Texas Southwestern Medical Center and other investigators have hypothesized that clinically evident lung cancers have accumulated 10–20 different genetic abnormalities in dominant oncogenes, or tumor suppressor genes. If this is true, it may be possible to discover carcinogen-exposed respiratory epithelial cells with only a subset of these changes and to intervene at an early stage with treatment or chemoprevention. Similarly, it is also hypothesized that these changes are recurrent and common among different tumors, and this may have implications for directing the search for specific diagnostic and therapeutic targets. Many studies have been published on the search for genetic abnormalities in lung cancer. However, with few exceptions, these studies have not been global in nature, either in testing for genome-wide abnormalities or in testing for multiple abnormalities in the same individual lung cancer.

There are four major histologic types of lung cancer, and there are acquired genetic differences among these types. The general mechanisms that underlie their pathogenesis are similar; thus, it is important to identify the molecular changes that lead to these cancers. If 20 different changes are required for a lung cancer to develop, smoking-damaged respiratory epithelial cells could in theory be detected with only a few changes, allowing an early molecular diagnosis of lung cancer or prediction of which people are most likely to develop it.

Allelotyping—in this instance, comparing tumor and normal tissue for genetic change involving loss of one of the parental alleles—is one way to look for genetic changes. Minna and colleagues find that multiple small clonal or subclonal patches containing molecular abnormalities are present in histologically normal or slightly abnormal bronchial epithelium of patients with lung cancer and people who smoke cigarettes. In detailed studies of bronchial epithelium and bronchial biopsies from current or former smokers without lung cancer, they also found thousands of clonal patches showing allele loss in histologically normal-appearing respiratory epithelium. These patches can be detected more than 30 years after cessation of cigarette smoking, suggesting the potential for damaged stem cells to repopulate.

Small cell lung cancer (SCLC) has many morphological and biochemical features that distinguish it from non-small-cell lung cancer (NSCLC) histologic types, said Minna. These distinctions are of diagnostic importance and commit patients with different histologic types to different initial treatment regimens. With the exception of bronchoalveolar lung cancer, smoking and tobacco carcinogens are the major underlying etiologic factors. Clearly, SCLC etiology is strongly tied to cigarette smoking. Thus, the smoking-damaged, histologically normal epithelium associated with SCLC appears "genetically scrambled" and has incurred significantly more damage than the epithelium accompanying NSCLC. Minna concluded that SCLC and NSCLC do not differ significantly in the number of their genetic alterations. However, they do differ in the kinds of specific genetic alterations that occur. In addition, the smoking-damaged bronchial epithelium accompanying SCLCs appears to have undergone significantly more acquired genetic damage than that seen in NSCLCs. Minna called for studies to identify the specific genes involved at these multiple sites and to determine whether these provide new tools for early molecular detection, monitoring of chemoprevention efforts, and identification of specific targets for developing new therapies.

COLORECTAL CANCER

Risks for Colorectal Cancer

Colorectal cancer remains the third leading cause of cancer deaths in each sex and the second leading cause overall in the United States. Despite the fact that this disease is often preventable by screening, there are 130,000 new cases and about 55,000 deaths each year. Colorectal cancers arise primarily in adenomas. Because adenomas are usually asymptomatic, they are not readily detected. The prevalence of these lesions increases with age and is greater in men than women. Autopsy studies suggest that one-fifth to three-fifths of individuals have prevalent adenomas, and screening studies of average-risk populations have found that one-fourth to two-fifths of individuals have adenomas.

An important environmental role in the development of colorectal cancer is suggested by the variance in incidence of the disease. Moreover, there is a twentyfold variation in incidence rates in different geographic regions around the country, indicating that genetic, environmental, and lifestyle factors play a role in etiology. Because adenomatous polyps are precursors to colorectal cancer, assessing the effect of environmental and genetic factors in adenoma occurrence and recurrence might help identify relatively asymptomatic individuals who are at increased risk of cancer and who would benefit most from public health interventions.

Certain populations and individuals with particular genetic syndromes inherit germline mutations that increase the risk of colorectal cancer. The genetic basis for the development of colorectal cancer involves the accumulation of specific somatic mutations in proto-oncogenes and tumor suppressor genes with increasing age, said Raymond DuBois of the Vanderbilt University Medical Center (Figure 5-2). However, only a small proportion of colorectal cancers are attributable to inheritance of these rare, highly penetrant mutated genes.

It is also evident that variability in carcinogen-metabolizing genes influences the risk of colorectal cancer. These polymorphisms can be very common, such that even modestly increased relative risks may account for a higher popu-

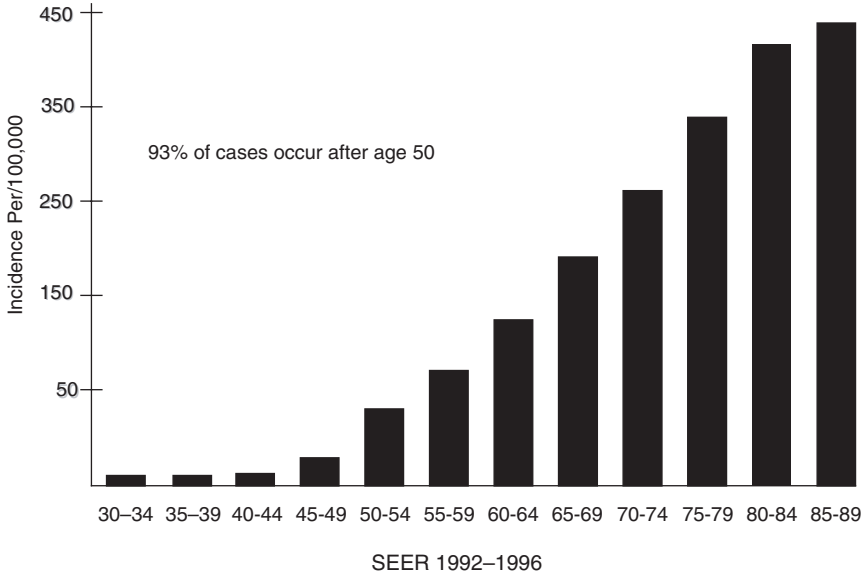

FIGURE 5-2 Incidence of colorectal cancer versus age. SOURCE: SEER (1992–1996). The SEER program collects and publishes cancer incidence and survival data from 11 population-based cancer registries and 3 supplemental registries covering approximately 14 percent of the U.S. population (see http://seer.cancer.gov for more information).

lation-attributable risk than that due to highly penetrant, but rare, genetic mutations. Epigenetic changes, such as alterations in DNA methylation and gene expression, also may play a critical role in the development of this malignancy. These alterations are important in both inherited syndromes such as familial adenomatous polyposis or hereditary nonpolyposis colorectal cancer and in sporadic tumors. It will be important to understand the roles of environmental exposure and host susceptibility to develop better screening, prevention, and treatment strategies, said David Alberts of the Arizona Cancer Center.

Susceptibility to colorectal cancer is related to interindividual variability in biotransformation of endogenous and exogenous substances, as well as in DNA repair and cell cycle control, according to Alberts. Genetic variation may increase susceptibility by altering the rates of activation and detoxification of carcinogens. In the future, risk assessment has to factor in susceptibility to certain classes of carcinogens in subpopulations. Interactions between specific polymorphisms in a metabolism gene and environmental exposures provide evidence that the gene substrate is a component relevant to colorectal cancer etiology or prevention. Environmental factors may interact with metabolic genetic polymorphisms via a model in which the exposure alone, but not the variant genotype alone, increases disease risk, and exposure interacts with the variant genotype to further increase risk in exposed individuals.

The same interaction can also modulate disease pathogenesis, in that exposure and susceptibility factors may alter the effects of other risk factors. Classification of subgroups of the population into those who may be more vulnerable to the effects of certain carcinogens may also have important implications beyond risk assessment. Through the identification of an increased risk in certain subgroups, disease risk factors may be better defined. However, to date, sample sizes for most studies attempting to uncover gene–environment interactions have been small, said Alberts, which limits the potential for detecting significant findings.

> Consumption of folic acid supplements, after a period of 15 or more years, may decrease risk of colon cancer by about 75 percent.
>
> *David Alberts*

Alcohol consumption and tobacco smoking are known to increase the risk of colorectal cancer. Other studies have found an increased risk of colorectal cancer recurrence with alcohol consumption.

Increased physical activity, dietary supplemental calcium intake, dietary iron intake, and hormone replacement therapy (for women) are all associated with a decreased risk of colorectal cancer. The role of a diet rich in fruits and vegetables in reducing this type of cancer is controversial. Further research will be needed to resolve this issue.

In addition, the chronic use of aspirin and other nonsteroidal anti-inflammatory drugs (NSAIDs) is associated with a 40–50 percent reduction in risk. Al-

though the pathways by which anti-inflammatories inhibit cancer growth are unknown, research efforts have focused on understanding the molecular basis for the chemoprotective effects associated with the use of NSAIDs. The activity of the enzymes cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) is inhibited by NSAIDs. Some researchers have reported an increase in COX-2 levels in a number of solid tumors, which suggests that this enzyme may serve as a molecular target for cancer prevention. Further, recent clinical studies indicate that the presence of COX-2 in human lung and colon cancers is associated with a negative clinical prognosis. Therefore, COX-2 inhibitors are presently being evaluated for the prevention or treatment of several cancers in humans, said Alberts.

Alberts also described an increasing body of epidemiological evidence from case-control and cohort studies supporting the role of folate in reducing the risk of colorectal cancer. Folate intake and blood levels have also been consistently associated with a lower risk of colon adenomas. Recent results indicate that the consumption of folic acid supplements, after a period of 15 or more years, may decrease the risk of colon cancer by about 75 percent. Additionally, these investigations suggest that alcohol consumption increases the risk of colorectal neoplasia by acting as a folate antagonist.

If colorectal cancer is treated surgically while still localized, the outcome is quite good, with a survival rate greater than 90 percent. Therefore, early detection could save about 28,000 lives each year, said DuBois. Development and characterization of accurate markers for adenomas are needed because this could identify the highest-risk group of patients that might benefit from early detection with colonoscopy and other screening interventions.

PROSTATE CANCER

Prostate Cancer: Epidemiology, Hormones, and Diet

Prostate cancer is the second leading cause of cancer deaths in men in the United States today. Currently, researchers have identified age as being one of the two most important risk factors for prostate cancer. Recent estimates by the National Cancer Institute suggest that one in four men has some cancerous cells in his prostate by age 50, which increases to one in two by age 80.

Race–ethicity is the second most important risk factor for prostate cancer. In the United States, African American men have the highest incidence of prostate cancer, while Asian Americans and Latinos have the lowest incident rates (Ross et al., 1998). Interestingly, one sees that the international rates for prostate cancer in men and breast cancer in women are remarkably similar and seem to be determined primarily by the environment in which one lives, said Donald Coffey of the Johns Hopkins School of Medicine (Figure 5-3). This risk can vary by more than tenfold among countries. Traditionally, native Japanese and Chinese

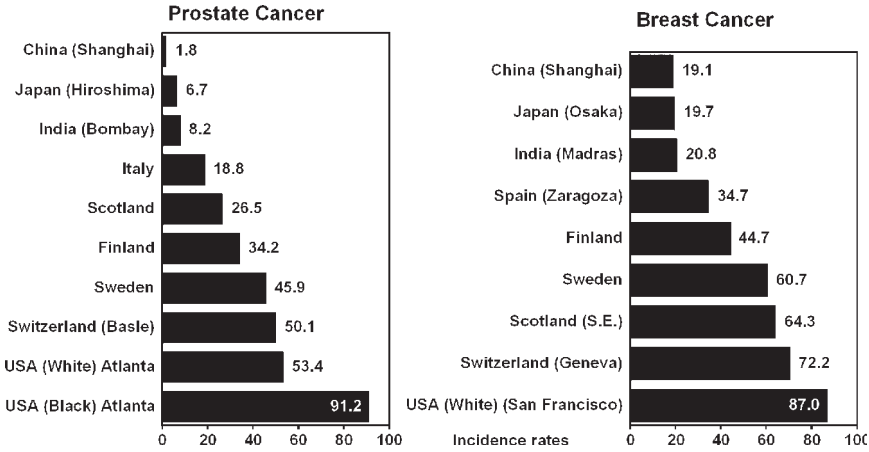

FIGURE 5-3 Comparison of prostate and breast cancer in different countries. SOURCE: Griffiths et al. (2001). Reprinted with permission by CompGraphics Services, UK.

men have the lowest incidence of prostate cancer. Similar to breast cancer, researchers have observed that migration can change a person's risk. For example, Asians who immigrate to the United States have a greater incidence of prostate cancer than those living in Asia. However, their rates never reach the incident rates of Caucasian Americans. The differences between various incidence rates may be due to testosterone biosynthesis or metabolism (see Ross et al., 1998), as well as environmental influences, genetic control, or a gene–environment interaction. Further research will help to us to understand these differences.

Hormones play a major role in the growth and function of the prostate gland including cellular growth and proliferation (Ross et al., 1998). For these reasons, hormones have attracted considerable research interest in cancer of the reproductive organs. Indeed, researchers have elucidated a role of hormones in both breast and prostate cancer. However, not all organs involved in reproduction have reported incidences of cancer. For example, there is little known risk for human seminal vesicle cancer. In addition, not all mammals have high rates of cancers. Only man and dog have been shown thus far to have a high naturally occuring incident rate of prostate cancer (Coffey, 1993). Some of the differences may be linked to the role of hormones in various organs. Androgen, estrogen, and progesterone receptors are present in prostate and in breast tissue, and cellular growth is driven by androgens or estrogens in either tissue, said Coffey. In the developing human, testosterone and androgen receptors inhibit breast development and induce the seminal vesicles. Studies in animal models have shown that hormonal treatment influences prostatic growth. Dihydrotestosterone and the androgen receptor induce prostate growth. Estrogens depress androgen production and depress prostate growth, whereas estrogens plus dihydrotestosterone enhance pros-

tate growth. Understanding and characterizing the differences between organs may help to understand the mechanisms underlying prostate cancer.

The question remains whether genetic or environmental factors may play a role in the development of prostate cancer. Research is still going on in this area; however, factors that alter the hormonal environment may have an effect on the incidence of cancer. Similar to breast cancer, dietary factors are hypothesized to influence prostate cancer. An association has been reported between prostate cancer and fat intake. Dietary fat is converted by the body to androgens, which may stimulate the growth of prostatic cancer cells. Men who eat high-fat diets have a higher rate of prostate cancer than those who adhere to diets low in fat and rich in yellow and green vegetables. Researchers are continuing to investigate the interplay between hormones, genetics, and the enviroment to develop a more complete picture of prostate cancer.

Herbal Therapies

Alternative medicine including the use of herbal therapies is an emerging area in the treatment of various diseases. Often, patients are looking for alternatives or complements to Western medicine. Some researchers have begun to investigate some of the herbal therapies to determine if there is a scientific backing for the claims. One such alternative, according to Robert DiPaola of the Cancer Institute of New Jersey, is PC-SPES, a commercially available combination of eight herbs used as a treatment for cancer of the prostate. Because other herbal medicines have estrogenic effects in vitro, DiPaola and colleagues tested the estrogenic activity of PC-SPES in yeast and mice and in men with prostate cancer. They assessed the clinical activity of PC-SPES in eight patients with hormone-sensitive prostate cancer by measuring serum prostate-specific antigen (PSA) and testosterone concentrations during and after treatment. In the men with prostate cancer, PC-SPES decreased serum testosterone concentrations, and in those and other patients, it decreased serum concentrations of PSA. The batches of PC-SPES studied contained estrogenic compounds that were distinct from diethylstilbestrol, estrone, and estradiol. Therefore, its use may confound the results of standard or experimental therapies or produce clinically significant adverse effects.

"The question remains, Why is it that mild phytoestrogens or mild estrogenic compounds within these particular plants might have a preventive possibility in terms of animal models but yet stronger estrogens would induce cancer?" said DiPaola. His laboratory has fractionated PC-SPES and found certain fractions with greater activity, suggesting that there are multiple estrogens in this product. Component herbs, such as licorice root, have weaker phytoestrogens. Since poor quality control exists in the manufacture of these unstandardized herbal mixtures, chemical analysis will be important to identify the chemical compounds responsible for this activity.

SUMMARY

The variation in cancer incidence among various population groups has long suggested a role for the environmnet in cancer development. The environmental hypothesis is further supported by shifting cancer incidences in migrant populations whose rates tend to approximate those of their current host country, by geographic differences in cancer rates within the United States, by the changing incidence of certain cancers over time, by ethnic–socioeconomic differentials, and by the epidemiologic evidence linking risks to a variety of lifestyle and other environmental exposures. Not long ago, the role of inherited susceptibility in human cancer was considered to be quite small. However, recent progress in identifying and characterizing highly penetrant, but relatively rare, susceptibility genes has furthered our understanding of genetic mechanisms and their role in cancer etiology. Further, the common polymorphisms of modifier genes that confer low relative and absolute risks, but high population-attributable risks in the presence of relevant environmental exposures, are becoming increasingly important to the public health burden of cancer.

Many speakers discussed the evidence suggesting a role for the environment, for genes, and for their interactions. Cancers such as breast, lung, and colorectal, which were thought to lack an inherited component in the past, are being linked to a number of rare but highly penetrant mutated genes. As many speakers asserted, understanding and characterizing these genes will be an important area of research for the next decade. They further emphasized the need to combine epidemiologic techniques with molecular biology. Cancer involves changes in DNA. We will have to determine which changes are germline and which are somatic and how environmental influences alter the mechanisms of DNA repair and replication.

Some conference participants identified several strategies for reducing the future incidence of and death from cancer, the most critical being the reduction of tobacco use by all segments of the population, since smoking causes an estimated 30 percent of all cancer deaths. Another strategy suggested by some speakers would be to increase the use of effective but currently underutilized cancer screening tools. Yet other strategies identified include developing and applying state-of-the-art diagnostic tests and treatments, as well as identifying and reducing health disparities across diverse populations. Behavioral change, perhaps the most challenging, but potentially the most effective strategy, should be a central element of a successful cancer prevention program regardless of genetic predisposition to cancer, said several speakers.

6

Special Address: A Novel Approach to Cancer Treatment Based on Immune Stimulation and Other Environmental Approaches

Steven Rosenberg

The history of cancer treatment has evolved over the years. Surgery, sometimes quite primitive, began more than 3,000 years ago. Just a year after the discovery of radiation therapy by Roentgen in 1895, a Chicago physician treated a patient with advanced breast cancer by exposing the chest wall to radiation and in fact saw tumor regression; thus began modern radiation therapy. Chemotherapy began about 60 years ago predominantly as a result of a 1942 laboratory accident involving the development of nerve gas that exposed workers to nitrogen mustard. It was noted that these individuals developed a lymphopenia and a decrease in circulating numbers of lymphocytes. This led a Yale physician, G.D. Lindskog, to use this material to treat a patient with what was then known as an "X-ray resistant lymphosarcoma," and he saw a dramatic regression of that lymphoma. This led to the realization that chemicals could be used to cause cancer regression, and that has led to a substantial amount of work in the last 60 years attempting to develop inorganic molecules that might be used for treating patients with a variety of malignancies.

Today, the appropriate application of surgery, radiation therapy, and chemotherapy can cure about half of all individuals who develop cancer. However, the half of cancer patients who cannot be cured by these methods accounted for almost 600,000 deaths in 2000, which points to the need for the development of new approaches to treat cancer. One of these approaches is biologic therapy, that is, treatments that act primarily through natural host defense mechanisms or by the administration of natural mammalian substances. In biologic therapies, we take advantage of the body's own natural defense mechanisms to reject the cancer.

An issue that has plagued the field of tumor immunology is whether it is possible to use the immune system to cause the regression of established human cancers. Historically, there has been a great deal of skepticism about this possi-

bility. However, studies of the cytokine interleukin-2, produced by human lymphocytes, reveal that it is a natural body substance that plays a predominant role in regulating the immune response in humans and other mammals.

Interleukin-2 has no direct impact on cancer cells. Cancer cells can be incubated in the highest achievable concentrations of interleukin-2 with no impact on the growth of the cells. All of the impact of interleukin-2 occurs because of its ability to mediate immune reactions in the body and alter the body's own natural defenses to attack the cancer. The National Cancer Institute has treated more than 400 metastatic melanoma and metastatic kidney cancer patients with interleukin-2 alone. About 15 percent of patients with metastatic melanoma show an objective regression, that is, at least 50 percent of the tumor disappears, and in half of those patients all of the cancer will disappear. In kidney cancer patients, about 20 percent show complete regressions. This then is the proof of principle that it is possible to use the body's immune system to cause cancer regression. If we can understand the mechanisms by which the body rejects these cancers, we might then be able to extend this to patients with other types of cancer.

Prior to these studies it was thought that large solid tumors could not be attacked by the immune system, but success in achieving complete regression of all metastatic disease in the lung and liver has been demonstrated. Bony metastases can also respond to this treatment.

What are the antigens involved in cancer regression? We identified a kind of cell, a tumor infiltrating lymphocyte (TIL), which invades tumors and is part of the body's reaction against these tumors. TILs obtained from patients with melanoma were then used to identify the genes encoding the antigens recognized by these TILs.

TILs have been identified that can recognize unique cancer antigens on murine and human cancers, including melanoma, breast cancer, colon cancer, and lymphoma. In clinical trials of TIL administration, 36 percent of patients with metastatic melanoma underwent objective cancer remission. To determine whether the identified genes actually encoded cancer regression antigens, patients were immunized with the gene products to determine whether the regression of growing cancers could be induced. Alternatively, in vitro lymphocytes sensitized against the putative cancer regression antigens were generated and adoptively transferred into patients to determine whether they could mediate cancer regression.

Through the use of a genetic technique that enables the expression cloning of genes encoding antigens recognized by CD4+ immune cells, we have been able to identify several new class II (MNC) restricted tumor antigens. Several of these are tissue-specific. It is thought that some of these tissue-specific proteins in tumors derived from nonessential organs can serve as the targets for immunotherapy.

Cancer antigens can arise from a variety of different cellular events. A single cancer antigen contains peptides that can be presented on many different

types of MNC molecules. Individual patients can react against multiple antigens. It is the cellular arm of the immune response that is predominantly involved in immune reactivities, and a key question is whether we can use this information to generate antitumor T cells by immunizing patients using a vaccine.

In a pilot trial, we demonstrated that a modified peptide could consistently immunize cancer patients and generate T cells in their circulation that can recognize the cancer. Work by us and others has opened new possibilities for the development of effective immunotherapies for the treatment of cancer.

7

Cancer and the Environment: A View from the Hill

John Porter

It is a pleasure to provide at this workshop a perspective of the policymaker regarding medical research and the relationship of cancer and the environment. Over the years, I've learned so much about public health and environmental health from the CDC, NIEHS, academicians, and others. We have made many advances, and conferences such as this one help to raise awareness in the Congress about cancer and the environment and suggest strategies to lay the groundwork for congressional support of additional investments in research and, where appropriate, in improving prevention efforts.

Environmental health concerns continue to be on the mind of the public. The greatest concern of most Americans, and this includes most members of Congress, is with the well-being of children in this country. I am sure that many of you had an opportunity to watch the Bill Moyer's special *Trade Secrets*, which accused the chemical industry of knowingly releasing carcinogens that exposed the American people, or the Julia Roberts film *Erin Brokovich*. Programs such as these continue to reach out to and educate the American public about the importance of the environment and their health.

My own particular interest in cancer and the environment began when I participated in the visit of a congressional delegation to Poland. We went to the children's hospital in Krakow, Poland, where I saw hundreds of children afflicted with cancer and learned that the probable cause was their exposure to heavy metals at apparently hundreds of times the permitted exposure in our own country. This interest led Congresswoman Nancy Pelosi and myself to jointly sponsor a special congressional hearing on children's health and the environment. While cancer was not the only illness covered, it was clearly the principal focus of the conference.

Congress and the administration have a strong commitment to funding basic medical research, including research in environmental health concerns. This is

evident from the continual increase in the budgets for basic research. We won't know the actual budget until the work comes out of the subcommittee, but one cannot assume that there will be another 15 percent increase for the National Institutes of Health. We continue to hope and work for this number, but it should not be viewed as a "done deal." Similarly, we need to be concerned about the budget numbers for the CDC and the Agency for Healthcare Research and Quality. The question also remains about funding for the physical sciences. We need researchers to step up to bat to defend the importance of both the life science and the physical science research budgets.

Environmental issues are ascendant in Washington. They will have a central focus in next year's political campaigns. Issues such as CO_2, arsenic in drinking water, drilling in the Arctic National Wildlife Refuge, and energy production will continue to be of interest to the American public. Governmental effects on health will continue to be of growing concern and will require further research. I would want to see new language in the reports that accompany the House and Senate appropriations bills this year. I would want to see that language carried over to the statement of the managers that accompanies the final conference report on the bills funding the Department of Health and Human Services, the Department of Veterans Affairs, and the Department of Housing and Urban Development (and, therefore, the National Science Foundation). I would want to see language that expresses congressional concern and urges additional research and greater attention to cancer and the environment, in particular a focus on the genetic component and its interaction with environmental factors. In order to accomplish the latter, the participants of this workshop should devote some attention to strategies for increasing government awareness and increasing resources for this research into gene–environment interactions.

8

Moving Forward

This year alone, approximately 560,000 Americans will die of cancer-related causes, and almost 1.4 million new cancer cases are expected to be diagnosed. Despite these dire numbers, the data presented at the workshop reinforced the fact that we are making progress in the war against cancer. Most speakers agreed that it was an exiciting time to be in cancer research as we reach a point where we can treat or cure most cancers far more effectively. This is great news; however, as one speaker pointed out, we would prefer not to get cancer in the first place. The workshop laid out a number of strategies for the future of cancer research. The views of the speakers, panelists, and participants do not necessarily reflect the views of the Institute of Medicine or the Roundtable on Environmental Health Sciences, Research, and Medicine.

DISPARITIES

The success we have begun to see in the war against cancer isn't equally accesible to all individuals or groups. As discussed by some speakers, several ethnic and racial populations have disturbingly high cancer incidence and mortality rates relative to the population at large, and these differences are even more pronounced for some cancer sites or organs. Reasons for the disparities might include the presence of specific genetic mutations, but they are more likely to reflect differences in environmental exposures, risk behaviors (e.g., tobacco use, diet), and utilization of prevention, screening, and treatment services. The populations with disparities include Hispanics, American Indians, Alaska Natives, Asian and Pacific Islanders, African Americans, Native Hawaiians, blue-collar workers, rural, elderly, and low-income and low-literacy groups. They not only carry a higher burden of cancer, but also are more prone to other diseases and societal problems. Moreover, the burden of cancer is disproportionately borne by the poor and the undereducated, as well as by populations at higher risk due to lifestyle, environmental exposure, or genetic susceptibility.

Achieving better cancer care and control within these underserved and high-risk populations is an extremely important goal. Even with additional emphasis, cancer will likely continue to be one of the leading causes of death in the early years of this century. Furthermore, the aging population will dramatically change the patterns of cancer in this century because cancer risks increase with age.

Thus, the need for research on special populations is greater than ever before because these populations are the most vulnerable to negative consequences from the rapidly changing health care system. As several speakers emphasized, "One size does not fit all." We must be creative in our approach to addressing cancer in various populations. As Dr. Lovell Jones emphasized, if we do what we have always done, then we will get what we always got. For these reasons, many panelists and speakers urged participants to conduct additional research on special populations to address disparities in incidence rates, mortality rates, and access to care in these populations and to put a greater emphasis on environmental disease surveillance including more complete cancer registries across all populations.

CANCER PREVENTION

Presentations throughout the two days highlighted the fact that many lifestyle and environmental carcinogens have been identified by investigating cancer in populations and that this knowledge has led to new approaches for reducing cancer risk. Yet there is still much to learn about the causes of cancer, particularly why one person with the same cancer-causing exposure (such as smoking or diet) develops cancer, whereas another does not. Individuals' genetic makeup can affect their risk for developing cancer in ways more subtle than those seen in familial cancer syndromes. Variations in genetic susceptibilities related to how individuals control and respond to endogenous hormone levels, diet, exposure to carcinogens, sun, and infectious agents are likely to influence a given individual's chance of developing cancer.

According to many speakers, the estimates of cancer incidence support the claim that in an ideal world, more than 50 percent of cancers could be prevented if what is already known about the etiology and early course of cancer were acted on and fully adopted. These speakers also noted that tobacco use accounts for 30 to 40 percent of preventable cancer mortality; diet for another 20 to 40 percent; and alcohol, occupational exposure, and pollution for the remaining 5 to 17 percent. We have seen decreases in some cancer rates in the past 50 years due to positive changes in society. For example, stomach cancer and cervical cancer declined from 1950 to 1993 due to changes in food preparation and storage and improvements in medical screening and early treatment, respectively. In the 1990s, there was a modest (1 to 3 percent) overall age-adjusted decrease in cancer death rates. This decrease can be attributed to changes in behavior and environment, for example, successful reductions in smoking and better early

detection of cancer. According to some speakers and panelists, we need additional research and public health focus on preclinical stages of cancer with an emphasis on early diagnosis and intervention.

FUTURE RESEARCH AREAS

Mounting evidence indicates that mutation patterns detected in certain tumors may be distinct enough to provide a molecular fingerprint that is traceable to specific environmental agents. In large, population-based studies now under way, investigators described how they are exploring the way in which genetic factors and environmental exposures, including those related to lifestyle and diet, interact to influence cancer risk. By using minute quantities of DNA, it is possible to detect gene mutations whose patterns, functions, or effects may point the way to environmental, nutritional, hormonal, and other factors that contribute to cancer. As more information about human genes becomes available, there will be novel opportunities to test the importance of newly discovered genes for both their relation to cancer susceptibility and clues to environmental carcinogens.

Development of multigenic models of cancer susceptibility will be an important future approach to predicting, preventing, and diagnosing some cancers, said participants. For example, prostate cancer is a common disease for which there are few well-established risk factors. Pedigree analyses suggest a genetic component for some individuals; however, the majority of prostate cancer cases cannot be explained by a single-gene model, suggesting multigenic etiology. Moreover, the international and racial–ethnic variations in prostate cancer incidence, combined with the effects of migration on risk patterns, suggest that genetic factors are likely to play a central role in determining prostate cancer risk.

Even when there is evidence of genetic predisposition, however, future research efforts must focus on gene–environment interactions to fully develop effective cancer prevention and treatment strategies, concluded participants. Thus, even though genetic polymorphisms that predispose some men to prostate cancer or women to breast cancer have been found, the environmental factors that contribute to the actual development of disease must be explored. Moreover, although cancer cases are often clustered in certain families, pedigree analysis indicates that only 5 to 10 percent of cancer patients have a genetic predisposition to the disease. The basic mechanisms for hereditary cancer have been outlined, and a large number of the genes involved have been identified.

CANCER REGISTRIES AND LARGE POPULATIONAL STUDIES

During the workshop, we heard about a number of cancer registries including the NCI's SEER program and the Kentucky Cancer Registry, and also about

large population studies (greater than 100,000 individuals) sponsored by the NCI and the American Cancer Society. Several participants suggested that the cancer registry data be combined with other databases to identify new etiologic leads. For example, cancer registry data could be combined with population survey data or environmental data sources such as pesticide usage or hospitalization data. Large epidemiologic consortia could pool data and publish results from several independent investigations simultaneously to quickly determine whether a given result in one study is supported by other studies. Such consortia would facilitate the pooling of data to assess rare subtypes, or combine data from multiply infected families, and would improve reproducibility and other quality control measures. Participants also noted the need for a linked environmental surveillance system. If cancer clusters were identified within the context of a nationwide childhood cancer registry, one could identify similar areas in the United States and look for similar types of clustering. Finally, there are approximately 80,000 industrial chemicals now registered for use, but very few have been tested for their health effects singly, synergistically, or with different kinds of genetic patterns. Hazard assessment for environmental chemicals is essential and would likely require the collaboration of many federal agencies and the private sector.

RESEARCH IMPLICATIONS

The workshop highlighted a number of potential research implications. Individuals discussed the needs for additional collaboration in multidiscliplinary research. One speaker discussed the need for more discovery-driven research to define the molecular landscape of cancer. This research has to be combined with epidemiological and animal research to fully understand the potential therapeutic implications.

We need to continue the trend of investing in research on the preclinical stages of cancer and on early diagnosis. For example, with recent technological advances in molecular biology, several speakers noted the potential for cancer screening—that is, the identification of markers for inherited disease susceptibility, markers for gene alterations suggesting the development of disease, and markers of existing disease. These have important implications for cancer research because they allow the targeting of interventions based on genetic status. Thus, the more that is understood about the fundamental properties of a tumor cell, the more likely it is that an effective intervention can be identified.

We also have to include the community in the problem, according to some speakers. If cancer is in the community, then the solution needs to be in the community. When the community is involved and has “buy-in,” we have access to greater amount of relevant data (e.g., lifestyle, exposure) that are critical to understanding cancer in a given geographic area. We need to take research from

the bench to the bedside, and then back to the bench, if we are to make progress, they suggested.

Finally, we have to enhance skills and obtain better technologies in the area of risk communication. A few speakers suggested that the media is looking to the scientific community for guidance, but the message must be clear. Individuals in the community are looking for answers but often are frustrated, according to some speakers.

POLICY IMPLICATIONS

Since the Cancer Act of 1972, many reports on cancer have discussed the policy implications of current cancer research. The Institute of Medicine has released a number of these, including *Ensuring Quality Cancer Care* and *The Unequal Burden of Cancer*. One speaker reminded individuals that these reports contained a number of recommendations that have not yet been implemented.

Additionally, we will have to allow broad access to population group data, but we must ensure patient consent and protection of patients' rights. There will be a number of ethical–legal–social implications of genetic research that have to be defined and debated. Handling issues related to technology transfer will continue to be important in order to advance research results. The science community will have to give guidance in these areas to Congress and the public.

Overall, many participants felt that we were making tremendous strides in the war on cancer. People felt that being diagnosed with cancer is no longer a death sentence and that the future holds promise for further progress in both treatment and prevention.

Abstracts of Talks

GENE–ENVIRONMENT INTERACTIONS RELATED TO COLON CANCER[2]

David Alberts, M.D. and M. Elena Martinez, M.D.

Rates of Colorectal Cancer and Adenomatous Polyps. Colorectal cancer remains the third leading cause of cancer deaths in each sex and second overall in the United States (Landis et al., 1999), despite the fact that it is largely a preventable disease. Approximately half of diagnosed individuals will die of this malignancy. Because adenomatous polyps are precursors to colorectal cancer, assessing the effect of environmental and genetic factors in adenoma occurrence and recurrence instead of cancer might help identify relatively asymptomatic individuals who are at increased risk of cancer and who would benefit most from an overall public health intervention. Additionally, the identification of risk factors for recurrence may help define follow-up screening protocols. Although we have obtained some clues regarding risk factors for newly diagnosed adenomas (Neugut et al., 1993), few data exist on predictors of adenoma recurrence among individuals with resected adenomas (Davidow et al., 1996; Tseng et al., 1997; Baron et al., 1998; Hyman et al., 1998; Whelan et al., 1999).

Genetic Basis for Colorectal Neoplasia. The genetic basis for the development of colorectal cancer involves the accumulation of specific somatic mutations in proto-oncogenes and tumor suppressor genes with increasing age

[2]Supported by a Cancer Research Foundation of America grant, Public Health Service grants CA-41108 (Colon Cancer Prevention Program Project) and CA-23074 (Arizona Cancer Center Core Grant) from the National Cancer Institute, and a Career Development Award (KO1 CA-79069-10) grant to M.E. Martinez from the National Cancer Institute

(Kinzler and Vogelstein, 1996). However, only a small proportion of colorectal cancers are attributable to inheritance of these rare, highly penetrant mutated genes. Epigenetic changes, such as alterations in DNA methylation (e.g., CpG island methylator phenotype, or CIMP) and gene expression, also may play a critical role in the development of this malignancy (Baylin et al., 1998). It is evident too that variability in carcinogen-metabolizing genes influences the risk of colorectal neoplasia in humans (Gertig and Hunter, 1998; Hussain and Harris, 1998a; Pereira, 1998).

It is clear that susceptibility to colorectal cancer is related to interindividual variability in biotransformation of endogenous and exogenous substances, as well as in DNA repair and cell cycle control. Common genetic variation may enhance susceptibility to environmental carcinogens by altering the rates of activation and detoxification of carcinogens. The interactions of environmental factors with metabolic polymorphisms may act via a model in which the exposure alone, but not the variant genotype alone, increases disease risk; however, exposure interacts with the variant genotype to further increase risk in the exposed individuals (Vineis, 1997). The same interaction can also modulate disease pathogenesis in that exposure and susceptibility factors may alter the effects of other risk factors, such as folate intake, methylenetetrahydrofolate reductase (*MTHFR)*, CIMP, selenium, or celecoxib intervention, and cyclooxygenase (COX) upregulation, on adenoma recurrence. An example of such an interaction is the relationship between alcohol intake, folate, and *MTHFR*. Classification of subgroups of the population into those who may be more vulnerable to the effects of certain carcinogens may also have important implications beyond risk assessment. Through the identification of an increased risk in certain subgroups, disease risk factors may be better defined. However, to date, sample sizes for most studies attempting to uncover gene–environment interactions have been small, limiting the potential for detecting significant findings.

CIMP as a Marker of Gene Methylation. As stated previously, a genetic basis for cancer has been established with the assumption that the age (and mutagen exposure) related accumulation of somatic mutations accounts for the increased incidence of cancer with age (Ames et al., 1993). The actual rate of mutation accumulation in aged tissues is more uncertain, with some investigators finding lower than expected mutation rates (Warner and Price, 1989; Bohr and Anson, 1995), possibly reflecting the presence of additional mechanisms for activation and/or inactivation of genes important in the carcinogenesis process. In the past few years, there has been renewed interest in epigenetic mechanisms in carcinogenesis (Jones, 1996; Baylin et al., 1998). Epigenetics refers to the study of changes in gene expression that can be mitotically inherited, without associated changes in the coding sequence of the affected genes. Aging and transformed cells show profound changes in gene expression, many of which cannot be accounted for genetically (Sager, 1997). Methylation of DNA within

promoter-associated CpG islands can be a powerful molecular mechanism for gene silencing (Razin and Riggs, 1980; Adams and Burdon, 1982).

In mammals, 5-cytosine methylation of CpG dinucleotides is the only naturally occurring modification of DNA. DNA methylation patterns form early in development with the establishment of gene and tissue-specific patterns of methylation, which are relatively stable (Razin and Riggs, 1980). In humans, approximately 70% of CpG dinucleotides are methylated in adult cells (Adams and Burdon, 1982; Bird, 1992). The function of normal DNA methylation remains controversial, as suggested by the fact that highly expressed genes tend to be hypomethylated, and that silent genes tend to be hypermethylated (Cedar, 1988; Bird, 1992).

Issa and coworkers (Toyota et al., 1999a, 1999b) have developed a definition for a methylated phenotype referred to as CIMP. This phenotype was developed after analysis of a number of colon cancers, colorectal adenomas, and normal colonic mucosa. The research group found that CIMP-positive colorectal cancers averaged 5.1 methylated loci (out of 7) *versus* 0.3 for CIMP-negative tumors. Additionally, some genes were methylated in an age-related manner, while others were more clearly associated with cancer. Based on this work, CIMP-positive adenomas were more likely to have Ki-*ras* mutations, while CIMP-negative adenomas were more likely to have mutations in p53. Furthermore, CIMP-positive adenomas were found to have lower levels of *COX2* expression because of promoter methylation, and their dependence on expression of DNA methyltransferase to maintain tumor suppressor gene silencing via hypermethylation.

Folate, MTHFR, and Gene Methylation. An increasing epidemiologic body of evidence from case-control (Ferraroni et al., 1994) and cohort studies (Giovannucci et al., 1995, 1998; Glynn et al., 1996) supports the important role of folate in reducing the risk of colorectal cancer. Another study (Ma et al., 1997), which did not have comprehensive dietary data, showed an inverse association between plasma folate and risk of colon cancer. Folate intake and blood levels have also been consistently associated with lower risk of colon adenomas (Giovannucci et al., 1993; Tseng et al., 1996). Recent results indicate that increased consumption of folic acid from supplements, after a period of 15 or more years, may decrease the risk of colon cancer by about 75% (Giovannucci et al., 1998). Giovannucci et al. (1993) have proposed that the increased risk associated with low folate levels is related to intracellular methylation defects. Additionally, these investigators proposed that alcohol consumption increases the risk of colorectal neoplasia by acting as a folate antagonist; this hypothesis is based on data demonstrating the modifying effect of folate and methionine on the alcohol and colorectal neoplasia relationship (Giovannucci et al., 1993, 1995; Ma et al., 1997).

Given the epidemiologic evidence for the proposed protective effect of folate on colorectal neoplasia, some studies have explored the mechanisms involved in

this association. Because folate is the primary methyl donor in cellular metabolism (Hoffman, 1985), markers of folate status are important factors to address in the etiology of gene methylation. A critical role of folate is in synthesizing methionine from homocysteine (Hoffman, 1985). Methionine, in turn, is converted to *S*-adenosylmethionine (SAM), the primary methyl donor in the reaction transferring a methyl group to the enzyme 5'-cytosine-DNA methyltransferase. Transfer of a methyl group from SAM to methyltransferase produces S-adenosylhomocysteine (SAH), which is then hydrolyzed to homocysteine. Folate is also essential for nucleotide biosynthesis (Eto and Krumdieck, 1986). In folate deficiency, thymidylate shortages cause an imbalance in the thymidylate–deoxyuridylate pool and a resultant incorporation of uridylate into DNA. Excess uridylate incorporation into DNA results in unstable chromosomes, decreased DNA repair, and increased chromosome breaks (Barclay et al., 1982; Reidy, 1987; Everson et al., 1988; Dianov et al., 1991; James et al., 1992).

Genetic Polymorphisms of MTHFR. A genetic factor that modifies the effects of folate status has recently been identified that includes the inherited variation in the activity of MTHFR, a critical enzyme involved in the production of the form of folate that supplies the methyl group for methionine synthesis (Kutzbach and Stokstad, 1971). Different endogenous forms of folate, 5-methyltetrahydrofolate, and 10-methylenetetrahydrofolate, are essential for DNA methylation and DNA synthesis, respectively. A common thermolabile polymorphism in the *MTHFR* gene (C677→T, alanine→valine) has been shown to be protective against colon cancer in some (Chen et al., 1996; Ma et al., 1997; Slattery et al., 1999; Ulrich et al., 1999) but not all (Chen et al, 1998) studies. Low MTHFR activity is thought to protect against colorectal cancer since less tetrahydrofolate is converted to 5-methyltetrahydrofolate, allowing more folate to be shunted toward DNA synthesis and repair. In these studies, an inverse association was shown for the presence of the *val*/*val* genotype and colorectal cancer among individuals with adequate folate intake, whereas this effect was not seen among those with low folate intake (Chen et al., 1996; Ma et al., 1997). Since MTHFR is required to convert 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, individuals with low MTHFR levels (*val*/*val* homozygotes) would be expected to have relatively high levels of 5,10-methylenetetrahydrofolate resulting from the low levels of the 5-methyltetrahydrofolate (Frosst et al., 1995). Therefore, in a low-folate environment where there are inadequate quantities of methyl groups for these pathways, individuals with the *val*/*val* genotype do not divert as much folate from the thymidylate pathway to the methylation pathway, resulting in lower SAM levels and high levels of homocysteine. Under conditions in which folic acid levels are insufficient to meet metabolic needs, *val*/*val* homozygotes would be less efficient at diverting folate metabolites into the 5-methyltetrahydrofolate product, resulting in a shortage of methyl groups. When levels of 5,10-methylenetetrahydrofolate (which is required

to convert deoxyuridylate to thymidylate) are low, misincorporation of uracil for thymidine may occur during DNA synthesis (Wickramasinghe and Fida, 1994), possibly increasing spontaneous mutation rates (Weinberg et al., 1981), sensitivity to DNA-damaging agents (Meuth, 1981), frequency of chromosomal aberrations (Sutherland, 1988; Fenech and Rinaldi, 1994), or errors in DNA replication (Hunting and Dresler, 1985; Fenech and Rinaldi, 1994; James et al., 1994).

Folate Deficiency and CpG Island Gene Methylation. The proposed mechanism for the above-reviewed studies relates to dietary factors that influence methyl group availability, which can in turn affect DNA methylation. DNA methylation is an essential mechanism of gene regulation, and disturbances may cause differential gene expression (Cedar, 1988). In animal models, folate deficiency can cause imbalances in DNA methylation (Wainfan and Poirier, 1992; Kim et al., 1996). Furthermore, folate deficiency in rats has been shown to induce DNA strand breaks and altered methylation within the p53 tumor suppressor gene (Kim et al., 1997) and to result in deoxynucleotide pool disturbances (James et al., 1992). Given this literature, support for the potential effect of folate status and methyl group availability in the etiology of CIMP status exists. Additional supporting evidence for this association derives from our own study (Martinez et al., 2001), in which a low intake of folate was associated with a significantly higher risk of Ki-*ras* mutations in adenomatous polyps. Previous work by Toyota et al. (1999a) indicates that CIMP-positive adenomas are more likely to harbor Ki-*ras* mutations than CIMP-negative adenomas. Thus, the potential role of folate in the etiology of Ki-*ras*, mutations along with data supporting the high rate of these mutations in CIMP-positive adenomas, suggests that folate may be involved in the etiology of CIMP-positive adenomas.

Dietary Folate Intake, MTHFR Status, and Colorectal Cancer. Published data on the interaction between folate and MTHFR in the etiology of colorectal neoplasia are inconsistent, suggesting that this interaction is more complex than originally proposed. In a recent report of more than 3,000 case and control participants (Slattery et al., 1999), a lower risk of colorectal cancer associated with higher intake of folate was shown among individuals with the *val*/*val* genotype as compared to those with the *ala*/*ala* genotype who had low folate intake (odds ratio (OR) = 0.6; 95% confidence interval = 0.4–1.0). Of particular interest, the effects of folate among the *val*/*val* genotypes appeared to be stronger for the proximal (OR = 0.5) compared to the distal (OR = 0.8) colon. Based on Issa's work, CIMP-positive adenomas were more prevalent in the proximal colon, which may be related to factors affecting folate metabolism and methyl group availability.

Plasma Homocysteine, MTHFR Status, and Polyp Recurrence. We prospectively examined whether plasma levels of homocysteine were associated

with the risk of recurrence of adenomatous polyps (Martinez et al., 2001). Analyses were conducted among 1,014 men and women, 40 to 80 years of age, enrolled in a Phase III trial testing the effects of a wheat bran fiber intervention on adenoma recurrence. We also examined whether the association between plasma homocysteine and adenoma recurrence was modified by the *MTHFR* genotype among 961 participants with genotype data. Homocysteine in plasma was analyzed at baseline by high-performance liquid chromatography (HPLC). *MTHFR* genotyping was performed by high-throughput microarray technology. Compared to participants with lower plasma homocysteine levels, those with higher levels were older, were more likely to be male, had lower intakes of total (dietary plus supplemental) folate, had higher alcohol intakes, and had lower plasma folate levels. After adjustment for age, gender, number of colonoscopies, and a history of previous polyps, the odds ratio for adenoma recurrence for individuals with homocysteine levels >11.6 mmol/l was 1.45 (95% CI = 0.98–2.14; *P*-trend = 0.02) compared to those with levels <7.8. When we assessed the relationship between homocysteine levels and adenoma recurrence according to *MTHFR* status, individuals with the *TT* genotype and homocysteine levels above the median (>9.4) had a higher risk of recurrence (relative risk = 1.96) compared to those with the *CC* genotype and homocysteine levels below the median. The results of these analyses suggest a modest effect of plasma homocysteine levels on adenoma recurrence and a risk-enhancing effect of high homocysteine levels on adenoma recurrence among individuals with the *TT MTHFR* genotype.

DIET AND RISK OF CHILDHOOD CANCER

Greta Bunin, Ph.D.

Epidemiologists often broadly define environment to encompass anything that is not genetics. Diet is an integral part of the environment. All solids including fish, foul, meat, grains, vegetables, and foods may contain trace contaminants (e.g., pesticides, heavy metals, polychlorinated biphenyls [PCBs]). The evidence is just beginning to emerge on the role of diet and cancer in children and, more specifically, the role of diet in the generation of cancer.

Fewer than 20 studies have looked for a link between childhood cancer and diet. Most commonly, researchers have investigated the link between brain cancer and diet because of a hypothesis based on animal data. The hypothesis postulates that children with greater exposure to *N*-nitroso compounds (NOCs) and their precursors are more likely to develop a brain tumor compared to other children. In many species of animals, NOCs are highly potent carcinogens. Some NOCs induce nervous system tumors, and for a few NOCs, the risk of tumor development is multiplied when the exposure occurs in utero.

Human exposure to NOCs is widespread, and they have been detected in many common products, including cigarette smoke, automobile interiors, and

cosmetics. Additionally, we are also exposed to precursors that combine to form NOCs in our stomachs and elsewhere in our bodies. In fact, most human exposure is thought to occur via synthesis in the body from precursors. Substances such as vitamins C and E inhibit the formation of NOCs and, thus, may be important for the prevention of brain tumors.

Diet is a major source of NOCs, NOC precursors, and NOC inhibitors. Meats cured with nitrites, such as hot dogs and lunch meat, contain NOCs and NOC precursors, while fruits, vegetables, and vitamin supplements contain NOC inhibitors. Therefore, researchers hypothesize that a mother's frequent eating of cured meats and infrequent eating of fruits and vegetables increase the risk of brain tumors in children.

Of the eight studies investigating the NOC hypothesis, four found a significant doubling of risk of brain cancer when the mother frequently consumed cured meats during pregnancy. In a fifth study, a similar association was observed but it was not significant. Two studies had small numbers of children with brain tumors, which may explain why they failed to detect a difference. The last study looked at a less common type of brain tumor and observed no association with cured meat. This tumor type has a different sex and age distribution than the most common type and therefore might have a different etiology as well. Overall, the data are fairly consistent for an association between frequent consumption of cured meats during gestation and childhood brain tumors. Further research will be needed to determine if the brain tumors are due to the NOCs in cured meats or to a nutrient such as high fat or low folate in the diet.

SIMILARITIES OF PROSTATE AND BREAST CANCER: EVOLUTION, DIET, AND ESTROGENS

Donald S. Coffey, Ph.D.

The risk of both prostate and breast cancer is similar and primarily determined by the environment in which one lives, and this risk can vary more than tenfold between countries. In contrast, no risk exists for human seminal vesicle cancer, thus demonstrating tissue specificity for cancer in the human. There is also species specificity because there is no risk for prostate cancer in any other of the thousand of aging mammal species except the dog. Evolution indicates that the prostate and breast appeared at the same time 65 million years ago with the development of mammals. All male mammals have a prostate; however, the presence of seminal vesicles is variable and is determined by the diet so that species primarily eating meat do not have seminal vesicles. The exception is the human, who has seminal vesicles and consumes meat, although this is a recent dietary change. Human lineage departed from other higher primates 8 million years ago. The closest existing primate to humans is the bonobo (pigmy chimpanzee), which does not eat meat but exists primarily on a high-fruit and fresh

vegetable diet. *Homo sapiens* evolved only about 150,000 years ago, and only in the last 10% of that time (10,000–15,000 years ago) did humans and dogs dramatically alter their diets. This is the time when humans domesticated the dog, bred animals, grew crops, and cooked, processed, and stored meats and vegetables. Current epidemiologic evidence and suggestions for preventing prostate and breast cancer in humans indicate that we should return to the original type of diets under which our ancestors evolved. The recent development of the Western-type diet is associated with breast and prostate cancer throughout the world. It is believed that the exposure to and metabolism of estrogen, and the dietary intake of phytoestrogens, combined with fat intake, obesity, and burned food processing, may all be related to hormonal carcinogenesis and oxidative DNA damage. An explanatory model is proposed. (For details see Coffey, 2001.)

EFFECT OF HERBAL THERAPIES ON PROSTATE CANCER

Robert S. DiPaola, M.D.

Background. Herbal mixtures are popular alternatives to demonstrated therapies. PC-SPES, a commercially available combination of eight herbs, is used as a nonestrogenic treatment for cancer of the prostate. Since other herbal medicines have estrogenic effects in vitro, we tested the estrogenic activity of PC-SPES in yeast and mice and in men with prostate cancer.

Methods. We measured the estrogenic activity of PC-SPES with transcriptional activation assays in yeast and biologic assay in mice. We assessed the clinical activity of PC-SPES in eight patients with hormone-sensitive prostate cancer by measuring serum prostate-specific antigen and testosterone concentrations during and after treatment.

Results. PC-SPES had estrogenic activity similar to that of 1 nM estradiol, and in ovariectomized CD-1 mice, the herbal mixture increased uterine weights substantially. In six of six men with prostate cancer, PC-SPES decreased serum testosterone concentrations ($P < 0.005$), and in eight of eight patients, it decreased serum concentrations of PSA. All eight patients had breast tenderness and loss of libido, and one had venous thrombosis. HPLC, gas chromatography, and mass spectometry showed that PC-SPES contains estrogenic organic compounds that are distinct from diethylstilbestrol, estrone, and estradiol.

Conclusions. PC-SPES has potent estrogenic activity. The use of this unregulated mixture of herbs may confound the results of standard or experimental therapies and may produce clinically significant adverse effects. Further studies to identify the estrogen(s) responsible for this activity are warranted.

COLORECTAL CANCER AND ENVIRONMENTAL RISK FACTORS

Raymond N. DuBois, M.D., Ph.D.

Risk factors for colorectal cancer include a positive family history, meat consumption, smoking, and alcohol consumption. A reduction in risk for the disease is associated with vegetable intake, use of nonsteroidal anti-inflammatory drugs (NSAIDs), hormone replacement therapy, and physical activity. There are several genetic and epigenetic alterations that are known to be involved in the development of colorectal cancer. These alterations are important in both inherited syndromes such as familial adenomatous polyposis (FAP) or hereditary nonpolyposis colorectal cancer (HNPCC) and in sporadic tumors. It will be important to understand the roles of environmental exposure and host susceptibility to develop better screening, prevention, and treatment strategies.

Population-based studies indicate a 40–50% reduction in mortality from colorectal cancer in persons using NSAIDs on a regular basis (Smalley and DuBois, 1997). Colorectal cancer is a major cause of death from cancer in Western civilizations, claiming more than 55,000 lives in the United States each year. Environmental and dietary factors play an important role in the etiology of this disease as well as the known genetic components. Research efforts have been focused on understanding the molecular basis for the chemoprotective effects associated with use of aspirin and other NSAIDs. NSAIDs inhibit both cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) activity (Williams and DuBois, 1996). Since COX-2 levels are increased in a number of solid tumors, this enzyme may serve as a molecular target for cancer prevention (Sheng et al., 1997). Recent clinical studies indicate that the presence of COX-2 in human lung and colon cancers is associated with a negative clinical prognosis (Achiwa et al., 1999; Sheehan et al., 1999). Therefore, COX-2 inhibitors are currently being evaluated for the prevention and/or treatment of cancer in humans (Steinbach et al., 1999).

GENES AND THE ENVIRONMENT IN CANCER ETIOLOGY

Joseph F. Fraumeni, Jr., M.D.

The importance of environmental factors in human cancer has long been evident from the striking international variation reported in cancer incidence, resulting in estimates that perhaps 80% of all cancer in the United States is potentially preventable or avoidable. Further indications of environmental cancer come from the shifts in the cancer experience of migrant populations whose rates tend to approximate those of the host country, the geographic patterns of cancer within the United States, the changing incidence of certain cancers over time, ethnic and socioeconomic differentials, and the abundant epidemiologic evidence linking carcinogenic risks to a variety of lifestyle and other environ-

mental exposures. Not long ago, the role of inherited susceptibility in human cancer was considered to be quite small given the rarity of hereditary cancer syndromes, but recent progress in identifying and characterizing highly penetrant but relatively rare susceptibility genes in familial cancer has revolutionized our understanding of genetic mechanisms and their critical importance in cancer etiology. Of special significance to the public health burden of cancer, however, are the common polymorphic susceptibility or modifier genes that confer low relative and absolute risks, but high population-attributable risks in the presence of relevant environmental exposures. The two classes of genes represent parts of a continuum because even the highly penetrant genes responsible for hereditary cancer may involve environmental exposures for expression, as illustrated by the susceptibility to carcinogens in hereditary retinoblastoma and Li–Fraumeni syndrome.

Especially exciting is the opportunity to parlay discoveries of polymorphic genes and their functions into a better understanding of environmental carcinogenesis. By incorporating careful exposure assessment and mechanistically plausible candidate susceptibility genes into epidemiological study designs, it should be possible to identify the more subtle risks due to specific dietary and nutritional factors, metabolic alterations, environmental pollutants, and other common exposures that have eluded traditional epidemiologic approaches. Although the molecular and statistical tools to examine complex gene–environment interactions are still in development, opportunities now exist for population and family-based studies using biomarkers that integrate the search for susceptibility genes and the exogenous and endogenous exposures that cause cancer. While the methodologic challenges of "molecular epidemiology" are formidable, this interdisciplinary approach to cancer etiology should provide unprecedented opportunities to enlarge our understanding of environmental and genetic risk factors and their biological pathways, and set the stage for new clinical and public health strategies aimed at preventing and controlling cancer.

CANCER DISPARITIES IN APPALACHIA

Gilbert H. Friedell, M.D.

Despite recent good news about decreasing U.S. cancer mortality rates, not all population subgroups are sharing in this success story. Progress toward meeting the cancer-related Healthy People 2010 goals will be hampered by the nation's inability to deal effectively with the greater cancer burden borne by certain vulnerable populations. These "special populations," defined as population groups at higher-than-average risk of death, disease, and disability, include people with low incomes, African Americans, Hispanics, American Indians, and other ethnic minorities.

In addition, the National Cancer Institute (NCI) has stated that it considers rural residents to constitute "a special population." Rural Americans tend to be older, poorer, less educated, and more likely to be uninsured than their urban counterparts. Rural communities have higher rates of chronic illness and disability, and report poorer overall health status than their urban neighbors. Residents in rural areas generally have less contact and fewer visits with physicians and, in general, lower levels of preventive care. In addition to factors related to rural health status and practices, there are systemic factors related to rural life in general (e.g., lack of public transportation and lower levels of other community services) that may also contribute to less than optimal cancer control.

All of these factors are evident in the largely rural and predominantly white population of Appalachia, particularly in the Central Highlands, including the Appalachian counties of Ohio, West Virginia, Kentucky, Tennessee, and Virginia. Lung cancer is a leading cause of male cancer deaths in central Appalachia, with the highest rate in Appalachian Kentucky, the geographic area where the Behavioral Risk Factor Surveillance Survey data (BRFSS) indicate the highest rates of cigarette smoking in the state. Cervical cancer mortality rates are also higher in central Appalachia than in the U.S. population as a whole.

Data from the Kentucky cancer registry showed that the incidence of invasive cervical cancer and lung cancer in eastern Kentucky is higher than the incidence of these cancers in the overall Kentucky population and in the population covered by the Surveillance, Epidemiology, and End Results (SEER) program. It is, however, quite similar to the incidence of lung cancer and cervical cancer in the predominantly urban, African American population of Kentucky.

Poverty is a common characteristic in much of this region. Some of the counties in Appalachian Kentucky, for example, are among the poorest in the country. In the same geographic areas, the level of literacy—indicated by the highest grade of formal schooling completed—is also lower than in most of the country. Problems associated with poverty are similar to those confronting poor populations in other parts of the country, but the latter are often characterized by race or ethnicity rather than by socioeconomic status (SES). This use of race and ethnicity as surrogates for poverty has obscured the fact that the problems related to cancer in the poor white population are comparable in many ways to those seen in recognizable minority populations.

Individuals living in poverty often do not receive quality health care, including cancer prevention, diagnosis, treatment, and appropriate follow-up care, because services are not available, accessible, and/or utilized. Behavioral risk factors, such as tobacco use, poor nutrition, obesity, and underutilization of cancer screening examinations are more evident in impoverished populations. The social environment in which poor people live also prevents the development of healthy behaviors. Freeman has pointed out that poverty "is a proxy for other elements of living, including lack of education, unemployment, substandard housing, poor nutrition, risk-promoting lifestyles and behaviors, and a dimin-

ished access to health care," all of which affect individual chances of developing cancer and surviving it. However, until cancer surveillance incorporates socioeconomic status into its database, the relationship between poverty and cancer in population groups will be difficult to sort out.

Data concerning income or other elements of SES generally are not collected by either hospital- or population-based cancer registries. It is therefore difficult to identify individuals whose income is below the poverty line. In Appalachia, however, and specifically in the defined geographic area of central Appalachia, a high proportion of the almost entirely white, largely rural population is poor.

Research is hampered by the lack of access to necessary data. Currently in eastern Kentucky, the *average levels* of income, education, and other elements of SES can be determined, but obtaining this information on an *individual basis* for patients at the present time is quite difficult. The necessary data are generally not available in the medical records of cancer patients. Moreover, to have meaningful, population-based data for the purposes of comparison, this information would necessarily have to be a part of the medical record for all patients at both in-patient and ambulatory facilities.

Some barriers to increased participation in cancer control programs exist at all socioeconomic levels, (e.g., lack of information about cancer and about both the availability and the benefit of cancer screening). Fear of what might be found during such an examination mitigates against women either gaining information about cancer or doing something with the information once it is obtained. Additionally, health literacy continues to be a problem. The average reading level in this region is approximately at the fifth or sixth grade, making it difficult for individuals to understand and correctly respond to higher-level printed materials. Addressing issues such as these at the community level will be necessary.

TUMOR SUPPRESSOR GENES: AT THE CROSSROADS OF MOLECULAR CARCINOGENESIS, MOLECULAR EPIDEMIOLOGY, AND HUMAN RISK ASSESSMENT

Curtis C. Harris, M.D.

Environmental, occupational, and recreational exposures to carcinogens contribute to cancer risk in humans. Cancer formation is a multistage process involving the activation of proto-oncogenes and the inactivation of tumor suppressor genes. Carcinogens can interact during any of these stages through genetic and epigenetic mechanisms.

Mutational spectra of cancer-related genes (e.g., p53, BRCA-1, and $p16^{INK4}$) may provide a molecular link between etiological agents and human cancer. Mutations in the evolutionarily conserved codons of the p53 tumor suppressor

gene are common in diverse types of human cancer (Hollstein et al., 1994), and the mutational spectra differ among cancers of the colon, lung, esophagus, breast, liver, brain, reticuloendothelial tissues, and hemopoietic tissues. Analysis of these mutations may provide clues to the mutagenic mechanisms and the function of specific regions of p53 and generate hypotheses for investigation (Hussain and Harris, 1998a). Most transversions in lung, breast, and esophageal carcinomas are dispersed among numerous evolutionarily conserved codons within the p53 domain responsible for sequence-specific DNA binding and transcriptional activity. Transitions predominate in colon, brain, and lymphoid malignancies. Mutational hotspots at CpG dinucleotides in codons 175, 248, 273 of the p53 gene and condon 282 may reflect an endogenous mutagenic mechanism, (e.g., the deamination of 5-methylcytosine to thymidine). Oxyradicals and nitrogen oxyradicals may enhance the rate of deamination. For example, we have observed that (1) an increased production of nitric oxide (NO) by nitric oxide synthase-2 (NOS2) is associated with p53 cytosine to thymidine (C to T) transitions during colon carcinogenesis (Ambs et al., 1999); (2) p53 transrepresses basal and cytokine-induced NOS2 expression in vitro (Forrester et al., 1996) and in vivo (Ambs et al., 1998a); and (3) NO increases both the expression of the vascular endothelial growth factor and angiogenesis (Ambs et al., 1998b). p53 G:C to T:A (where G = guanosine and A = adenosine) transversions are the most frequent substitutions observed in cancers of the lung, breast, stomach, and liver, and are more likely to be due to bulky carcinogen–DNA adducts. G:C to T:A transversions also are more common in lung cancers from smokers compared to never smokers (Takeshima et al., 1993; Hussain and Harris, 1998b) and are more frequent in lung cancers from women compared to men (Guinee et al., 1995). The high frequency of G to T p53 mutations in the nontranscribed DNA strand is a reflection of strand-specific repair of the transcribed strand (Evans et al., 1993). The p53 gene may also contribute to DNA repair and apoptosis by protein–protein interactions with transcription–repair factors, XPB (ERCC3) and XPD (ERCC2), and in TFIIH (Wang et al., 1994, 1995a, 1996). A p53 mutation, allelic deletion and/or posttranslationally modified protein can be an early event in bronchial, mammary, or esophageal carcinogenesis (Bartek et al., 1990; Davidoff et al., 1991; Bennett et al., 1992; Sozzi et al., 1992; Sundaresan et al., 1992; Vahakangas et al., 1992; Nuorva et al., 1993) and may prove useful in the early diagnosis of cancer.

In liver tumors from persons living in geographic areas where aflatoxin B_1 (AFB) and hepatitis B virus (HBV) are cancer risk factors, most p53 mutations are at the third nucleotide pair of codon 249 (Hsu et al., 1991). A dose-dependent relationship between dietary AFB intake and codon 249^{ser} p53 mutations is observed in hepatocellular carcinoma. Exposure of AFB to human liver cells in vitro produces 249^{ser} (AGG to AGT) p53 mutants (Aguilar et al., 1993; Mace et al., 1997). The mutation load of 249^{ser} mutant cells in nontumorous liver also is positively correlated with dietary AFB exposure (Aguilar et al., 1994). These

results indicate that the expression of the 249^{ser} mutant p53 protein provides a specific growth and/or survival advantage to liver cells. Because cellular context may influence the pathobiological effects of specific mutants of p53, the 249^{ser} mutant may be especially potent in hepatocytes by the enhanced growth rate of p53-null HEP-3B cells by transfected 249^{ser} mutant p53, and indicates a gain of oncogenic function (Ponchel et al., 1994). The 249^{ser} mutant p53 is more effective than other p53 mutants (143^{ala}, 175^{his}, 248^{trp}, and 282^{his}) in inhibiting wild-type p53 transcriptional activity in human liver cells (Forrester et al., 1995). One model concerning the generation of liver cancers with the 49^{ser} mutation is the following: (1) AFB is metabolically activated to form the promutagenic N7dG adduct; and (2) enhanced cell proliferation due to chronic active viral hepatitis allows both the fixation of the G:C to T:A transversion in codon 249 of the p53 gene and selective clonal expansion of the cells containing this mutant p53 gene. HBV also has significant pathobiological effects. For example, the HBVX gene is frequently integrated and expressed in human hepatocellular carcinomas from high-risk geographic areas (Unsal et al., 1994; Paterlini et al., 1995). Hepatitis B viral gene products may form complexes with cellular transcription factors (e.g., ATF2; Maguire et al., 1991), upregulate transcription of cellular and viral genes (Twu and Schloemer, 1987; Spandau and Lee, 1988; Shirakata et al., 1989; Caselmann et al., 1990; Kekule et al., 1990) including NOS2 (Elmore et al., 1997), or activate the ras-raf-MAP kinase signaling cascade (Benn and Schneider, 1994). Inactivation of the p53 tumor suppressor gene functions, including DNA repair and apoptosis, may be another consequence of the cellular protein–HBV oncoprotein complex formation. The HBVX protein binds to p53 (Pirisi et al., 1987; Wang et al., 1994; Ueda et al., 1995), sequesters it in the cytoplasm (Elmore et al., 1997), and inhibits its sequence-specific DNA binding and transcriptional activity (Wang et al., 1994). The HBVX protein also inhibits p53-dependent apoptosis (Wang et al., 1995b). In nucleotide excision DNA repair, the HBVX protein may modulate p53 function (Wang et al., 1995a; Jia et al., 1999), including the repair of AFB_1–DNA adducts. HBV integration also could increase genomic instability, including abnormal chromosomal segregation, and increase the rates of DNA recombination (Hino et al., 1989, 1991).

Three other associations between the p53 mutational spectra and carcinogen exposure have been observed. The induction of skin carcinoma by ultraviolet light is indicated by the occurrence of p53 mutations at dipyrimidine sites, including CC to TT double-base changes (Brash et al., 1991; Ziegler et al., 1994). The p53 mutational spectrum in radon-associated lung cancer from uranium miners also differs from lung cancer caused by tobacco smoking alone (Vahakangas et al., 1992; Taylor et al., 1994). Hepatic angiosarcomas induced by occupational exposure to vinyl chloride have a high frequency of A:T to T:A p53 mutations when compared with sporatic angiosarcoma (Hollstein et al., 1994; unpublished results). In summary, these differences in mutational frequency and spectra among human cancer types indicate the following: (1) the etiological

contributions of both exogenous and endogenous factors to human carcinogenesis; (2) specific proliferative effects conferred by different mutant p53 genes in different human cell types; and (3) hypotheses for investigation (Hussain and Harris, 1998b). These genetic changes in the tumor suppressor genes also have implications for cancer diagnosis, prognosis, and therapy (Harris and Hollstein, 1993; Harris, 1996).

The association of a suspected carcinogenic exposure and cancer risk can be studied in populations by classic epidemiologic techniques. However, these techniques are not applicable to the assessment of risk in individuals. A goal of molecular epidemiology is to integrate molecular biology, in vitro and in vivo laboratory models, biochemistry, and epidemiology to infer individual cancer risk (Harris, 1991; Shields and Harris, 1991; Perera and Santella, 1993; Perela, 1997; Ponder, 1997). Carcinogen–macromolecular adduct levels and somatic cell mutations can be measured to determine the biologically effective dose of a carcinogen. Molecular epidemiology also explores host cancer susceptibilities, such as carcinogen metabolic activation, DNA repair, endogenous mutation rates, and inheritance of mutated tumor suppressor genes. Substantial interindividual variation for each of these biological end points has been shown (Harris, 1991) and highlights the need for assessing cancer risk on an individual basis. Given the pace of the past decade, it is feasible that future advances will allow molecular epidemiologists to develop a cancer risk profile for an individual that includes assessment of a number of exposure and host factors. This will help focus preventive strategies and strengthen quantitative risk assessments.

GENETIC EPIDEMIOLOGY AS A TOOL FOR GENE–ENVIRONMENT INTERACTIONS

Kari Hemminki, M.D., Ph.D.

Age-incidence relationships and experimental evidence suggest that cancer is a polygenic multifactorial disease (Armitage and Doll, 1954; Kinzler and Vogelstain, 1996). Tumors are monoclonal, implying that multiple hits need to affect a single clone of cells. Multifactorial diseases include an environmental component, which has been assumed to be the main cause in most types of cancer. The support for multistage carcinogenesis in vivo is limited. Almost all the known cancer syndromes are monogenic, and they conform to a two-stage model in requiring inactivation of the two copies of the tumor suppressor gene (Vogelstein and Kinzler, 1998). In this presentation, I consider the effects of multistage carcinogenesis for study of familial cancer, based on Swedish population and cancer registries. These sources of data allow estimations of the environmental and heritable contributions in the causation of cancers.

It is estimated that some 1% of cancer is caused by the currently known cancer syndromes and up to 5% by highly penetrant single-gene mutations

(Lynch et al., 1995; Vogelstein and Kinzler, 1998). These data apply to dominant Mendelian conditions, which can be assessed in family studies covering two or more generations. However, such studies provide no data on recessive Mendelian conditions and have a limited resolving power on polygenic conditions (Fearon, 1997). Consequently, apart from highly penetrant single-gene mutations, the estimation of the total hereditary contribution is extremely difficult. The risks posed by low-penetrance single-gene mutations, polygenes, and recessive genes are poorly understood.

Twin Model: Nonparametric Approach. Studies among twins are traditional tools for dissecting questions about disease etiology, genes, and environment. The twin model is particularly valuable because the mode of inheritance need not to postulated, (i.e., the approach is nonparametric, and the results are informative of the overall genetic effects). Polygenic effects are diluted among dizygotic twins but not among monozygotic twins. Twin studies invite genetic interpretation because monozygotic twins are genetically identical and dizygotic twins share half of their segregating genes. Thus, if monozygotic twins are more similar for a trait than dizygotic twins, genetic effects are likely to be involved. If there is twin similarity not accounted for by genetic effects, this indicates that shared environmental effects, (e.g., shared childhood experiences such as diet), contribute to variance in the trait. Yet the rareness of twinning has limited this approach to a few publications addressing the relative importance of genetic and environmental effects in cancer, the largest of these studies originating from the Nordic countries.

A cancer study was carried out by pooling data from the Swedish, Finnish, and Danish twin registries for joint analysis (Lichtenstein et al., 2000). The aim of this study was to provide reliable estimates of genetic and environmental effects for the most common cancer sites and to assess the modification of such estimates by age at diagnosis. Data from 90,000 twins were combined to assess the cancer risks at 28 sites for co-twins of twins with cancer. A structural equation model, MX, was applied in estimating the proportions of variation in cancers due to environmental and inherited causes. The nonshared random environmental effect was the largest factor for all cancers, accounting for 58–82% of the total variation. Statistically significant heritability estimates were detected for cancers of the colorectum (35%), breast (27%), and prostate (42%). Estimates for the shared environmental effects ranged from 0 to 20%, but none were statistically significant. There were no significant differences between sexes at any of the sites.

The Family-Cancer Database. The Swedish Family-Cancer Database now contains data on 10 million people, organized in families, and their 1 million cancers, retrieved from the Swedish Cancer Registry (Hemminki and Vaittinen, 1999). The Family-Cancer Database is the largest population-based data set ever

used for studies on familial cancer. The database has been used in some 80 studies characterizing familial risks of various cancers. It has been used to model for cancer causation, using the same MX program employed in the above-mentioned twin study (Hemminki et al., 2001). Because of the overwhelming size of the data, most estimates for environmental and heritable causes were statistically significant. However, the results were not different from the twin studies. Environmental causes explained most of the total variation for all neoplasms except thyroid cancer, for which heritable causes were largest. For example, the estimate for heritable causes of 25% in breast cancer was in line with the estimate from twins.

Multiple Cancers. An increased occurrence of second primary cancers can result from intensive medical surveillance after first diagnosis, therapy-induced exposure to X-rays and carcinogens, and shared environmental and hereditary causes between the first and second cancer. I will take up only the aspect relating to genetic epidemiology, the hereditary risks possibly revealed in second cancers. The risks for second cancer tend to be much higher than those for the first cancer. Very high risks for second cancer were noted for nose, skin (squamous cell carcinoma), connective tissue, and leukemia. We have analyzed the effect of family history on some cancers, such as breast cancer, and it is an important factor but affects a relatively small proportion of patients with second breast cancer (Dong and Hemminki, 2001; Vaittinen and Hemminki, 2000). The data suggest that patients with second cancer include a subgroup with a strong genetic predisposition to cancer, which often cannot be predicted by a family history. Such risks would be typical of polygenic diseases, and our tenet in the recent work on second cancers has been that they may serve as a unique population model for polygenic cancers (Dong and Hemminki, 2001; Hemminki and Mutanen, 2001).

Interpretations. There is a consensus on the predominant importance of environmental factors and somatic events in human cancer, and the present results on the twin and family sets quantified the effect of nonshared environment to range from 40 to 90% for different cancers. Nonshared environment encompasses anything that is not hereditary and not shared between the relatives (sporadic causes of cancer). It is of interest to note that this effect was large for some cancers of identified environmental causes, such as lung and cervical cancers. Shared environment, summing up common family experiences and habits of family member, accounted for 0–30% of etiology. The structural equation modeling carried out can accommodate both dominant and recessive Mendelian modes and polygenic modes of inheritance. Thus, the results on heritability summarize the total genetic effects. The recent data identified significant heritable effects for colorectal, breast, and prostate cancer. Heritabilities for these cancers

were estimated to be between 15 and 40%, challenging previous estimates of the magnitude of genetic effects, based mainly on high-penetrance dominant conditions. For all cancer the genetic effect was 26%. Moreover, we found evidence for heritability of all main cancers, ranging from 1 to 50%. The frequencies of mutations in the known high-risk susceptibility genes *BRCA1* and *BRCA2* in breast cancer and DNA mismatch repair genes in HNPCC are so low that they explain at most 10% of the genetic effects noted (Peto et al., 1999; Salovaara et al., 2000). For prostate cancer, candidate genes have been mapped but not identified. These findings suggest that other genes are yet to be identified, but because they are likely to be relatively common and of moderate risk only, the incrimination will be difficult.

We conclude that the overwhelming cause of cancer in these twin populations was nonshared environment, accounting for some 70% of all cancer. Analytical and molecular epidemiological studies provide tools to identify and quantify risk factors contributing to environmental effects. Etiologic clues may even be found in childhood environment or in long-lasting family habits, because the shared environment appeared to contribute to some forms of cancer in which common environmental risk factors have not been identified, including those of the gallbladder and corpus uteri. The most challenging result, however, was the large heritability of all cancer and of colorectal, breast, and prostate cancer in particular. The twin method probably detected recessive and polygenic cancers that are difficult to detect in other types of family studies. If these proportions were reliable, they would reveal major gaps in our understanding of the molecular basis of heritable cancer.

Polygenic Cancer and Future Study Designs. The data presented on twins, families, and second cancers provide additional support for the multistage theory of carcinogenesis. However, overall, direct support for the theory is limited even though a common belief is that it has been proven long since. If most cancers are indeed polygenic, this should be adequately considered in study designs for gene-mapping approaches. Linkage analysis in families of multiple affected individuals does not suit polygenic diseases well, but it is still the main approach in attempts to identify cancer-related genes. Instead, large case-control studies on well-defined patient series may be more powerful (Risch and Merikangas, 1996). The studies can be carried out on patients without regard to family history because otherwise the selection causes bias toward monogenic or oligogenic dominant cancers. Monozygotic twins and patients with multiple cancers would be very suitable study population, but identification of such patients may be cumbersome.

EPIDEMIOLOGY AND GENETIC SUSCEPTIBILITY TO BREAST CANCER[3]

Brian E. Henderson, M.D. and Kenneth T. Norris, Jr.

In 2000, it is estimated that 184,200 women in the United States will be diagnosed with breast cancer, reflecting the high incidence rates now experienced by many racial–ethnic groups including African American, Japanese, and white women. Much lower rates of breast cancer are found in multiethnic cohort (MEC) Latina women, due especially to its extremely low incidence in first-generation migrants. The increase in breast cancer rates for Asian women born in the United States compared to traditional Asian women (Parkin et al., 1992; Zeigler et al., 1993) is striking. While much of this increase may be explained by their transition to a more Western lifestyle (e.g., decrease in age of menarche and use of hormone replacement therapy), the fact that their cancer rate is as high as other racial–ethnic groups despite their much lower postmenopausal weight (Probst-Hensch et al., 2000) suggests that both genetic and environmental differences may be important.

We have had a long-standing interest in the role of sex steroids in the etiology of breast cancer, especially related to estrogen and, more recently, progestin stimulation of the breast. This interest has been driven by the premise that estrogen and progestin are the primary determinants of cell proliferation in breast epithelium and that cell proliferation is a prerequisite for many of the genetic changes necessary for a cell to transform to a malignant phenotype. The strong, consistent association between a woman's menstrual history and breast cancer risk implicates lifetime exposure to sex steroid hormones as a major factor in the causation of breast cancer. A recent meta-analysis of epidemiological studies implicated estrogens more directly, by showing that circulating levels of the biologically most potent estrogen, estradiol (E_2), are significantly higher in breast cancer patients compared to controls (Thomas et al., 1997). Moreover, we have found that plasma estrogen levels differ by racial–ethnic group and that these differences appear to contribute to racial–ethnic variation in breast cancer rates (Probst-Hensch et al., 1999). We (and others) have recently found that exogenous exposure to these steroids, as combined estrogen and progestin replacement therapy, also substantially increases the risk of breast cancer (Ross et al., 2000). Androgens, as a precursor of estrogen biosynthesis or by direct action, also may contribute to breast carcinogenesis (Dorgan et al., 1997; Hankinson et al., 1998b).

Three biosynthesis genes (*CYP17*, *CYP19*, *HSD17B1*) form the foundation of our current activities. These genes are important rate-limiting factors in estro-

[3]This work is support by NCI grant numbers CA63464 and CA54281.

gen production. While our current focus is on estrogen biosynthesis, variants in other candidate genes in estrogen and progesterone biosynthesis, transport, metabolism, and binding are being explored jointly with our colleagues at the Whitehead Institute–Massachusetts Institute of Technology (MIT).

The *CYP17* C allele is associated with elevated endogenous estrogen levels in both pre- and postmenopausal women, and based on data from the MEC we found that women carrying the C allele were at an increased risk of advanced breast cancer (Feigelson et al., 1997). Several polymorphisms have been identified in *HSD17B1*, including a common polymorphism in exon 6 that results in an amino acid change from serine (allele *S*) to glycine (allele *G*) at position 312 (Normand et al., 1993; Mannermaa et al., 1994). Although current evidence indicates that this amino acid change may not affect the catalytic or immunologic properties of the enzyme (Puranen et al., 1994), an early report suggested that individuals who are homozygous for *A* are at increased risk of breast cancer (Mannermaa et al., 1994). Among MEC participants, in a preliminary analysis, women homozygous for allele *A* have been found to be 38% more likely to develop advanced-stage breast cancer than women homozygous for *G*.

CYP17 and HSD17B1. In a combined analysis of *CYP17* and *HSD17B1*, we found that carrying one or more "high-risk" allele(s) increases the risk of advanced breast cancer in a dose–response fashion and that this relationship could be explained by endogenous serum estrogen levels (Siegelmann-Danieli and Buetow, 1999). The relative risk among women carrying four high-risk alleles for *CYP17* and *HSD17B1 (C/C/S/S)* was 2.76 compared to those carrying no high-risk alleles. Using an alternative model in which the risk of breast cancer was estimated per unit change in serum estrogen as predicted by genotype (using other data sources), we found an RR of 1.76 for the *C/C/S/S* genotype with comparable adequacy of fit to our data. We plan to extend these studies in order to evaluate this relationship within each racial–ethnic group and in relationship to circulating levels of sex steroid hormones and binding proteins (E_1[estrone], E_2, bioavailable E_2, SHBG, estrone sulfate, androstenedione, dehydroepiandrosterone [DHEA], DHEA sulfate, 5-androstene-, and androstenediol) in prediagnostic bloods collected in both Los Angeles and Hawaii.

CYP19. The *CYP19* gene codes for the aromatase enzyme. Aromatase catalyzes the irreversible conversion of androstenedione to E_1 and testosterone to E_2. Tissue-specific aromatase expression is determined in part by tissue-specific promoters, which give rise to transcripts with unique 5'-noncoding termini that correspond to untranslated sections of exon I. These specific 5'-termini sequences are all spliced onto exon II at the common 3'-splice junction. The association of obesity with postmenopausal blood estrogen levels as well as breast cancer risk may at least in part be mediated by an increased aromatization of androstenedi-

one in the adipose tissue of obese women (Huang et al., 1997; Siegelmann-Danieli and Buetow, 1999). Several polymorphisms have been described in the *CYP19* gene (Sourdaine et al., 1994; Probst-Hensch et al., 1999; Healy et al., 2000). The functional relevance is unknown for all of the polymorphisms identified so far. The only polymorphism in the coding region of *CYP19* leading to a nonconservative amino acid substitution (exon VII, *Arg264Cys*) has no apparent effect on aromatase activity or response to aromatase inhibitors (Watanabe et al., 1997; Kristensen et al., 1998), nor has it been associated with breast cancer risk (Kristensen et al., 1998; Probst-Hensch et al., 1999). However, a possible role of a tetranucleotide repeat polymorphism of unknown functional relevance in breast cancer has been suggested by several studies (Rasmussen and Cullen, 1998; Haiman et al., 1999; Siegelmann-Danieli and Buetow, 1999), but we and others could not confirm this (Sourdaine et al., 1994; Probst-Hensch et al., 1999). The search for functionally relevant polymorphisms in *CYP19* is currently focused on the regulatory regions of the gene, and we are studying these regions through collaborations with Dr. Nicole Probst at the University of Basel and Dr. Sue Ingles at the University of Southern California.

GH–IGF Pathway. Insulin-like growth factors (e.g., IGF-1) also cause epithelial breast cells to divide, and it is hypothesized that the mitogenic effect of estrogen may be mediated through an IGF-1 signaling pathway (Holly, 1998). Members of the growth hormone–IGF axis (GH–IGF) may exert a direct effect on breast cancer risk or potentiate the effects of estrogens on breast epithelium (Oh, 1998). IGFs have been recognized as major regulators of mammary epithelium and breast cancer cell growth (Clarke et al., 1997) and act as mitogens, as well as potent survival factors. In experimental studies, E_2 has been found to stimulate breast epithelial cell proliferation in normal human breast tissue xenografted into athymic mice via a paracrine mechanism involving IGFs (Hankinson et al., 1998a). Epidemiologic studies support a strong positive association between IGF-1, IGF-binding protein-3 (IGFBP-3), and breast cancer risk (Bohlke et al., 1998; Byrne et al., 2000), with increased mammographic density being an important breast cancer risk factor (Rosen et al., 1998). Initial findings have been limited to premenopausal breast cancer. Among postmenopausal control women in the MEC, we found an association between circulating levels of IGF-1 and breast cancer incidence by racial–ethnic group. In the MEC, we will explore the relationship between breast cancer risk, plasma IGF (free IGF-1, IGF-1, IGF-2, IGFBP-3) levels in prediagnostic bloods, and genetic variants in the GH–IGF axis. A microsatellite repeat *(CA)* in the *IGF-1* gene approximately 1 kilobase (kb) upstream from the transcription start site has been associated with lower mean levels of plasma IGF-1 (Rosen et al., 1998), and we are currently exploring the association between plasma IGF-1, the microsatellite repeat, and breast cancer risk among women in the MEC. We also will genotype these same women for a *G* to *C* transversion (exon I, position 2132) in the *IGFBP-3* gene, of

yet unknown functional relevance, that may serve as a marker for IGFBP-3 plasma levels. Moreover, we will expand our investigation of candidate genes in the GH–IGF pathway through our collaboration with the Whitehead Institute–MIT.

While we have focused our major research effort on estrogen biosynthesis, genetic variation, and breast cancer risk, we have concomitantly evaluated germline missense variants in genes involved in breast cancer progression. We have confirmed a previous observation that a missense variant (Ile 655 Val) in Her-2/neu is associated with breast cancer risk and that this variant affects stages at diagnosis (McKean-Cowdin et al., in progress). We are pursuing similar missense variants in BRCA1, BRCA2, and AT.

MIGRANT FARMWORKER CHILDREN AT HIGH RISK FOR PESTICIDE EXPOSURE

María A. Hernández-Valero, Dr.P.H.

There are approximately 3–6 million migrant and/or seasonal farmworkers (MSFs) in the United States, appoximately 85–90% are from ethnic and racial minorities (e.g., Hispanics, African Americans). Hispanics of Mexican descent constitute the majority (approximately 92%) of the MSF population, with children and adolescents comprising 20–25% of the total number.

The majority of the agricultural studies have been conducted among farm owners and operators, while cancer research among MSF is almost nonexistent. The lack of research is often attributed to the perceived difficulty in conducting epidemiological studies among this underrepresented population. This is true even though MSFs are chronically exposed to pesticides and other agricultural exposures, sometimes starting at a very young age when susceptibility may be of great importance.

MSF children are chronically exposed to pesticides via their parents' occupation, including application drift; overspray; carry-home exposures from parents; exposure in utero; breast-feeding; going to or working in the fields with their parents; and the foods they eat.

A pilot feasibility study was conducted to measure 21 organochlorine pesticides (OCPs) and correlate the measured levels with reported exposures in 62 Mexican American MSFs (36 children and 26 adults) home-based in the Houston metropolitan area. The pilot sutdy objectives were to (1) provide quantitative measures of OCP levels in the serum of MSF adults and children; (2) obtain epidemiological data on work histories, field exposures, pregnancy, lactation, medical histories, and other factors from the standardized bilingual Migrant Farmworker Questionnaire developed by the National Cancer Institute; and (3) correlate the epidemiological data with the measured OCP levels. The referent laboratory's detection limit for OCPs was 0.3 ng/ml (parts per billion [ppb]).

The study population was composed of 42% males and 78% females. The majority of the children or adolescents in the study were born in the United States (89%), with an average age of 12.4 ± 7.5. The opposite was observed among the adults who for the most part were born in Mexico (73%), with an average age of 45.4 ± 12.1. Two OCPs and/or metabolites (DDE and mirex) were detected in most of the samples tested. Five other OCPs were also detected in adults only (DDT, b-HBC, d-HBC, g-chlordane and oxychlordane). The average DDE serum level for the children was low (1.6 ± 1.6 ppb); the adults' level was higher than expected (15.4 ± 17.2 ppb), almost five times higher than the referent laboratory population's average (3.2 ± 1.8). The average mirex levels among both adults and children were almost identical (adults 1.8 ± 0.6, children 1.7 ± 0.7) and were also higher than expected, approximately eight times higher than the mean levels measured in the referent population (<0.3, nondetectable).

In conclusion, traces of OCPs that had been banned in the United States for many decades are still measured in the serum of MSFs. OCPs, although decreasing in use, may still pose a threat to MSF children through their continued use and their persistence in the environment and body tissues. Since MSF children are potentially exposed to pesticides, both occupationally and environmentally through several pathways, there is a need to monitor this high-risk population. It may be necessary to legislate stricter public health measures aimed at reducing pesticide exposure among MSF children, and restricting these children from working or entering the fields with their parents. In regards to future research, (1) MSF children need to be included in prospective cohort studies to prove scientifically whether or not chronic exposure to pesticides from childhood into adulthood places humans at risk of developing deleterious health outcomes, including cancer during childhood and later in life, (2) the measurement of OCP levels should be included in ongoing and future studies; and also (3) the dietary intake of MSF children should be studied to evaluate the possible synergistic action of diet and pesticide exposure.

CHEMICALS AND CHROMOSOMES, CHILDREN AND CANCER, CLUSTERS AND COHORTS IN A NEW CENTURY

Richard Jackson, M.D., M.P.H.

Eighty-six percent of the U.S. population says that environmental factors are either important or very important in causing disease. Thus, it shouldn't be surprising to researchers and policymakers that a cancer cluster is assumed (by the general public) to be environmental until proven otherwise. Cancer clusters can be the bane of existence for many state and local health officers and have been called the epidemiologists fool's gold—the idea being that you shouldn't spend your time chasing them because they never pan out. However, if one uses good

communication skills, science, medicine, and policy, there are tremendous opportunities to meet public health needs and achieve good outcomes.

I studied many disease clusters during my time in the Public Health Department in California, a state that uses many types of pesticides. In fact, California uses about a quarter of the nation's pesticides, and at least 5% of all the pesticides in the world. The uses of these pesticides become important as we discuss water pollution, air pollution, agriculture, economics, and workers' health. We as researchers and government officials have had and continue to have many problems that hamper our ability to handle clusters effectively, including: lack of obligatory reporting of pesticides, unless they are Category One—extremely toxic; data gaps on particular chemicals—chemicals that were thought to be safe but turned out to have health effects; and lack of disease registries to ascertain the baseline levels of a disease in a given region. Without this information, it is hard to be able to answer fundamental questions. Clusters also require a community relations specialists who not only speaks their language, but is able to understand their issues.

In the future, approaching disease clusters will require collection of disease rates in registries because if we don't know the baseline level of any disease, it is impossible to know if the reported cluster is a statistical aberration or not. We also need better tracking of exposures, because the more accurately you document people's precise level of exposures, the more accurately you can calibrate risks. The Centers for Disease Control and Prevention (CDC) has just started to release a report on a series of chemicals in the American people. We will continue adding 25 chemicals each year until we have a total of 100 in four years. We are moving beyond the traditional two-by-two table investigations where we look at exposed, unexposed, disease, no disease. The era of looking at exposures and not looking at the genetic makeup of the individual in our epidemic studies is over. Trying to tease out nature versus nurture is a recipe for disaster. We need to be able to look at both of these at the same time.

HEALTH DISPARITIES: DO GENE–ENVIRONMENT INTERACTIONS PLAY A ROLE?

Lovell Jones, Ph.D.

When we discuss health disparities, I often remember the saying "if you always do what you have always done, we will always get what we already got." When we approach the efforts to address the lack of measurable progress in tackling health disparities, we tend to fall back on what we did before. It may be under a different name or it may be packaged in a different box, but ultimately it is the same strategy.

In a recent study, people questioned whether health disparities are "real."

Consider the fact that breast cancer in Hispanic females has tripled over the last 15 years. African American females under the age of 35 have a 50% higher incidence of breast cancer than white females. These are just two examples. We need to understand that these are not access issues—access to health care does not play a role in incidence. It is not an issue of poverty because it crosses all socioeconomic sectors. These disparities are growing and are becoming a major problem in this nation. If these trends and incidences were reported for white females, every alarm in this nation would be going off.

- What about other ethnic groups and other forms of cancer? Here are a few examples:
- Vietnamese women have cervical cancers at nearly five times the rate of white females.
- African American men under age 65 have nearly twice the rate of prostate cancer of white males.
- Hispanics, Native Americans, and Alaskan Natives are nearly twice as likely as Caucasians to have diabetes.
- In Pacific Islanders, certain cancers are 60 times more prevalent than in Caucasians.

As we begin to address these disparities in terms of genetics and gene–environment interactions, we will have to answer what role race and ethnicity will play. We need to understand that racial classifications are a social construct and not a biological construct. Yet racial classification still has an impact on the health of this nation. In the United States, we use the one-drop rule. If you have one drop of blood or if someone could trace one drop of African American bloods, you are African American—no matter your phenotype. Just as not all African Americans, Hispanics, and Asians may be similar culturally (within their ethnic groups), they may not have the same phenotypes. Most importantly, as we continue our research into gene–environment interactions, we need to remember that one size does not fit all.

DIET AND OTHER ENVIRONMENTAL FACTORS AS MODIFIERS OF CANCER RISK

John A. Milner, Ph.D.

The last decade has witnessed important advances in the understanding of factors that influence cancer risk. Several environmental factors continue to surface as potentially instrumental in explaining the wide global variation in the incidence and biological behavior of tumors. Undeniably, exposure to environmental agents and/or ultraviolet radiation may contribute to oxidative stress or other biochemical events that foster uncontrolled cell proliferation. Clearly, diet

is a significant environmental factor to which an individual is continually exposed. Although an individual's diet may serve as a protector against the potentially lethal effects of reactive oxygen species and toxic environmental chemicals, under some circumstances it may also be a significant source of deleterious compounds. Thus, an individual's diet may increase or decrease cancer risk depending on its composition.

A variety of linkages between diet habits and cancer risk have surfaced in both epidemiological and preclinical studies. Some of the most compelling evidence linking diet and cancer comes from the epidemiological observation that increased vegetable and fruit consumption is associated with a reduction in the risk for cancers of the mouth and pharynx, esophagus, lung, stomach, colon, and rectum. Likewise considerable preclinical evidence points to a host of essential and nonessential nutrients as modifiers of cancer risk at a variety of sites. Part of this variation in cancer risk may arise from variation in the intake of one or more essential nutrients supplied by either plant or animal food sources. Vegetables derived from various parts of plants including roots (e.g., carrots, parsnips), leaves (e.g., spinach, lettuce), flowers (e.g., artichoke, broccoli), stalks (e.g., celery, rhubarb), and seeds (e.g., corn, peas), as well as a host of fruits, provide thousands of chemically diverse phytonutrients that may contribute to these observations. Some of these phytonutrients, including flavonoids, carotenoids, organosulfides, and isothiocyanates, have been the focus of recent research to determine both their effects on risk and their mechanism(s) of action.

While the risk of developing several cancers has been linked with dietary patterns, frequent inconsistencies are noted. These inconsistencies may reflect the multifactorial and complex nature of cancer, the specificity that individual dietary constituents have in modifying specific genetic pathways, and the temporal relationship between dietary intervention and phenotypic changes in tumor incidence or behavior. Again, the complexity of defining the precise role of diet is magnified by the numerous and diverse essential (i.e., folate, selenium, vitamin E, n-3 fatty acids, and calcium) and nonessential (i.e., oligofructose, allyl sulfurs, carotenoids, flavonoids, and isothiocyanates) components that may alter one or more phases of the cancer process and the temporal and compensatory responses to these dietary constituents. Because of the chemical and biological diversity of dietary components and the range of molecular targets, the elucidation of the importance of diet is proving to be not only an exciting undertaking, but also an immense challenge.

The past decade has witnessed great strides in understanding the biological basis of cancer. Discoveries that both essential and nonessential dietary nutrients can markedly influence several key biological events including cell cycle regulation, processes involved with replication/or transcription, immunocompetence, and factors involved with apoptosis have strengthened convictions that specific foods and/or components may markedly influence cancer risk. Unraveling which dietary component is most important in establishing cancer risk is a daunting

task but should be easier with new gene and protein technologies. For instance, limonene (a monoterpene found particularly in citrus fruits) addition to tumor cells has been reported to enhance 42 genes and suppress another 58 genes. Since several of the identified genes are involved in the mitoinhibitory transforming growth factor (TGF) signal transduction pathway, this provides support for the hypothesis that monoterpenes may initiate mitoinhibitory and apoptotic signaling through a signal transduction-related mechanism. Similarly, studies have been undertaken with a variety of other nutrients including selenium, isothiocyanates and allyl sulfide. Dietary isothiocyanates; have been reported to modify at least 20 different gene products associated with cancer prevention. Knockout and transgenic animals are beginning to provide clues to the specific site of action of specific dietary components and should be used more extensively as tools for probing mechanisms of action. It should be noted that dietary allyl sulfur compounds can lead to a number of changes that may influence overall cancer risk including blocking nitrosamine formation, retarding carcinogen bioactivation, blocking cell division, promoting apoptosis, and retarding angiogenesis. Recent studies from our laboratory found that diallyl disulfide compounds lead to marked changes in more than 20 genes associated with the cancer process. Collectively these studies reveal that individual dietary components are capable of bringing about a host of intracellular changes that may influence cancer risk. The utilization of genomic technologies to evaluate the effects of nutrients offers hope in determining which cellular change is most important in bringing about a change in the incidence or behavior of a tumor. It should be noted that preclinical evidence suggests that diverse dietary constituents including selenium, allyl sulfur, genistein, and resveratrol can influence the same genetic pathways. Such common effects raise concerns about potential interactive and cumulative effects among nutrients. Thus, a reductionist approach to understanding the role of diet in cancer prevention may produce oversimplifications and confusion.

Preclinical evidence exists that such diverse dietary components as folate, allyl sulfur, genistein, and resveratrol can alter genes and pathways associated with tumor cell proliferation and apoptosis. Part of this protection may relate to their ability to prevent oxidative damage. Compounds suppressing oxidative stress have been reported to produce changes in c-fos, c-jun, and c-myc and the tumor suppressor gene p53, as well as genes coding for the syntheses of protective molecules such as metallothioneins, glutathione, and stress proteins. Astonishing strides have been made in understanding how molecules and genetic pathways differ in precancerous and malignant cells and from their normal counterparts. Capitalizing on the differences in cellular signatures that are characterized by active and inactive genes and cellular products should assist in determining who should and should not benefit from intervention strategies. Clearly such information will help clarify the reason for discrepancies among preclinical, epidemiological, and intervention studies.

At least part of this variation in response to dietary components may relate to the consumer's genetic profile. It is now becoming apparent that the prevalence of polymorphisms is variable among studied populations and these differences could influence the response to diet. For example, in a random sample of participants in the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study (ATBC Study), there was a low prevalence of polymorphisms in genes coding for activation (phase I) enzymes CYP1A1 (0.07) and CYP2E1 (0.02) and a high prevalence in genes coding for detoxification (phase II) enzymes GSTM1 (0.40) and NQO1 (0.20). Evidence exists that several genetic polymorphisms may modulate cancer risk through their influence on folate metabolism, including two polymorphisms of the *MTHFR* gene C677 C–T (alanine –valine) and 1298 A–C (glutamate–alanine) and a polymorphism of *MTR*, the gene that codes for methionine synthase C2756 A–G (aspartate–glycine); all of these polymorphisms reduce enzyme activity. Epidemiologic studies have reported that when folate intake was adequate, colorectal cancer risk was reduced (abou 50%) in individuals with the *MTHFR* 677TT genotype compared with the *MTHFR* 677CC genotype, and the risk of adult acute lymphocytic leukemia (ALL) was reduced by 77%. Variation in the response to folate metabolism is not unique since other studies suggest that variation in receptors for vitamin D may also be linked to cancer risk. Considerably more information is needed about how genetic polymorphisms influence the response to dietary components and ultimately cancer risk.

Unquestionably cancer is intertwined with environmental factors including diet. Strategies to prevent cancer through modification of either diet or specific dietary patterns, although intriguing and likely a low-cost health care strategy, will probably not be uniformly effective for all individuals. A better understanding of gene–nutrient interactions will be needed to unravel who might benefit most from dietary intervention and who might be placed at risk. Future research in nutrition and cancer prevention must give top priority to studies that seek to understand the basic molecular and genetic mechanisms by which nutrients influence the various steps in carcinogenesis. While the challenges to researchers will be enormous, the potential rewards in terms of reducing cancer morbidity and mortality will be of an equally great magnitude.

MOLECULAR PATHOGENESIS OF LUNG CANCER

John D. Minna, M.D.

We and others have hypothesized that clinically evident lung cancers have accumulated 10–20 different genetic abnormalities in dominant oncogenes and/or tumor suppressor genes (TSGs) (Sekido et al., 1998; Fong et al., 1999). If true, this hypothesis has important ramifications for the clinic. For example, it should be possible to discover carcinogen-exposed respiratory epithelial cells

with only a subset of these changes and intervene with very early treatment and/or chemoprevention. A related hypothesis is that these changes are recurrent and common between different tumors. If true, this has implications for directing the search for specific diagnostic and therapeutic targets and indicating the likelihood that all of the changes are required for the malignant phenotype. There have been many studies published on searching for genetic abnormalities in lung cancer (Sekido et al., 1998; Fong et al., 1999). However, with few exceptions, these studies have not been global in nature either in testing for genome-wide abnormalities or in testing for multiple abnormalities in the same individual lung cancer. To approach these two hypothesis in a global and quantitative fashion, we have performed a high resolution (10 cM) genome-wide search for loss of heterozygosity (LOH, allele loss) in 36 lung cancer cell lines using 399 polymorphic markers. Individual tumors averaged 17–22 chromosomal regions involved in frequent, recurrent allele loss ("hot spots"), and these regions were significantly different between small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC) (Girard et al., 2000). On average, 35% of the markers showed allele loss in individual tumors, with an average size of subchromosomal regions of loss of 50–60 cM. We found 22 different regions with more than 60% LOH, 13 with a preference for SCLC, 7 for NSCLC, and 2 affecting both histologic types. This provides clear evidence on a genome-wide scale that SCLC and NSCLC differ significantly in the TSGs that are inactivated during their pathogenesis. However, in all other aspects (e.g. fractional allele loss, number of breakpoints, number of microsatellite alterations) SCLC and NSCLC were not significantly different. The chromosomal arms with the most frequent LOH were 1p, 3p, 4p,4q, 5q, 8p, 9p (*p16*), 9q, 10p, 10q, 13q (*Rb*), 15q, 17p (*p53*), 18q, 19p, Xp, and Xq.

We next conducted detailed high-density allelotyping (~5-cm level) on chromosome arms 3p, 4p, 4q, and 8p (average of 25 markers per chromosome arm) in 66 microdissected primary archival lung cancers (22 SCLC, 21 squamous, 22 adenocarcinomas), as well as microdissected respiratory preneoplastic lesions from patients with lung cancers and from cigarette smokers (Shivapurkar et al., 1999; Wistuba et al., 1999b, 2000a). Allelic losses of 3p were found in 96% of lung cancers and in 78% of the preneoplastic/or preinvasive lesions. The 3p allele losses were often multiple and discontinuous, with areas of LOH interspersed with areas of retention of heterozygosity. There was progressive increase in the frequency and size of 3p allele loss regions with increasing severity of histopathological preneoplastic/preinvasive changes. Analysis of all of the data indicated multiple regions of localized 3p allele loss. A panel of six markers in the 600-kb 3p21.3 homozygous deletion region showed loss in 77% of lung cancers (100% SCLC, 100% squamous, 90% adenocarcinomas), 70% of normal or preneoplastic/preinvasive lesions associated with lung cancer, and 49% of 47 normal, mildly abnormal, or preneoplastic/preinvasive lesions found in smokers without lung cancer (Wistuba et al., 2000a). This was in contrast to 0% loss in 18

epithelial samples from seven never smokers. We have identified all of the genes in this completely sequenced region, and several of them appear to suppress the tumorigenic phenotype when introduced back into lung cancers with multiple other genetic lesions (Lerman and Minna, 2000). Of these, the best studied is the *RASSF1A* mRNA isoform of the RASSF1 locus (Dammann et al., 2000; Burbee et al., 2001). This gene is rarely mutated in lung cancer, but its expression is lost by promoter-acquired hypermethylation in ~90% of SCLCs and 30–40% of NSCLCs. Methylation in NSCLCs is associated with adverse survival and treatment of lung cancer cells with 5-azacytidine reactivates RASSF1A expression. This isoform contains a RAS binding domain and a putative diacylglycerol binding domain and suppresses the tumorigenic phenotype of lung cancer cells (Dammann et al., 2000; Burbee et al., 2001). This 600-kb region and the 3p14.2 (*FHIT*/*FRA3B*) and 3p12 (U2020/*DUTT1*) regions were common, independent sites of breakpoints (Wistuba et al, 2000a). We conclude that 3p allele loss is nearly universal in lung cancer pathogenesis; involves multiple, discrete, 3p LOH sites that often show a "discontinuous LOH" pattern in individual tumors; occurs in preneoplastic/preinvasive lesions of smokers with and without lung cancer; and frequently involves breakpoints in at least three very small, defined genomic regions. These findings are consistent with previously reported LOH studies in a variety of tumors showing allele loss occurring by mitotic recombination and induced by oxidative damage. Similarly, high frequencies of LOH (86% SCLC, 100% squamous, 81% adenocarcinomas) for the 8p21–23 regions were detected in primary lung cancers (Wistuba et al., 1999b). The LOH commenced early during the multistage development of lung cancer at the hyperplasia/metaplasia stage in cancer patients and in smokers without cancer. Of interest, 8p21–23 allelic losses always followed 3p and usually followed 9p allele loss. In contrast to 3p LOH, no 8p LOH was found in histologically normal epithelium; however, 15% of mildly abnormal, 50% of dysplastic, and 92% of carcinoma in situ lesions had 8p21–23 LOH. Allelic loss occurred in 65% of smokers without cancer and persisted for up to 48 years after smoking cessation. Frequent LOH of 4p and 4q markers was seen in SCLC and mesotheliomas (Region 1 4q33–34 >80%, Region 2 4q25–26 >60%, and Region 3 4p15.1–15.3 >50%) but was much less frequent (20–30%) in NSCLC where the most frequent pattern was loss of Region 3 alone (Shivapurkar et al., 1999). For 3p, the regions of loss in SCLC and squamous cancers were usually quite large, often involving multiple markers, whereas those in adenocarcinomas were much smaller and usually involved only one or two markers. SCLC had significantly higher frequency of 4p and 4q allele loss (two separate regions on each arm) than did NSCLC (Shivapurkar et al., 1999). The converse was seen with the 8p allelotype (Wistuba et al., 1999b).

Tumor-acquired promoter methylation is a new, important mutational mechanism for inactivating TSGs. We found tumor-acquired aberrant promoter methylation in nine genes in 107 resected primary NSCLCs (*RAR beta* 40%, *TIMP-3* 26%, *p16ink4a* 25%, *MGMT* 21%, *FHIT* 37%, *DAPK* 19%, *ECAD* 18%,

p14ARF 8%, and *GSTP1* 7%) (Park et al., 1999; Virmani et al., 2000; Zochbauer-Muller, et al., 2001a, 2001b). At least one gene was methylated in 86% of NSCLCs, whereas normal lung from these same patients was methylated in only a few patients. In addition, we found 63/87 (72%) of SCLCs to exhibit *RAR* promoter hypermethylation. The methylation events occurred independently of one another. However, about 13% of the NSCLCs exhibited more frequent promoter hypermethylation and thus are candidates for having a "global CpG island methylator phenotype."

In nonmalignant bronchial epithelium 218 foci (195 of histologically normal or slightly abnormal epithelium and 23 of dysplastic epithelium) were studied from 19 surgically resected lobectomy specimens (Park et al., 1999). Thirteen (68%) of the nineteen specimens had at least one focus of bronchial epithelium with molecular changes. At least one molecular abnormality was detected in 32% of the 195 histologically normal or slightly abnormal foci and in 52% of the 23 dysplastic foci. Extrapolating from a two-dimensional analysis, we estimate that most clonal patches contain approximately 90,000 cells. Although, in a given individual, tumors appeared homogeneous with respect to molecular changes, the clonally altered patches of mildly abnormal epithelium were heterogeneous. Our findings indicate that multiple small clonal or subclonal patches containing molecular abnormalities are present in normal or slightly abnormal bronchial epithelium of patients with lung cancer. In detailed studies of bronchial epithelium and bronchial biopsies from current or former smokers without lung cancer, we also find thousands of clonal patches showing allele loss in histologically normal-appearing respiratory epithelium. In fact, these patches can be detected more than 30 years after cessation of cigarette smoking. This would suggest the potential for damaged stem cells to repopulate.

We also investigated the relationship between the amount of smoking and the degree of methylation of the *p16*, *RASSF1A*, *RAR, APC*, and *HCAD(CDH13)* genes in more than 200 resected NSCLCs from Japan with known smoking history. We found that p16 and RASSF1A developed promoter hypermethylation related to the amount of cigarette smoking, while *RAR*, *APC*, and *HCAD(CDH13)* methylation occurred independently of the amount of cigarette smoking (Zöchbauer-Müller et al., in preparation).

To investigate whether methylation of genes such as *RAR*, *HCAD(CDH13)*, *p16*, *RASSF1A*, and *FHIT* occurs in smoking-damaged epithelium before lung cancer develops, we analyzed oropharyngeal brushes, sputum samples, bronchial brushes, and bronchoalveolar lavage (BAL) samples from more than 100 heavy smokers without evidence of cancer (Zochbauer-Muller et al., 2001a). Methylation of at least one gene was present in one or more specimens from nearly 50% of the smokers. However, the frequency of methylation of these genes found in the epithelial samples from heavy smokers was lower than the frequency found in primary lung cancers. These findings indicate that promoter-acquired methy-

lation should be tested as an intermediate marker of risk assessment and response to chemoprevention.

Small cell lung cancer has many distinct morphologic and biochemical features (such as neuroendocrine phenotype) distinguishing it from the non-small cell lung cancer histologic types (Sekido et al., 1998, 2001; Fong et al., 1999). These distinctions are of diagnostic importance and commit patients with different histologic types to quite different initial treatment regimens. With the exception of bronchoalveolar lung cancer, all histologic types have smoking and tobacco carcinogens as the major underlying etiologic factor. Clearly, SCLC etiology is strongly tied to cigarette smoking. Therefore, we have sought to answer the following major questions: Are there differences in the number or type of acquired molecular abnormalities between SCLC and NSLC; what are the specific genes involved; and what is the nature of the molecular changes that are found in smoking-damaged bronchial epithelium accompanying SCLC and NSLC? Finally, are there gene expression profiles that distinguish these two major histologic types?

There is a wealth of information concerning molecular abnormalities in SCLC (Fong et al., 1999; Sekido et al., 2001). *Ras* mutations represent an obvious difference, they are found in ~30% of NSCLCs (predominantly adenocarcinomas) but, to our knowledge, have never been found in SCLCs (with more than 100 tumors analyzed). In fact, introducing a mutant *ras* allele into SCLCs in vitro has led to an alteration of the cellular phenotype to one more like NSCLC. A related component in the same signal transduction pathway is Her2/neu, which is overexpressed in ~30% of NSCLCs but rarely overexpressed in SCLCs. We have recently found a related signal pathway activation difference for the ERK/MAP kinase pathway. While ERK1 and ERK2 proteins are expressed in all histologic types of lung cancer, we find constitutive activation (detection of the "active" phosphorylated forms of ERK1 and ERK2 using specific antibodies) in 80% of NSCLCs but <5% of SCLCs. Autocrine growth factors involving neuroendocrine regulatory peptides (e.g., bombesin–gastrin-releasing peptide) were first described for SCLC. However, recently it has become clear that both SCLC and NSCLC can express these peptides and their specific receptors (Sekido et al., 1998, 2001; Fong et al., 1999). While there are some differences related to histology (e.g., expression of neuromedin B in NSCLCs), it appears that both histologic types use this mechanisms. *Myc* oncogenes are overexpressed frequently in both SCLC and NSCLC. *C-myc* is overexpressed in both SCLC and NSCLC, but the overexpression of *myc* family members L-*myc* and N-*myc* is usually only found in SCLC.

The p53 gene is frequently mutated in both SCLC and NSCLC, but this occurs in >90% of SCLCs and ~50% of NSCLCs. The other components of the p53 pathway (such as MDM2 and p14ARF) need to be studied. In comparing the type of mutations occurring in p53, there appear to be no differences (e.g., in nucleotide-type change or location in the p53 open reading frame) between SCLC

and NSCLC, providing evidence that the carcinogenic insult was similar. Another major difference is seen in the RB/p16 signaling pathway. This pathway is inactivated in the vast majority of all histologic types of lung cancer. However, the target mutated various dramatically between histologic types. In SCLC, *Rb* is inactivated in >90% of cases, with loss of protein expression usually occurring with truncating mutations. It is very rare for SCLCs to have mutations inactivating the expression of *p16*. In contrast, NSCLCs inactivate p16 expression in ~50% of cases, while loss of expression of Rb protein occurs in <20% (Sekido et al., 1998). There appear to be no differences in mutational frequencies for inactivation of *FHIT* occurring in 50–70% of all lung cancers (Fong et al., 1999; Sekido et al., 2001).

Finally, we have looked at the bronchial epithelium accompanying SCLC and NSCLC for the occurrence of clonal alterations using precise laser capture microdissection with subsequent allelotyping for polymorphic markers (Wistuba et al., 2000b). In NSCLC, we frequently find clones of cells with molecular abnormalities in histologically affected epithelium (e.g., carcinoma in situ, dysplasia, hyperplasia) and occasionally in normal-appearing epithelium in the case of current or former smokers. In SCLC, these histologic preneoplastic changes were minimal. However, in studies of histologically normal respiratory epithelium, we found a severalfold increased rate of allele loss in SCLC compared to NSCLC patients. Thus, the smoking-damaged histologically normal epithelium associated with SCLC appeared "genetically scrambled" and had incurred significantly more damage than the epithelium accompanying NSCLCs. We conclude that SCLCs and NSCLCs do not differ significantly in the number of genetic alterations that occur, however SCLCs do differ significantly from NSCLCs in the specific genetic alterations that occur. In addition, smoking-damaged bronchial epithelium accompanying SCLCs appears to have undergone significantly more acquired genetic damage than that accompanying NSCLCs. Future studies need to identify the specific genes involved at these multiple sites and determine whether these provide new tools for early molecular detection, for monitoring of chemoprevention efforts, and as specific targets for developing new therapies.

We conclude from our global and quantitative studies that clinically evident lung cancers have acquired 20 or more clonal genetic alterations; SCLC and NSCLC have acquired different genetic lesions; alterations in 3p TSGs appear especially early, followed by changes in 9p, 8p, and then multiple other sites; tumor-acquired promoter hypermethylation is a frequent mutational mechanism in lung cancer; changes consistent with oxidative damage leading to mitotic recombination are frequently seen; smoking-damaged histologically normal epithelium, as well as epithelium with preneoplastic/preinvasive changes, has thousands of clonal patches containing genetic alterations; and correcting even single genetic abnormalities can reverse the malignant phenotype. All of these observa-

tions are ready for translation into the clinic for new methods of diagnosis, risk assessment, prevention, and treatment.

BREAST CANCER GENETICS: BRCA1 AND BRCA2 GENES

Olufunmilayo Olopade, M.D.

Breast cancer is a genetic disease, caused by spontaneous mutations in somatic cells or by germline inheritance of mutations in breast cancer susceptibility genes. Germline mutations in the *BRCA1* or *BRCA2* susceptibility genes result in breast cancers characterized by young age of onset, bilaterality, association with ovarian cancer and other tumor types, vertical transmission, and distinct tumor phenotypes. Because breast cancer develops in 37–85% of women that carry *BRCA1* or *BRCA2* mutations, genetic testing of individuals with a high risk of familial breast cancer is an important part of a cancer control effort. Somatic genomic rearrangements that cause breast cancer include amplification of the *HER-2*/neu gene, which is associated with a poor prognosis, relative resistance to chemotherapy and tamoxifen, and sensitivity to Herceptin. Detection of *HER-2*/neu amplification in tumors is therefore an important factor in prognosis and choice of therapies. These examples reveal the clinical value of addressing the genetic basis of cancer and illustrate the importance of understanding genetic mechanisms in developing methods of cancer prevention, early detection, and targeted therapies.

TYPES AND TRENDS OF CHILDHOOD CANCER; CANCER IN CHILDHOOD CANCER SURVIVORS

Leslie Robison, Ph.D.

In the United States, cancer is the leading cause of death due to disease among individuals between the ages of 1 and 20. The annual incidence rate is 15/100,000, which translates into a cumulative risk of cancer equivalent to 1 in 300 by the age of 20 years. The types of malignancies in children and adolescents differ from adults and include (annual rate per million and proportion): leukemia (37, 24.8%); lymphoma (24, 16.1%); brain and central nervous system (CNS) (25, 16.8%); neuroblastomoa (7, 4.7%); retinoblastoma (3, 2.0%); kidney, predominantly Wilms' tumor (6, 4.0%); liver, predominaently hepatoblastoma (2, 1.3%); bone, primarily osteoscarcoma; and Ewing's sarcoma (9, 6.0%); soft tissue, predominaently rhabdomyosarcoma (11, 7.4%); germ cell (10, 6.7%); and others (15, 10.0%).

There are distinct age-specific patterns of incidence; most notable are the peaks in incidence of acute lymphoblastic leukemia that occur between the age

of 3 and 6 years; the aggregation of neuroblastoma, retinoblastoma, and Wilms' tumor in children below the age of 5; the increasing incidence with age of lymphoma; and the relatively constant incidence of brain and CNS malignancies. Overall, males have a higher rate of malignancies than females, which is attributable primarily to a higher incidence among males of lymphomas and acute lympohoblastic leukemia. In the 15–20 years of age group, females have a higher incidence of cancer than males. Further, the annual incidence (per million population) of childhood and adolescent cancers differs by race: Caucasian (161.7), black (124.6), Hispanic (145.6), Asian/Pacific Islander (136.8), and Native American (79.6).

Observations during the past several decades have identified a modest, but consistent, increase in the incidence of childhood cancers. Secular trends have varied with specific categories, but the most consistent increases have been seen in acute leukemia and tumors of the central nervous system.

The survival rate for childhood and adolescent cancer has increased dramatically during the past three decades. Currently, more than 70% of individuals diagnosed with cancer before age 15 will survive five or more years from diagnosis, with the majority being cured of their original malignancy. With these improvements in treatment and survival, it is estimated that approximately 1 in every 900 individuals in the United States between the age of 15 and 45 is now a survivor of childhood cancer. These survivors are, however, at increased risk for long-term complications of their initial cancer and subsequent therapy. Late sequelae of childhood cancer can include an increased risk of second and subsequent malignancies, as well as serious organ dysfunction and psychosocial effects. As more patients survive and the length of follow-up grows, patterns of second and subsequent malignancies are being identified in survivors, including increased rates of breast cancer, thyroid malignancies, CNS tumors, and leukemia.

CANCER TREATMENT BASED ON IMMUNE STIMULATION

Steven Rosenberg, M.D., Ph.D.

Tumor infiltrating lymphocytes (TILs) obtained from patients with melanoma have been used to clone the genes encoding the antigens recognized by these TILs. TILs have been identified that can recognize unique cancer antigens on murine and human cancers including melanoma, breast cancer, colon cancer, and lymphoma. The major histocompatibility complex MHC restricted recognition of human cancer antigens was detected by assaying panels of human leukocyte antigen (HLA)-typed target cells and by transfection into target cells of genes encoding the appropriate HLA specificities. In clinical trials of TILs administration, 36% of patients with metastatic melanoma underwent objective cancer remission. TILs trafficked to and accumulated in cancer deposits.

GENETIC SUSCEPTIBILITY TO LUNG CANCER[4]

Margaret R. Spitz, M.D.

Less than 20% of long-term smokers develop lung cancer by age 75. Genetically determined factors that abrogate the effects of environmental carcinogens may explain differences in susceptibility. The challenge in quantitative risk assessment is to account for this interindividual variation in susceptibility to carcinogens. Evidence of familial aggregation of lung cancer provides indirect support for the role of genetic predisposition to lung cancer. These patterns of inheritance studies suggest that a small proportion of lung cancer is due to "lung cancer genes" that are probably of low frequency, but high penetrance. However, the study of low-penetrance, high-frequency genes is likely to be more useful in elucidating the causal pathways for the vast majority of lung cancers.

Lung cancer risk is dependent on the dose of tobacco carcinogens, which in turn is modulated by genetic polymorphisms in the enzymes responsible for carcinogen activation (e.g., myeloperoxidase) and detoxification (e.g., glutathione s-transferases), as well as by the efficiency of the host cells in monitoring and repairing tobacco carcinogen DNA damage. Individuals with susceptible genotypes (or adverse phenotypes) tend to develop lung cancer at earlier ages and with lower levels of tobacco exposure. On the other hand, the genetic component in risk tends to be lower at high dose levels, when environmental influences overpower genetic predisposition.

We have applied phenotypic assays to measure DNA repair capacity (DRC) by means of the in vitro host cell reactivation assay using plasmids damaged with benzo[*a*]pyrene. DRC was significantly lower in cases than controls, lower in women compared to men, and lower in younger compared to older cases. There was a statistically significant trend for increasing risk with decreasing DRC and an odds ratio (OR) of 2.54 ($P < 0.05$) for lung cancer in the least efficient repair stratum. The mutagen sensitivity assay, in which in vitro mutagen-induced breaks are quantitated as a measure of carcinogen sensitivity, has also been identified as a significant risk factor for lung cancer. Mutagens used are bleomycin and benzo[*a*]pyrene. Higher risk estimates are evident for current compared to former smokers and lighter smokers (less than one pack per day) compared to heavier smokers. There was a dose–response relationship with adjusted ORs for increasing quartiles of induced chromatid breaks for both bleomycin sensitivity and benzo[*a*]pyrene sensitivity (trend $P < 0.0001$). On stratified analyses, the risk for both adverse phenotypes (suboptimal DRC and mutagen sensitivity) was fivefold.

[4]Supported by National Cancer Institute grants CA55769 (to M.R.S.) and CA 68437 (to W.K.H.).

Genotype–phenotype and diet–gene interactions are also being studied intensively. For example, while the GSTM1 null genotype was not an apparent independent risk factor for lung cancer, in the presence of low isothiocyanate intake, the OR for the GSTM1 null genotype was 2.33. There was no increased risk in any stratum for former smokers.

It is most likely that multiple susceptibility factors must be accounted for to represent the true dimensions of gene–environment interactions. In the near future, microarray technology will facilitate the performance of large-scale, low-cost genotyping. The ethical, educational, social, and informatics considerations that will result are challenging. However, the ability to identify smokers with the highest risks of developing cancer has substantial preventive implications for intensive screening and smoking cessation interventions and for enrollment into chemoprevention trials.

ENVIRONMENT AND BREAST CANCER

Mary S. Wolff, Ph.D.

Wide variations are seen in the incidence of breast cancer, both internationally and nationally among ethnic groups. In the United States, Hispanic women have lower rates of breast cancer than black and white women, and lower rates than Japanese Americans but higher than other women of Asian ancestry. Genetics, diet, and reproductive factors do not explain all of the differences. Indeed, women with inherited genetic predisposition may never suffer from breast cancer, just as not all smokers get lung cancer. Environmental exposures have been implicated, but few specific agents are strongly related to breast cancer. However, individuals respond differently to exposures in terms of their innate ability to metabolize chemicals. Therefore, diet, lifestyle, and adverse exposures must collaborate with susceptibility factors to incur risk for breast cancer.

Breast cancer etiology is complex because tumorigenesis can arise from a combination of many different mechanisms over a very long time. Because breast cancer risk is strongly associated with reproductive hormones, any role for environmental exposures must act in concert with endogenous hormones. Environment, genes, and hormones must work together at specific end points—extending from perinatal mammary cell development, to onset of puberty, through birth, lactation, and menopause—following the course of tumor progression and, after diagnosis, prognosis for recurrence.

In this age of generally low exposures, relative risks for main effects are low, and many environmental exposures and genetic variants alone are not strong risk factors for breast cancer. Combinations of exposures may obscure exposure–disease relationships. Crucial exposures as well as critical reproductive end points may have happened years before a tumor is found. The second generation of studies will address whether complex genetic factors, hormonal milieu, or

dietary intakes alter environmental risk factors. Such effects may be responsible for differences in breast cancer among racial and ethnic groups who may have risks related to genetic polymorphisms and excessive exposures.

PRIORITIES AND SPECIAL POPULATIONS: TIES THAT BIND

Armin Weinberg, Ph.D.

It becomes clear as we examine charts and data on health such as the those in Healthy People 2000 that socioeconomic status (SES) plays a role in cancer and many other health care issues. Individuals and groups with a higher SES (1) can obtain better housing, (2) can live in better neighborhoods, (3) have opportunities to engage in healthy behaviors, (4) have better access to health care, and (5) can more readily participate in clinical trials. Thus, in order to understand clinical data as it relates to gene–environment interactions, we need to include analysis of the communities and SES.

Studies today use descriptive phrases such as "special populations," "priority populations," and "vulnerable populations." These terms are used extensively in research and discussions, but there is a great diversity of opinion as to their definition. As we continue to use these phrases in our communities, research will have to refine the definitions to better describe—not label—these groups. Additionally, they will have to provide a certain degree of flexibility to accommodate the subgroups that emerge.

More than 2,500 individuals immigrate to the United States each day—a trend that complicates gene–environment research. Many of these individuals are from Latin America and Mexico, as well as other countries throughout the world. As we start to include these foreign-born residents in our studies, we must pay attention to the fact that these individuals are mostly young and have had different exposures in their country of origin at a time when they were most vulnerable. Additionally, migratory patterns have shifted in the United States as individuals from different countries or regions of a country immigrate distinctly to geographic regions in the United States.

The NCI's national special population networks have been formed to consider factors related to these and other issues. The steering committee for one network Radas En Acum, which has research sites in California, Illinois, New York, Florida, and Texas, was formed to help establish both a research agenda and research priorities. In addition to the genetics and the gene–environment issues, language and health literacy will require special attention. As we try to close the gap in enrollment in clinical trials, these other issues will become more important. We will have to be sensitive to cultural differences in these communities and ensure that material is available in many languages. Further, as we talk to communities about gene–environment interactions, we must communicate what this means, what we want, and what we hope to learn.

References

Achiwa H, Yatabe Y, Hida T, Kuroishi T, Kozaki K, Nakamura S, Ogawa M, Sugiura T, Mitsudomi T, Takahashi T. 1999. Prognostic significance of elevated cyclooxygenase 2 expression in primary, resected lung adenocarcinomas. *Clinical Cancer Research* 5(5):1001–1005.

Adams RL, Burdon RH. 1982. DNA methylation in eukaryotes. *Critical Reviews in Biochemistry and Molecular Biology* 13:349–384.

Aguilar F, Hussain SP, Cerutti P. 1993. Aflatoxin B1 induces the transversion of GÆT in codon 249 of the p53 tumor suppressor gene in human hepatocytes. *Proceedings of the National Academy of Sciences* 90(18):8586–8590.

Aguilar F, Harris CC, Sun T, Hollstein M, Cemtti, P. 1994. Geographic variation of p53 mutational profile in nonmalignant human liver. *Science* 264:1317–1319.

Ambs S, Merriam WG, Ogunfusika MO, Bennett WP, Ishibe N, Hussain SP, Tzeng EE, Geller DA, Billiar TR, Hanis CC. 1998a. p53 and vascular endothelial growth factor regulate tumour growth of NOS2-expressing human carcinoma cells. *Nature Medicine* 4:1371–1376.

Ambs S, Ogunfusika MO, Merriam WG, Bennett WP, Billiar TR, Harris CC. 1998b. Upregulation of NOS2 expression in cancer-prone p53 knockout mice. *Proceedings of the National Academy of Sciences* 95:8823–8828.

Ambs S, Bennett WP, Merriam WG, Ogunfusika MO, Oser SM, Shields PG, Felley-Bosco E, Hussain SP, Ranis CC. 1999. Characteristic p53 mutations correlate with inducible NO synthase expression in human colorectal cancer. *Journal of the National Cancer Institute* 91:86–88.

Ames BN, Shigenaga MK, Gold LS. 1993. DNA lesions, inducible DNA repair, and cell division: Three key factors in mutagenesis and carcinogenesis. *Environmental Health Perspectives* 101(5):35–44.

Armitage P, Doll R. 1954. The age distribution of cancer and a multi-stage theory of carcinogenesis. *British Journal of Cancer* 8:1–12.

Barclay BJ, Kunz BA, Little JG, Haynes RH. 1982. Genetic and biochemical consequences of thymidylate stress. *Canadian Journal of Biochemistry* 60:172–184.

Baron JA, Sandler RS, Haile RW, Mandel JS, Mott LA, Greenberg ER. 1998. Folate intake, alcohol consumption, cigarette smoking, and risk of colorectal adenomas. *Journal of the National Cancer Institute* 90:57–62.

Bartek J, Bartkova J, Vojtesek B, Staskova Z, Rejthar A, Kovarik J, Lane DP. 1990. Patterns of expression of the p53 tumour suppressor in human breast tissues and tumours in situ and in vitro. *International Journal of Cancer* 46(5):839–844.

Baylin SB, Herman JG, Graff JR, Vertino PM, Issa JP. 1998. Alterations in DNA methylation: A fundamental aspect of neoplasia. *Advances in Cancer Research* 72:141–196.

Benn J, Schneider RJ. 1994. Hepatitis B virus HBx protein activates Ras-GTP complex formation and establishes a Ras, Raf, MAP kinase signaling cascade. *Proceedings of the National Academy of Sciences* 91:10350–10354.

Bennett WP, Hollstein MC, Metcalf RA, Welsh JA, He A, Zhu S, Kusters I, Resau JH, Trump BF, Lane DP, Harris CC. 1992. p53 mutation and protein accumulation during multistage human esophageal carcinogenesis. *Cancer Research* 52:6092–6097.

Bird A. 1992. The essentials of DNA methylation. *Cell* 70:5–8.

Bohlke K, Cramer DW, Trichopoulos D, Mantzoros CS. 1998. Insulin-like growth factor-I in relation to premenopausal ductal carcinoma in situ of the breast. *Epidemiology* 9(5):570–573.

Bohr VA, Anson RM. 1995. DNA damage, mutation and fine structure DNA repair in aging. *Mutational Research* 338:25–34.

Brash DE, Rudolpht JA, Simon JA, Lin A, McKenna GJ, Badent HP, Halperin AI, Ponten J. 1991. A role for sunlight in skin cancer: UV-induced p53 mutations in squamous cell carcinoma. *Proceedings of the National Academy of Sciences* 88:10124–10128.

Burbee D, Forgacs E, Zöchbauer-Müller S, Shivakuma L, Fong K, Gao B, Randle D, Virmani A, Bader S, Sekido Y, Latif F, Milchgrub S, Gazdar A, Lerman M, Zabarovsky E, White M, Minna J. 2001. RASSF1A in the 3p21.3 homozygous deletion region: Epigenetic inactivation in lung and breast cancer and suppression of the malignant phenotype. *Journal of the National Cancer Institute* 93:(in press).

Byrne C, Colditz G, Willett F, Speizer F, Pollak M, Hankinson S. 2000. Plasma insulin-like growth factor (IGF) I, IGF-binding protein-3, and mammographic density. *Cancer Research* 60(14): 3744–3748.

Caselmann WH, Meyer M, Kekule AS, Lauer U, Hofschneider PH, Koshy R, 1990. A trans-activator function is generated by integration of hepatitis B virus preS/S sequences in human hepatocellular carcinoma DNA. *Proceedings of the National Academy of Sciences* 87:2970–2974.

Cedar H. 1988. DNA methylation and gene activity. *Cell* 53:3–4.

Chen J, Giovannucci E, Kelsey K, Rimm EB, Stampfer MJ, Colditz GA, Spiegelman D, Willett WC, Hunter DJ. 1996. A methylenetetrahydrofolate reductase polymorphism and the risk of colorectal cancer. *Cancer Research* 56:4862–4864.

Chen J, Giovannucci E, Hankinson SE, Ma J, Willett WC, Spiegelman D, Kelsy KT, Hunter DJ. 1998. A prospective study of methylenetetrahydrofolate reductase and methionine synthase gene polymorphims, and risk of colorectal adenoma. *Carcinogenesis* 19:2129–2132.

Clarke RB, Howell A, Anderson E. 1997. Type I insulin-like growth factor receptor gene expression in normal human breast tissue treated with oestrogen and progesterone. *British Journal of Cancer* 75:251–257.

Coffey DS. 1993. Prostate cancer. An overview of an increasing dilemma. *Cancer* 71(3):880–886.

Coffey DS. 2001. Similarities of prostate and breast cancer: Evolution, diet and estrogens. *Urology* 57(4A):31–38.

Dammann R, Li C, Yoon JH, Chin PL, Bates S, Pfeifer GP. 2000. Epigenetic inactivation of a RAS association domain family protein from the lung tumour suppressor locus 3p21.3. *Nature Genetics* 25:315–319.

Davidoff AM, Humphrey PA, Iglehar ID, Marks JR. 1991. Genetic basis for p53 overexpression in human breast cancer. *Proceedings of the National Academy of Sciences* 88:5006–5010.

Davidow AL, Neugut AI, Jacobson JS, Habibul A, Garbowski GC, Forde KA, Treat MR, Waye JD. 1996. Recurrent adenomatous polyps and body mass index. *Cancer Epidemiology, Biomarkers, and Prevention* 5:313–315.

Dianov GL, Timchencko TV, Sinitsina OI, Kuzminov AV, Medvedev OA, Salganik RI. 1991. Repair of uracil residues closely spaced on opposite strands of plasmid DNA results in double-strand break and deletion formation. *Molecular Genetics and Genomics* 225:448–452.

Dong C, Hemminki K. 2001. Multiple primary cancers at colon, breast and skin (melanoma) as models for polygenic cancers. *International Journal of Cancer* (in press).

Dorgan JF, Stanczyk FZ, Longcope C, Stephenson HEJ, Chang L, Miller R, Franz C, Falk RT, Kahle L. 1997. Relationship of serum dehyroepiandrosterone (DHEA), DHEA sulfate, and 5-androstene-3 beta, 17 beta-diol to risk of breast cancer in postmenopausal women. *Cancer Epidemiology, Biomarkers, and Prevention* 6:177–181.

Elmore LW, Hancock AR, Chang SF, Wang XW, Chang S, Callahan CP, Geller DA, Will H, Harris CC. 1997. Hepatitis B virus X protein and p53 tumor suppressor interactions in the modulation of apoptosis. *Proceedings of the National Academy of Sciences* 94:14707–14712.

Eto I, Krumdieck CL. 1986. Role of vitamin B12 and folate deficiencies in carcinogenesis. *Advances in Experimental Medicine and Biology* 206:313–330.

Evans MK, Taffe BG, Harris CC, Bohr VA. 1993. DNA strand bias in the repair of the p53 gene in normal human and xeroderma pigmentosum group C fibroblasts. *Cancer Research* 53:5377–5381.

Everson RB, Wehr CM, Erexson GL, MacGregor JT. 1988. Association of marginal folate depletion with increased human chromosomal damage in vivo: Demonstration by analysis of micronucleated erythrocytes. *Journal of the National Cancer Institute* 80:525–529.

Fearon ER. 1997. Human cancer syndromes: Clues to the origin and nature of cancer. *Science* 278:1043–1050.

Feigelson HS, Coetzee GA, Kolonel LN, Ross RK, Henderson BE. 1997. A polymorphism in the CYP17 gene increases the risk of breast cancer. *Cancer Research* 57:1063–1065.

Fenech M, Rinaldi J. 1994. The relationship between micronuclei in human lymphocytes and plasma levels of vitamin C, vitamin E, vitamin B12 and folic acid. *Carcinogenesis* 15:1405–1411.

Ferraroni M, La Vecchia C, D'Avanzo B, Negri E, Franceschi S, Decarli A. 1994. Selected micronutrient intake and the risk of colorectal cancer. *British Journal of Cancer* 70:1150–1155.

Fong KM, Sekido Y, Minna JD. 1999. Molecular pathogenesis of lung cancer. *Journal of Thoracic and Cardiovascular Surgery* 118:1136–1152.

Forrester K, Lupold SE, Ott VL, Chay CH, Band V, Wang XW, Harris CC. 1995. Effects of p53 mutants on wild-type p53-mediated transactivation are cell type dependent. *Oncogene* 10:2103–2111.

Forrester K, Ambs S, Lupold SE, Kapust RB, Spillare EA, Weinberg WC, Felley-Bosco E, Wang XW, Geller DA, Billiar TR, Harris CC. 1996. Nitric oxide-induced p53 accumulation and regulation of inducible nitric oxide synthase (NOS2) expression by wild-type p53. *Proceedings of the National Academy of Sciences* 93:2442–2447.

Friedell GH, Tucker TC, Ross FE. 1999. The impact of poverty and education on lung and cervical cancer in Appalachian Kentucky. *Journal of Registry Management* 26:125–127.

Frosst P, Blom HJ, Milos R, Goyette P, Sheppard CA, Matthews RG, Boers GJH, den Heijer M, Kluijtmans LAJ, van den Heuvel LP, Rozen R. 1995. A candidate genetic risk factor for vascular disease: A common mutation in methylenetetrahydrofolate reductase (letter). *Nature Genetics* 10:111–113.

Gertig DM, Hunter DJ. 1998. Genes and environment in the etiology of colorectal cancer. *Seminars in Cancer Biology* 8:285–298.

Giovannucci E, Stampfer MJ, Colditz GA, Rimm EB, Trichopolous D, Rosner BA, Speizer FE, Willett WC. 1993. Folate, methionine, and alcohol intake and risk of colorectal adenoma. *Journal of the National Cancer Institute* 85:875–884.

Giovannucci E, Rimm EB, Ascherio A, Stampfer MJ, Colditz GA, Willett WC. 1995. Alcohol, low-methionine–low-folate diets, and risk of colon cancer in men. *Journal of the National Cancer Institute* 87:265–273.

Giovannucci E, Stampfer MJ, Colditz GA, Hunter DJ, Fuchs C, Rosner BA, Speizer FE, Willett WC. 1998. Multivitamin use, folate, and colon cancer in women in the Nurses' Health Study. *Annals of Internal Medicine* 129:517–524.

Girard L, Zöchbauer-Muller S, Virmani A, Gazdar A, Minna J. 2000. Genome-wide allelotyping of lung cancer identifies new regions of allelic loss, differences between SCLC and NSCLC, and loci clustering. *Cancer Research* 60:4894–4906.

Glynn SA, Albanes D, Pietinen P, Brown CC, Rautalahti M, Tangrea JA, Gunter EW, Barrett MJ, Virtamo J, Taylor PR. 1996. Colorectal cancer and folate status: A nested case-control study among male smokers. *Cancer Epidemiololgy, Biomarkers, and Prevention* 5:487–494.

Griffiths K, Morton MS, and Dennis LJ (eds). Diet and Prostate Disease. 1998. Oxford, United Kingdom, International Prostate Council.

Guinee DG, Travis WD, Trivers GE, DeBenedetti VM, Cawley HM, Welsh IA, Bennett WP, Jett I, Colby TV, Tazelaar H, Abbonanzo SL, Pairolero P, Trastek V, Caporaso NE, Liotta LA, Harris CC. 1995. Gender comparisons in human lung cancer: Analysis of p53 mutations, anti-p53 serum antibodies and C-erbB-2 expression. *Carcinogenesis* 16:993–1002.

Haiman CA, Hankinson SE, Spiegelman D, Colditz G, Willett WC, Speizer FE, Kelsey KT, Hunter D. 1999. The relationship between a polymorphism in CYP17 with plasma hormone levels and breast cancer. *Cancer Research* 59:1015–1020.

Hankinson SE, Willet WC, Colditz GA, Hunter DJ, Michaud DS, Deroo B, Rosner B, Speizer FE, Pollak M. 1998a. Circulating concentrations of insulin-like growth factor-1 and risk of breast cancer. *Lancet* 351(9113):1393–1398.

Hankinson SE, Willett WC, Manson JE, Colditz GA, Hunter DJ, Spiegelman D, Barbieri RL, Speizer FE. 1998b. Plasma sex steroid levels and risk of breast cancer in postmenopausal women. *Journal of the National Cancer Institute* 90:1292–1299.

Harris CC. 1991. Chemical and physical carcinogenesis: Advances and perspectives. *Cancer Research* 51:5023s–5044s.

Harris CC. 1996. Structure and function of the p53 tumor suppressor gene:clues for rational cancer therapeutic strategies. *Journal of the National Cancer Institute* 88:1442–1455.

Harris CC, Hollstein M. 1993. Clinical implications of the p53 tumor-suppressor gene. *New England Journal of Medicine* 329:1318–1327.

Healey CS, Dunning AM, Durocher F, Teare D, Pharoah PDP, Luben RN, Easton DF, Ponder BA. 2000. Polymorphisms in the human aromatase cytochrome P450 gene (CYP19) and breast cancer risk. *Carcinogenesis* 21:189–193.

Hemminki K, Mutanen P. 2001. Genetic epidemiology of multistage carcinogenesis. *Mutatational Research* 473:11–21.

Hemminki K, Vaittinen P. 1999. Familial cancers in a nation-wide family-cancer database: Age distribution and prevalence. *European Journal of Cancer* 35:1109–1111.

Hemminki K, Lönnstedt I, Vaittinen P, Lichtenstein P. 2001. Estimation of genetic and environmental components in colorectal and lung cancer and melanoma. *Genetic Epidemiology* 20:107–116.

Hino O, Nomura K, Ohtake K, Kawaguchi T, Sugano H, Kitagawa T. 1989. Instability of integrated hepatitis B virus DNA with inverted repeat structure in a transgenic mouse. *Cancer Genetics and Cytogenetics* 37:273–278.

Hino O, Tabata S, Hotta Y. 1991. Evidence for increased in vitro recombination with insertion of human hepatitis B virus DNA. *Proceedings of the National Academy of Sciences* 88:9248–9252.

Hoffman RM. 1985. Altered methionine metabolism and transmethylation in cancer. *Anticancer Research* 5:1–30.

Hollstein M, Marion MJ, Lehman T, Welsh J, Harris CC, Martel-Planche G, Kusters I, Montesano R. 1994. p53 mutations at A:T base pairs in angiosarcomas of vinyl chloride-exposed factory workers. *Carcinogenesis* 15:1–3.

Holly J. 1998. Insulin-like growth factor-I and new opportunities for cancer prevention. *Lancet* 351:1373–1375.

Hsu IC, Metcalf RA, Sun T, Welsh JA, Wang NJ, Harris CC. 1991. Mutational hotspot in the p53 gene in human hepatocellular carcinomas. *Nature* 350(6317):427–428.

Huang, Z., Hankinson SE, Colditz G, Stampfer MJ, Hunter DJ, Manson JE, Hennekens CH, Rosner B, Speizer FE, Willett WC. 1997. Dual effects of weight and weight gain on breast cancer risk. *Journal of the American Medical Association* 278:1407–1411.

Hunting D, Dresler S. 1985. Dependence of u.v.-induced DNA excision repair in deoxyribonucleoside triphosphate concentrations in permeable human fibroblasts: A model for the inhibition of repair by hydroxyurea. *Carcinogenesis* (London) 6:1525–1528.

Hussain SP, Harris CC. 1998a. Molecular epidemiology of human cancer. *Recent Results in Cancer Research* 154:22–36.

Hussain SP, Harris CC. 1998b. Molecular epidemiology of human cancer: Contribution of mutation spectra studies of tumor suppressor genes. *Cancer Research* 58:4023–4037.

Hyman J, Baron JA, Dain BJ, Sandler RS, Haile RW, Mandel JS, Mott LA, Greenberg EG. 1998. Dietary and supplemental calcium and the recurrence of colorectal adenomas. *Cancer Epidemiology, Biomarkers, and Prevention* 7:291–295.

James SJ, Cross DR, Miller BJ. 1992. Alterations in nucleotide pools in rats fed diets deficient in choline, methionine and/or folic acid. *Carcinogenesis* 13:2471–2474.

James S, Basnakian A, Miller B. 1994. In vitro folate deficiency induces deoxynucleotide pool imbalance, apoptosis, and mutagenesis in Chinese hamster ovary cells. *Cancer Research* 54:5075–5080.

Jia L, Wang XW, Harris CC. 1999. Hepatitis B virus X protein inhibits nucleotide excision repair. *International Journal of Cancer* 80:875–879.

Jones PA. 1996. DNA methylation errors and cancer. *Cancer Research* 56:2463–2467.

Kekule AS, Lauer U, Meyer M, Caselmann WH, Hofschneider PH, Koshy R. 1990. The preS2/S region of integrated hepatitis B virus DNA encodes a transcriptional transactivator. *Nature* 343:457–461.

Kim Y-I, Pogribny IP, Salomon RN, Choi SW, Smith DE, James SJ, Mason JB. 1996. Exon-specific DNA hypomethylation of the p53 gene of rat colon induced by dimethylhydrazine. Modulation by dietary folate. *American Journal of Pathology* 149:1129–1137.

Kim Y-I., Pogribny IP, Basnakian AG, Miller JW, Selhub J, James SJ, Mason JB. 1997. Folate deficiency in rats induces DNA strand breaks and hyp omethylation within the p53 tumor suppressor gene. *The American Journal of Clinical Nutrition* 65:46–52.

Kinzler K, Vogelstain B. 1996. Lessons from hereditary colorectal cancer. *Cell* 87:159–170.

Knudson AG. 1996. Hereditary Cancer: Two hits revisited. *Journal of Cancer Research and Clinical Oncology* 122(3):135–140.

Kristensen VN, Andersen TI, Lindblom A, Erikstein B, Magnus P, Borresen-Dale AL. 1998. A rare CYP19 (aromatase) variant may increase the risk of breast cancer. *Pharmacogenetics* 8:43–48.

Kutzbach C, Stokstad E. 1971. Mammalian methylenetetrahydrofolate reductase. Partial purification, properties, and inhibition by S-adenosylmethionine. *Biochimica et Biophysica Acta* 250:459–477.

Landis SH, Murray T, Bolden S, Wingo PA. 1999. Cancer statistics. *CA:A Cancer Journal for Clinicians* 49:8–11.

Lerman M, Minna J. 2000. The 630-kb lung cancer homozygous deletion region on human chromosome 3p21.3: Identification and evaluation of the resident candidate tumor suppressor genes. The International Lung Cancer Chromosome 3p21.3 Tumor Suppressor Gene Consortium. *Cancer Research* 60:6116–6133.

Lichtenstein P, Holm N, Verkasalo P, Illiado A, Kaprio J, Koskenvuo M, Pukkala E, Skytthe A, Hemminki K. 2000. Environmental and heritable factors in the causation of cancer. *New England Journal of Medicine* 343:78–85.

Lynch H, Fusaro R, Lynch J. 1995, Hereditary cancer in adults. *Cancer Detection and Prevention* 19:219–233.

Ma J, Stampfer MJ, Giovannucci E, Artigas C, Hunter DJ, Fuchs C, Willett WC, Selhub J, Hennekens CH, Rozen R. 1997. Methylenetetrahydrofolate reductase polymorphism, dietary interactions and risk of colorectal cancer. *Cancer Research* 57:1098–1102.

Mace K, Aguilar F, Wang JS, Vautravers P, Gomez-Lechon M, Gonzalea FJ, Groopman J, Harris CC, Pfeifer AMA. 1997. Aflatoxin B1 induced DNA adduct formation and p53 mutations in CYP450-expressing human liver cell lines. *Carcinogenesis* 18:1291–1297.

Maguire HF, Hoeffler JP, Siddiqui A. 1991. HBV X protein alters the DNA binding specificity of CREB and ATF-2 by protein-protein interactions. *Science* 252:842–844.

Mannermaa A, Peltoketo H, Winqvist R, Ponder B, Kiviniemi H, Easton D, Poutanen M, Isomaa V, Vihko R. 1994. Human familial and sporadic breast cancer: Analysis of the coding regions of the 17beta-hydroxysteroid dehydrogenase 2 gene (EDH17B2) using a single-strand conformation polymorphism assay. *Human Genetics* 93:319–324.

Martinez ME, Giuliano AR, Marshall JR, Kramer CB, Alberts DS. 2001 Plasma homocysteine and recurrence of adenomatous polyps. *Proceedings of the American Association of Cancer Research* 42:821.

Mayr E. 1982. *The Growth of Biological Thought*. Cambridge, MA: Belknap Press.

McKean-Cowdin R, Kolonel LN, Press M, Pike MC, Henderson BE. 2001. Germline Her-2 variant and breast cancer risk by stage of disease. *Cancer Research* 6:8393–8396.

Meuth M. 1981. Role of deoxynucleoside triphosphate pools in the cytotoxic and mutagenic effects of DNA alkylating agents. *Somatic Cell Genetics* 7:89–102.

NCI (National Cancer Institute). 2001. *Bypass Budget Proposal for Fiscal Year 2002*. Bethesda, MD: NCI.

Neugut A, Garbowski G, Lee W, Murray T, Nieves J, Forde K, Treat M, Waye J, Fenoglio-Preiser C. 1993. Dietary risk factors for the incidence and recurrence of colorectal adenomatous polyps: A case-control study. *Annals of Internal Medicine* 118:91–95.

Newell, GR. 1988. Social characteristics of minorities and the underprivileged. *Cancer Bulletin* 40: 105–107.

Normand T, Narod S, Labrie F, Simard J. 1993. Detection of polymorphisms in the estradiol 17beta-hydroxysteroid dehydrogenase 2 gene at the EDH17B2 locus on 17q11-q21. *Human Molecular Genetics* 2:479–483.

Nuorva K, Soini Y, Kamel D, Autio-Harmainen H, Risteli L, Risteli J, Vahakangas K, Paakko P. 1993. Concurrent p53 expression in bronchial dysplasias and squamous cell lung carcinomas. *American Journal of Pathology* 142:725–732.

Oh Y. 1998. IGF-independent regulation of breast cancer growth by IGF binding proteins. *Breast Cancer Research and Treatment* 47:283–293.

Park IW, Wistuba II, Maitra A, Milchgrub S, Virmani AK, Minna JD, Gazdar AF. 1999. Multiple clonal abnormalities in the bronchial epithelium of patients with lung cancer. *Journal of the National Cancer Institute* 91:1863–1868.

Parkin, DM, Muir CS, Whelan SL (eds). 1992. *Cancer Incidence in Five Continents*. Vol. VI. Lyon: IARC.

Paterlini P, Poussin K, Kew M, Franco D, Brechot C. 1995. Selective accumulation of the X transcript of hepatitis B virus in patients negative for hepatitis B surface antigen with hepatocellular carcinoma. *Hepatology* 21:313–321.

Pereira, FP. 1998. Molecular epidemiology of environmental carcinogenesis. *Recent Results in Cancer Research*, 154:39–46.

Perela FP. 1997. Environment and cancer: Who are susceptible? *Science* 278:1068–1073.

Perera FP, Santella R. 1993. *Carcinogenesis, Molecular Epidemiology: Principles and Practices*. Schulte P, Perera FP (eds.). New York: Academic Press.

Peto J, Collins N, Barfoot R, Seal S, Warren W, Rahman N, Easton D, Evans C, Deacon J, Stratton M. 1999. Prevalence of BRCA1 and BRCA2 gene mutations in patients with early-onset breast cancer. *Journal of the National Cancer Institute* 91:943–949.

Pirisi L, Yasumoto S, Feller M, Doniger J, DiPaolo JA. 1987. Transformation of human fibroblasts and keratinocytes with human papillomavirus type 16 DNA. *Journal of Virology* 61:1061–1066.

Ponchel F, Puisieux A, Tabone E, Michot JP, Froschl G, Morel AP, Frebourg T, Fontaniere B, Oberhammer F, Ozturk M. 1994. Hepatocarcinoma-specific mutant p53-249ser induces mitotic activity but has no effect on transforming growth factor beta 1-mediated apoptosis. *Cancer Research* 54:2064–2068.

Ponder B. 1997. Genetic testing for cancer risk. *Science* 278:1050–1054.

Probst-Hensch NM, Ingles SA, Diep AT, Haile RW, Stanczyk FZ, Kolonel LN, Henderson BE. 1999. Aromatase and breast cancer susceptibility. *Endocrine-Related Cancer* 6:165–173.

Probst-Hensch NM, McKean-Cowdin R, De Lellis K, Ingles SA, Stanczyk FZ, Kolonel LN, Henderson BE. 2000. IGF system and breast cancer in postmenopausal women of different ethnic backgrounds. *Proceedings of the American Assocication for Cancer Research* 41:816.

Puranen T, Poutanen M, Peltoketo H, Vihko P, Vihko R. 1994. Site-directed mutagenesis of the putative active site of human 17beta-hydroxysteroid dehydrogenase type 1. *The Biochemical Journal* 304:289–293.

Rasmussen AA, Cullen KJ. 1998. Paracrine/autocrine regulation of breast cancer by the insulin-like growth factors. *Breast Cancer Research and Treatment* 47(3):219–233.

Razin A, Riggs AD. 1980. DNA methylation and gene function. *Science* 210:604–610.

Reidy JA. 1987. Deoxyuridine increases folate-sensitive fragile site expression in human lymphocytes. *American Journal of Medical Genetics* 26:1–5.

Risch N, Merikangas K. 1996, The future of genetic studies of complex diseases. *Science* 273:1516–1572.

Rosen CJ, Kurland ES, Vereault D, Adler RA, Rackoff PJ, Craig WY, Witte S, Rogers J, Bilezikian JP. 1998. Association between serum insulin growth factor-I (IGF-I) and a simple sequence repeat in IGF-I gene: Implications for genetic studies of bone mineral density. *The Journal of Clinical Endocrinology and Metabolism* 83:2286–2290.

Ross RK, Pike MC, Coetzee GA, Reichardt JK, Yu MC, Feigelson H, Stanczyk FZ, Kolonel LN, Henderson BE. 1998. Androgen metabolism and prostate cancer: Establishing a model of genetic susceptibility. *Cancer Research* 58(20):4497–4504.

Ross RK, Paganini-Hill A, Wan PC, Pike MC. 2000. Effect of hormone replacement therapy on breast cancer risk: Estrogen versus estrogen plus progestin. *Journal of the National Cancer Institute* 92(4):328–332.

Sager R. 1997. Expression genetics in cancer: Shifting the focus from DNA to RNA. *Proceedings of the National Academy of Sciences* 94:952–955.

Salovaara R, Loukola A, Kristo P, Kaariainen H, Ahtola H, Eskelinen M, Harkonen N, Julkunen R, Kangas E, Ojala S, Tulikoura J, Valkamo E, Jarvinen H, Mecklin J, Aaltonen L, de la Chapelle A. 2000. Population-based molecular detection of hereditary nonpolyposis colorectal cancer. *Journal of Clinical Oncology* 18:2193–2000.

Sekido Y, Fong K, Minna J. 2001. Molecular biology of lung cancer. *Cancer: Principles and Practice of Oncology*. DeVita Jr VT, Hellman S, Rosenberg SA (eds.). Sixth edition. Chapter 31.1. Philadelphia: Lippincott-Raven.

Sekido Y, Fong K, Minna J. 1998. Progress in understanding the molecular pathogenesis of human lung cancer. *Biochimica et Biophysica Acta* 1378:F21–F59.

Sheehan, KM, Sheahan K, O'Donoghue DP, MacSweeney F, Conroy RM, Fitzgerald DJ, Murray FE. 1999. The relationship between cyclooxygenase-2 expression and colorectal cancer. *JAMA* 282:1254–1257.

Sheng H, Shao J, Kirkland SC, Isakson P, Coffey R, Morrow J, Beauchamp RD, DuBois RN. 1997. Inhibition of human colon cancer cell growth by selective inhibition of cyclooxygenase-2. *Journal of Clinical Investigation* 99:2254–2259.

Shields PG, Harris CC. 1991. Molecular epidemiology and the genetics of environmental cancer. *The Journal of the American Medical Association* 266:681–687.

Shirakata Y, Kawada M, Fujiki Y, Sano H, Oda M, Yaginuma K, Kobayashi M, Koike K. 1989. The X gene of hepatitis B virus induced growth stimulation and tumorigenic transformation of mouse NIH3T3 cells. *Japanese Journal of Cancer Research* 80:617–621.

Shivapurkar N, Virmani AK, Wistuba II, Milchgrub S, Mackay B, Minna JD, Gazdar AF. 1999. Deletions of chromosome 4 at multiple sites are frequent in malignant mesothelioma and small cell lung carcinoma. *Clinical Cancer Research: An official journal of the American Association for Cancer Research* 5:17–23.

Siegelmann-Danieli N, Buetow KH. 1999. Constitutional genetic variation at the human aromatase gene (Cyp 19) and breast cancer risk. *British Journal of Cancer* 79:456–463.

Slattery ML, Potter JD, Samowitz W, Schaffer D, Leppert M. 1999. Methylenetetrahydrofolate reductase, diet, and risk of colon cancer. *Cancer Epidemiology, Biomarkers, and Prevention* 8:513–518.

Smalley WE, DuBois RN. 1997. Colorectal cancer and nonsteroidal anti-inflammatory drugs. *Advanced Pharmacology* 39:1–20.

Snedeker SM. 2001. Pesticides and breast cancer risk: A review of DDT, DDE, and dieldrin. *Environmental Health Perspectives* 109(1):35–47.

Sourdaine P, Parker MG, Telford J, Miller WR. 1994. Analysis of the aromatase cytochrome P450 gene in human breast cancers. *Journal of Molecular Endocrinology* 13:331–337.

Sozzi G, Miozzo M, Donghi R, Pilotti S, Cariani CT, Pastorino U, Della Porta G, Pierotti MA. 1992. Deletions of 17p and p53 mutations in preneoplastic lesions of the lung. *Cancer Research* 52:6079–6082.

Spandau DF, Lee CH. 1988. Trans-activation of viral enhancers by the hepatitis B virus X protein. *Journal of Virology* 62:427–434.

Steinbach G, Lynch PM, Phillips R, Wallace M, Hawk E, Gordon G, Sherman J, Wakabayashi N, Saunders B, Shen Y, Godio L, Patterson S, Abbruzzese J, Jester S, King K, Zimmerman S, Levin B. 1999. Effect of celecoxib on colorectal polyps in patients with familial adenomatous polyposis (FAP). *American Journal of Gastroenterology* 94:2687.

Sundaresan V, Ganly P, Hasleton P, Rudd R, Sinha G, Bleehen NM, Rabbitts P. 1992. p53 and chromosome 3 abnormalities, characteristic of malignant lung tumours, are detectable in preinvasive lesions of the bronchus. *Oncogene* 7:1989–1997.

Sutherland G. 1988. The role of nucleotides in human fragile site expression. *Mutation Research* 200:207–213.

Takeshima Y, Seyama T, Bennett WP, Akiyama M, Tokuoka S, Inai K, Mabuchi K, Land CE, Harris CC. 1993. p53 mutations in lung cancers from non-smoking atomic-bomb survivors. *Lancet* 342:1520–1521.

Taylor JA, Watson MA, Devereux TR, Michelst RY, Saccomanno G, Anderson M. 1994. Mutational hotspot in the p53 gene in radon-associated lung tumors from uranium miners. *Lancet* 343:86–87.

Thomas HV, Reeves GK, Key TJA. 1997. Endogenous estrogen and postmenopausal breast cancer: A quantitative review. *Cancer Causes and Control* 8(6):922–928.

Toyota M, Ahuja N, Ohe-Toyota M, Herman JG, Baylin SB, Issa JP. 1999a. CpG island methylator phenotype in colorectal cancer. *Proceedings of the National Academy of Sciences* 96:8681–8686.

Toyota M, Ho C, Ahuja N, Jair KW, Li Q, Ohe-Toyota M, Baylin SB, Issa JP. 1999b. Identification of differentially methylated sequences in colorectal cancer by methylated CpG island amplification. *Cancer Research* 59:2307–2312.

Tseng M, Sandler RS, Greenberg ER, Mandel JS, Haile RW, Baron JA. 1997. Dietary iron and recurrence of colorectal adenomas. *Cancer Epidemiology, Biomarkers, and Prevention* 6:1029–1032.

Twu JS, Schloemer RH. 1987. Transcriptional trans-activating function of hepatitis B virus. *Journal of Virology* 61:3448–3453.

Ueda H, Ullrich SJ, Gangemi ID, Kappel CA, Ngo L, Feitelson MA, Jay G. 1995. Functional inactivation but not structural mutation of p53 causes liver cancer. *Nature Genetics* 9:41–47.

Ulrich CM, Kampman E, Bigler J, Schwartz SM, Chen C, Bostick R, Fosdick S, Beresford SAA, Yasui Y, Potter JD. 1999. Colorectal adenomas and the C677T MTHFR Polymorphism: Evidence for gene-environment interaction? *Cancer Epidemiology, Biomarkers, and Prevention* 8:659–668.

Unsal H, Yakicier C, Marcais C, Kew M, Volkmann M, Zentgraf H, Isselbacher KJ, Ozturk M. 1994. Genetic heterogeneity of hepatocellular carcinoma. *Proceedings of the National Academy of Sciences* 91:822–826.

Vahakangas KH, Samet JM, Metcalf RA, Welsh JA, Bennett WP, Lane DP, Harris CC. 1992. Mutations of p53 and ras genes in radon-associated lung cancer from uranium miners. *Lancet* 339:576–580.

Vaittinen P, Hemminki K. 2000. Risk factors and age–incidence relationships for contralateral breast cancer. *International Journal of Cancer* 88:998–1002.

Vineis, P. Molecular epidemiology: Low-dose carcinogens and genetic susceptibility. 1997. *International Journal of Cancer* 71:1–3.

Virmani AK, Rathi A, Zochbauer-Muller S, Sacchi N, Fukuyama Y, Bryant D, Maitra A, Heda S, Fong KM, Thunnissen F, Minna JD, Gazdar AF. 2000. Promoter methylation and silencing of the retinoic acid receptor-beta gene in lung carcinomas. *Journal of the National Cancer Institute* 92:1303–1307.

Vogelstein B, Kinzler KW. 1998. The genetic basis of human cancer. New York: McGraw-Hill.

Wainfan E, Poirier L. 1992. Methyl groups in carcinogenesis: Effects on DNA methylation and gene expression. *Cancer Research* 52:2071s–2077s.

Wang XW, Harris CC. 1997. p53 tumor-suppressor gene: Clues to molecular carcinogenesis. *Journal of Cellular Physiology* 173:247–255.

Wang XW, Forrester K, Yeh, H, Feitelson MA, Gu JR, Harris CC. 1994. Hepatitis B virus X protein inhibits p53 sequence-specific DNA binding, transcriptional activity, and association with transcription factor ERCC3. *Proceedings of the National Academy of Sciences* 91:2230–2234.

Wang XW, Vermeulen W, Coursen JD, Gibson M, Lupold SE, Forrester K, Xu G, Elmore L, Yeh H, Hoeijmakers JHJ, Harris CC. 1996. The XPB and XPD helicases are components of the p53-mediated apoptosis pathway. *Genes Development* 10:1219–1232.

Wang XW, Gibson MK, Vermeulen W, Yeh H, Forrester K, Sturzbecher HW, Hoeijmakers JHJ, Harris CC. 1995a. Abrogation of p53-induced apoptosis by the hepatitis B virus X gene. *Cancer Research* 55:6012–6016.

Wang XW, Yeh H, Schaeffer L, Roy R, Moncollin V, Egly IM, Wang Z, Friedberg EC, Evans MK, Taffe BG, Bohr VA, Hoeijmakers JH, Forrester K, Harris CC. 1995b. p53 Modulation of TFIIH-associated nucleotide excision repair activity. *Nature Genetics* 10:188–195.

Warner HR, Price AR. 1989. Involvement of DNA repair in cancer and aging. *Journal of Gerontology* 44:45–54.

Watanabe J, Harada N, Suemasu K, Higashi Y, Gotoh O, Karajiri K. 1997. Arginine-cysteine polymorphism at codon 264 of the human CYP19 gene does not affect aromatase activity. *Pharmacogenetics* 7:419–424.

Weinberg G, Ullman B, Martin Jr. D. 1981. Mutator phenotypes in mammalian cell mutants with distinct biochemical defects and abnormal deoxyribonucleoside triphosphate pools. *Proceedings of the National Academy of Sciences* 78:2447–2451.

Whelan RL, Horvath KD, Gleason NR, Forde KA, Treat MD, Teitelbaum SL, Bertram A, Neugut AI. 1999. Vitamin and calcium supplement use is associated with decreased adenoma recurrence in patients with a previous history of neoplasia. *Disease of the Colon and Rectum* 42(2):212–217.

Wickramasinghe S, Fida S. 1994. Bone marrow cells from vitamin B12- and folate-deficient patients misincorporate uracil into DNA. *Blood* 83:1656–1661.

Williams, C. W., and DuBois, R. N. 1996. Prostaglandin endoperoxide synthase: why two isoforms? *American Journal of Physicians* 270:G393–G400.

Wistuba I, Behrens C, Milchgrub S, Bryant D, Hung J, Minna JD, Gazdar AF. 1999a. Sequential molecular abnormalities are involved in the multistage development of squamous cell lung carcinoma. *Oncogene* 18:643–650.

Wistuba II, Behrens C, Virmani AK, Milchgrub S, Syed S, Lam S, Mackay B, Minna JD, Gazdar AF. 1999b. Allelic losses at chromosome 8p21-23 are early and frequent events in the pathogenesis of lung cancer. *Cancer Research* 59:1973–1979.

Wistuba I, Behrens C, Virmani AK, Mele G, Milchgrub S, Girard L, Fondon 3rd JW, Garner HR, McKay B, Latif F, Lerman MI, Lam S, Gazdar AF, Minna JD. 2000a. High resolution chromosome 3p allelotyping of human lung cancer and preneoplastic/preinvasive bronchial epithelium reveals multiple, discontinuous sites of 3p allele loss and three regions of frequent breakpoints. *Cancer Research* 60:1949–1960.

Wistuba II, Berry J, Behrens C, Maitra A, Shivapurkar N, Milchgrub S, Mackay B, Minna JD, Gazdar AF. 2000b. Molecular changes in the bronchial epithelium of patients with small cell lung cancer. *Clinical Cancer Research* 6:2604–2610.

Zeigler RG, Hoover RN, Pike MC, Hildesheim A, Nomura MY, West DW, Wu-Williams A, Kolonel LN, Horn PL, Ross JF, Rosenthal, Hyer MB. 1993. Migration patterns and breast cancer risk in Asian-American women. *Journal of the National Cancer Institute* 85:1819–1827.

Ziegler A, Jonason AS, Leffell DJ, Simon JA, Sharma HW, Kimmelman J, Remington L, Jacks T, Brash DE. 1994. Sunburn and p53 in the onset of skin cancer. *Nature* 372:773–776.

Zöchbauer-Müller S, Fong KM, Maitra A, Lam S, Geradts J, Ashfaq R, Virmani AK, Milchgrub S, Gazdar A, Minna J. 2001a. 5'CpG Island methylation of the FHIT gene is correlated with loss of gene exprssion in lung and breast cancer. *Cancer Research* 61(9):3581–3585.

Zöchbauer-Müller S, Fong KM, Virmani AK, Geradts J, Gazdar AF, Minna JD. 2001b. Aberrant promoter methylation of multiple genes in non-small cell lung cancers *Cancer Research* 61:249–255.

Zöchbauer-Müller S, Gazdar AF, Minna JD. 2002. Molecular pathogenesis of lung cancer. *Annual Reviews of Physiology* 64:681–708.

Appendix A

Glossary[5]

Adduct. Addition of new chemical structure into DNA.

Adenocarcinoma. Cancer that begins in cells that line certain internal organs.

Adenoma. A noncancerous epithelial tumor.

Adenomatous. Pertaining to adenoma.

Allelotype. The protein product (or the result of its activity) of an allele that may be detected as an antigen in another member of the same species.(e.g., histocompatibility antigens, immunoglobulins), obeying the rules of simple Mendelian inheritance.

Aneuploidy. Any deviation from an exact multiple of the haploid number of chromosomes, whether fewer (hypoploidy, as in Turner's syndrome) or more (hyperploidy, as in Down's syndrome).

Angiosarcoma. A type of cancer that begins in the lining of blood vessels.

Antigen. A molecule whose shape triggers the production of antibodies (immunoglobulins) that will bind to the antigen. A foreign substance capable of triggering an immune response in an organism.

Apoptosis. A normal series of events in a cell that lead to its death.

Biomarker. A biological molecule used as a marker for the substance or process of interest.

BRCA1 gene. A gene located on chromosome 17 that normally helps to sup-

[5]Sources of definitions include *Dorland's Illustrated Medical Dictionary* (Philadelphia: W.B. Saunders Co., 2000), The On-line Medical Dictionary (http://www.graylab.ac.uk), The Biology Teaching Organisation's On-line Glossary of Genetic Terms (http://helios.bto.ed.ac.uk/bto/glossary), and Lung-Cancer Option.com Glossary (http://www.lungcanceroption.com/glossary)

press cell growth. Inheriting an altered version of BRCA1 predisposes an individual to breast, ovarian, or prostate cancer.

BRCA2 gene. A breast cancer gene located on chromosome 13.

Carcinogen. Any substance that causes cancer.

Carcinoma. Cancer that begins in the skin or in tissues that line or cover internal organs.

Codon. A section of DNA (three nucleotide pairs in length) or RNA (three nucleotides in length) that codes for a single amino acid. A sequence of three RNA or DNA nucleotides that specifies (codes for) either an amino acid or the termination of translation.

DNA methylation. Modification of a DNA molecule by the addition of a methyl group.

Environment. The sum total of all the conditions and elements that make up the surroundings and influence the development and actions of an individual.

Epigenetic. Altering the activity of genes without changing their structure.

Ewing's sarcoma. A highly malignant, metastasizing, primitive small round cell tumor of bone, usually occurring in the diaphyses of long bones, ribs, and flat bones of children or adolescents.

Familial adenomatous polyposis (FAP). Multiple adenomatous polyps with high malignant potential, lining the mucous membrane of the intestine, particularly the colon, beginning at about puberty.

Genome. The total set of genes carried by an individual or cell.

Genotype. The specific allelic composition of a cell—either of the entire cell or, more commonly, of a certain gene or set of genes. The genes that an organism possesses.

Hemangioma. An extremely common benign tumor, occurring most commonly in infancy and childhood, made up of newly formed blood vessels and resulting from malformation of angioblastic tissue of fetal life.

Hepatocellular carcinoma. A type of adenocarcinoma, the most common type of liver tumor.

Immunocompetence. The ability or capacity to develop an immune response (i.e., antibody production and/or cell-mediated immunity) following exposure to an antigen.

Li–Fraumeni syndrome. Li–Fraumeni syndrome (LFS) is an inherited form of cancer, affecting children and young adults and characterized by a wide

spectrum of tumors, including soft-tissue and bone sarcomas, brain tumors, adenocortical tumors and premenopausal breast cancers.

Low-penetrance gene. A gene that produces a low incidence of a trait.

Modifier gene. A nonallelic gene that controls or changes the manifestation of a gene by interfering with its transcription.

Neoplasia. Abnormal and uncontrolled cell growth.

Neoplasm. A new growth of benign or malignant tissue.

Non-Hodgkin's lymphoma. A heterogeneous group of malignant lymphomas, whose only common feature is being an absence of the giant Reed–Sternberg cells characteristic of Hodgkin's disease.

Non-small cell lung cancer (NSCLC). A group of lung cancers that includes squamous cell carcinoma, adenocarcinoma, and large cell carcinoma.

Osteogenic sarcoma. A malignant primary neoplasm of bone composed of a malignant connective tissue stroma with evidence of malignant, osteoid, bone, or cartilage formation. Also called osteosarcoma.

Osteoma. A benign, slow-growing tumor composed of well-differentiated, densely sclerotic, compact bone, usually arising in bones, particularly the skull and facial bones.

Phytonutrient. A plant-derived nutrient.

Polyp. A growth that protrudes from a mucous membrane.

Proliferation. The reproduction or multiplication of similar forms, especially of cells and morbid cysts.

Proto-oncogene. A normal cellular gene that, with alteration, such as mutation, DNA rearrangement, or nearby insertion of viral DNA, becomes an active oncogene; most proto-oncogenes are believed to function normally in cell growth and differentiation.

Renal adenocarcinoma. Cancer that develops in the lining of the renal tubules, which filter the blood and produce urine.

Rhabdomyosarcoma. A malignant tumor of muscle tissue.

Sarcoma. A cancer of the bone, cartilage, fat, muscle, blood vessels, or other connective or supportive tissue.

Small cell lung cancer (SCLC). A type of lung cancer in which the cells appear small and round when viewed under the microscope, also called oat cell lung cancer.

Sqamous cell carcinoma. Carcinoma developed from squamous epithelium, having cuboid cells and characterized by keratinization and often by preservation of intercellular bridges. Initially local and superficial, the lesion may later invade and metastasize.

Syndrome. A set of signs or a series of events occurring together that often point to a single disease or condition as the cause.

Wilms' tumor. A rapidly developing malignant mixed tumor of the kidneys, made up of embryonal elements; it usually affects children before the fifth year but may occur in the fetus and rarely in later life.

Appendix B

Workshop Agenda

CANCER AND THE ENVIRONMENT:
GENE–ENVIRONMENT INTERACTIONS

Sponsored by

The Roundtable on Environmental Health Sciences, Research, and Medicine

---

National Academy of Sciences Auditorium.
2101 Constitution Avenue, N.W., Washington, D.C.
May 16–17, 2001

Wednesday, May 16, 2001

8:30 a.m.	Welcome and Opening Remarks The Honorable Paul G. Rogers (Roundtable Chair)
8:40 a.m.	Remarks from the IOM President Kenneth Shine, M.D.
8:55 a.m.	Remarks and Charge to Participants Samuel Wilson, M.D. Deputy Director, NIEHS
9:10 a.m.	Statement of Workshop Objectives Franklin Mirer, Ph.D. (Workshop Co-chair)
9:20 a.m.	Cancer, the Environment, and the Media Mr. Samuel Donaldson (ABC News)

SESSION I: LINK BETWEEN ENVIRONMENTAL FACTORS AND THE DEVELOPMENT OF CANCER

Moderator: Woodie Kessel, M.D., M.P.H.

9:50 a.m.	Genes and the Environment in Cancer Etiology Joseph Fraumeni, Jr., M.D. (NCI)
10:30 a.m.	Diet and Other Environmental Factors as Modifiers of Cancer Risk John Milner, Ph.D. (Pennsylvania State University and NCI)
11:10 a.m.	Break
11:25 a.m.	Genetic Epidemiology as a Tool for Gene–Environment Interactions Kari Hemminki, M.D., Ph.D. (Karolinska Institute)
12:05 a.m.	The p53 Pathway: At the Crossroads of Molecular Carcinogenesis and Molecular Epidemiology Curtis C. Harris, M.D. (NCI)
12:45 p.m.	General Discussion
1:00 p.m.	Lunch

SESSION II: GENETIC–ENVIRONMENTAL INTERACTION IN SPECIAL POPULATIONS

Moderator: Lovell A. Jones, Ph.D.

Vulnerable Populations

1:45 p.m.	Health Disparities: Do Gene–Environment Interactions Play a Role? Lovell A. Jones, Ph.D. (M.D. Anderson Cancer Center)
2:05 p.m.	Cancer Disparities in Appalachia Gilbert Friedell, M.D. (Markey Cancer Center)
2:20 p.m.	Migrant Farmworkers' Children and Pesticides: A High-Risk Population María A. Hernández-Valero, Dr.P.H. (M.D. Anderson Cancer Center)

2:35 p.m. Priorities and Special Populations: Ties That Bind
Armin Weinberg, Ph.D. (Baylor College of Medicine)

2:50 p.m. General Discussion

3:05 p.m. Break

Cancer in Children

3:20 p.m. Childhood Cancer and Diet: Preliminary Evidence
Greta Bunin, Ph.D. (Children's Hospital of Philadelphia)

3:35 p.m. Trends: Incidents and Survivorship
Leslie Robison, Ph.D. (University of Minnesota)

3:50 p.m. Chemicals and Chromosomes, Children and Cancer, Clusters and Cohorts in a New Century
Richard Jackson, M.D., M.P.H. (CDC)

4:10 p.m. General Discussion

Women and Cancer

4:25 p.m. Epidemiology and Genetic Susceptibility to Breast Cancer
Brian Henderson, M.D. (University of Southern California, Keck School of Medicine)

4:40 p.m. Gene-Environment Risk Factors for Breast Cancer
Mary Wolff, Ph.D. (Derald H. Ruttenberg Cancer Center, Mount Sinai School of Medicine)

4:55 p.m. Breast Cancer Genetics: *BRCA1* and *BRCA2* Genes
Olufunmilayo Olopade, M.D. (The University of Chicago, Pritzker School of Medicine)

5:10 p.m. General Discussion

5:30 p.m. Reception

Thursday, May 17, 2001

8:30 a.m. Welcome Back
The Honorable Paul G. Rogers, Roundtable Chair
Special Address

8:35 a.m. Cancer Treatment Based on Immune Stimulation
Steven Rosenberg, M.D., Ph.D. (NCI)

SESSION III: GENETIC–ENVIRONMENTAL INTERACTION IN SPECIFIC CANCERS

Moderator: John Minna, M.D.

Lung Cancer

9:30 a.m. Molecular Pathogenesis of Lung Cancer
John Minna, M.D. (University of Texas Southwestern Medical Center)

9: 50 a.m. Genetic Susceptibility to Lung Cancer
Margaret Spitz, M.D., M.P.H. (M.D. Anderson Cancer Center)

10:10 a.m. General Discussion

10:30 a.m. Break

Colon Cancer

10:50 a.m. Environmental and Genetic Factors Involved in Colorectal Carcinogenesis
Raymond DuBois, M.D., Ph.D. (Vanderbilt University—Ingram Cancer Center)

11:10 a.m. Environmental Issues Related to Colon Cancer
David Alberts, M.D. (Arizona Cancer Center)

11:30 a.m. General Discussion

Prostate Cancer

11:50 a.m. Similarities of Prostate and Breast Cancer: Evolution, Diet, and Estrogens
Donald Coffey, Ph.D. (Johns Hopkins University)

12:10 a.m. The Effect of Herbal Therapies in Prostate Cancer
Robert S. DiPaola, M.D. (Cancer Institute of New Jersey)

12:30 p.m. General Discussion

12:50 p.m. Lunch

SESSION IV: MOVING FORWARD

Moderator: Samuel Wilson, M.D.

1:30 p.m. Introduction and Remarks
The Honorable Paul G. Rogers, J.D.

2:00 p.m. Cancer and the Environment: A View from "the Hill"
The Honorable John E. Porter, J.D.

Roundtable Summation and General Discussion

2:30 p.m. Summation of the Workshop
Lovell A. Jones, Ph.D.
Roundtable Member

2:45 p.m. Roundtable Discussion
Discussion of the workshop and future needs of cancer research

Ms. Susan Braun
President and CEO
Susan G. Komen Breast Cancer Foundation

Donald Coffey, Ph.D.
Johns Hopkins University

Ms. Linda Frame, R.N., M.S., ACON
Senior Clinical Advisor
Susan G. Komen Breast Cancer Foundation

Lovell A. Jones, Ph.D.
Professor, Gynecologic Oncology
M.D. Anderson Cancer Center

Martha Linet, M.D., M.P.H.
National Cancer Institute

David Ringer, Ph.D., M.P.H.
Scientific Program Director
American Cancer Society

4:00 p.m. Adjournment

Appendix C

Speakers and Panelists

David S. Alberts, M.D.
Professor of Medicine Pharmacology and Public Health
Arizona Cancer Center

Susan Braun
President and CEO
Susan G. Komen Breast Cancer Foundation

Greta Bunin, Ph.D.
Research Associate Professor of Pediatrics
University of Pennsylvania School of Medicine and Children's Hospital of Philadelphia

Donald S. Coffey, Ph.D.
Professor of Urology, Oncology, Pathology, and Pharmacology and Molecular Sciences
Johns Hopkins University School of Medicine

Robert DiPaola, M.D.
Associate Professor of Medicine and Executive Director of the Dean and Betty Gallo Prostate Cancer Institute of New Jersey

Sam Donaldson
ABC News Anchor
ABC *PrimeTime Live*

Raymond DuBois, M.D., Ph.D.
Associate Director
Vanderbilt-Ingram Cancer Center for Cancer Prevention
Director of Gastroenterology, Hepatology and Nutrition
Vanderbilt University Medical Center

Linda Frame, R.N., M.S., AOCN
Senior Clinical Advisor
Susan G. Komen Breast Cancer Foundation

Joseph Fraumeni, M.D.
Director of the Division of Cancer Epidemiology and Genetics
National Cancer Institute

Gilbert H. Friedell, M.D.
Director Emeritus
Markey Cancer Center
Professor of Pathology Emeritus
University of Kentucky in Lexington

Curt Harris, M.D.
Chief
Laboratory of Human Carcinogenesis
National Cancer Institute
Clinical Professor of Medicine and Oncology
Georgetown University School of Medicine

Kari Hemminki, M.D., Ph.D.
Professor of Epidemiology
Karolinska Institute, Department of Biosciences at Novum Huddinge
Sweden

Brian E. Henderson, M.D.
Professor of Preventive Medicine
University of South California
Keck School of Medicine, Los Angeles
California

María A.Hernández-Valero, Dr.P.H.
Postdoctoral Fellow
Department of Gynecologic Oncology
Division of Surgery
University of Texas
M.D. Anderson Cancer Center
Houston, Texas

Richard Jackson, M.D., M.P.H.
Director
National Center for Environmental Health
Centers for Disease Control and Prevention

Lovell Jones, Ph.D.
Professor, Gynecologic Oncology
M.D. Anderson Cancer Center
University of Texas

Woodie Kessel, M.D., M.P.H.
Senior Child Health Science Advisor
Department of Health and Human Services

Martha S. Linet, M.D., M.P.H.
Chief
Population Studies Section, Radiation Epidemiology Branch
National Cancer Institute

John Milner, Ph.D.
Professor
Department of Nutrition
Director of the Graduate Program in Nutrition
University of Pennsylvania

John Minna, M.D.
Professor of Internal Medicine and Pharmacology
Director, Hamon Center for Therapeutic Oncology Research
University of Texas
Southwestern Medical Center

Olufunmilayo Olopade, M.D.
Director, Center for Clinical Cancer Genetics
Pritzker School of Medicine
University of Chicago

The Honorable John Porter
Partner
Hogan & Hartson

David Ringer, Ph.D., M.P.H.
Scientific Program Director
American Cancer Society

Leslie Robison, Ph.D.
Professor of Pediatrics
University of Minnesota
Division of Pediatric Epidemiology

The Honorable Paul Rogers
Partner
Hogan & Hartson

Steven Rosenberg, M.D., Ph.D.
Chief of Surgery
National Cancer Institute

Margaret R. Spitz, M.D., M.P.H.
Professor, Chair, Epidemiology
University of Texas
M.D. Anderson Cancer Center

Armin Weinberg, Ph.D.
Professor of Medicine
Baylor College of Medicine

Mary Wolff, Ph.D.
Professor
Mount Sinai School of Medicine

Appendix D

Workshop Participants

Christian Abnet
National Cancer Institute

Nicole African
Research Assistant
Georgetown University Lombardi Cancer Center

Karim Ahmed
Director, International Programs
National Council for Science and the Environment

Julie Albertus
National Institutes of Health

Linda Anderson
Director of Communications
Epidemiology and Genetics Research Program
National Cancer Institute
National Institutes of Health

Yutaka Aoki
Graduate Student
Johns Hopkins School of Public Health

Martin Apple
President
Council of Scientific Society Presidents

Mahboob Atique
Epidemiologist
Maryland Department of Health and Mental Hygiene

John Balbus
Associate Professor
George Washington University
School of Public Health

Donald Barnes
Staff Director
Scientific Advisory Board

Ervind Bhogte
CRTA-2 Fellow
National Cancer Institute
National Institutes of Health

Laura Bonetta
Freelance Writer
BioMedNet News

Lynn Bradley
Director
Environmental Health Association of Public Health Laboratories

Anstice Brand
Program Analyst
U.S. Department of Health and Human Services Centers for Disease Control and Prevention

Margaret Breida
Manager
Technical Committee Support
American Industrial Hygiene Association

Saba Brelvi
Program Associate
Grantmakers in Health

Joel Breman
Deputy Director
Division of International Training and Research
Fogarty International Center

Mark Brown
Director
Environmental Agents Service
Department of Veterans Affairs

Pamela Brown
Director
Cancer Prevention and Control
West Virginia University
Mary Babb Randolph Cancer Center

Stacye Bruckbauer
Legislative Officer
National Cancer Institute

William Burk
Deputy Director
Huntsman Cancer Institute

Ritva Butrum
Vice President for Research
American Institute for Cancer Research

Valere Byrd
Child Health Fellow
U.S. Department of Health and Human Services

Richard Calvert
Research Medical Officer
U.S. Food and Drug Administration

Victor Caravello
Consultant
Air Force Institute for Environment Safety and Occupational Health Risk Analysis

Jill Center
Health Policy Analyst
American Public Health Association

Amy Chapin
Acting Director
Office of Environmental Health
Maryland Department of Health and Mental Hygiene

Margaret Chu
Toxicologist
U.S. Environmental Protection Agency

Rebecca Cohen

Susan Kay Cummins
Senior Health Policy Advisor
National Center for Environmental Health
Centers for Disease Control and Prevention

Susan Daniels
Intern
National Academy of Sciences

Kathleen DeBettencourt
Executive Director
Environmental Literacy Council

Catherine Estes
Project Coordinator
The National Environmental Education and Training Foundation
The EnvironMentors Project

Bernadette A. Estrada
CRTA Fellow
National Cancer Institute

Geroncio Fajardo
Division of Environmental Health Assessment
Bureau of Epidemiology
Pennsylvania Department of Health

Praveen Fernandes
Bennett, Turner and Coleman, LLP

Barry Finnette
Associate Professor
Pediatrics, Microbiology, and Molecular Biology
University of Vermont College of Medicine

Rachael Flynn
Public Health Fellow
National Center for Policy Research for Women and Families

Herbert Fockler
Executive Director
Global Children's Health Fund

Elaine Francis
National Program Director
Endocrine Disruptors Research Program
U.S. Environmental Protection Agency

Molly French
Director of Policy Research
Partnership for Prevention

Kathy Gallagher
Program Analyst
National Cancer Institute
National Institutes of Health

Mary Gant
Assistant to the Director for Legislation
National Institute of Environmental Health Sciences

Jill Gay
Consultant
National Council for Science and the Environment

Ronald Gerste
Science Correspondent

Roger Giese
Director
Environmental Cancer Research Program
Northeastern University

Radoslav Goldman
Research Instructor
Georgetown University
Lombardi Cancer Center

Pauline Green
Associate Professor
Howard University

June Green
Consultant
National Alliance of Children's Trust and Prevention Funds

Marcia Greenblum
Nutrition and Food Safety Specialist
Egg Nutrition Center

William M. Grigg
Director of Communications
National Institute of Environmental Health Sciences

Mary Guinan
State Health Officer
Nevada State Health Division

Jhumka Gupta
Service Fellow
Office on Women's Health
U.S. Department of Health and Human Services

Janet Guthrie
Program Analyst
National Institute of Environmental Health Sciences

Bassem Haddad
Assistant Professor
Georgetown University Medical Center

Susanne B. Haga
National Institutes of Health

Deborah Hamernik
Scientific Review Administrator
National Institutes of Health

James Hanson
Acting Chief
Clinical and Genetic Epidemiology Research Branch
National Cancer Institute

Scott Hawkins
Intercet, Ltd.

Phil Heard
Maryland Department of the Environment

Richard Hegner
Senior Research Associate
National Health Policy Forum

Roger Herdman
Director
National Cancer Policy Board
Institute of Medicine

Ella Hinson
Student
Metabolism Branch
National Cancer Institute

Constance Horden
Writer
American Association for the Advancement of Science

Jennifer Hu
Assistant Professor
Wake Forest University School of Medicine

Charmaine Hutchinson
Clinical Nurse
Howard University Hospital

Yi-Ting Hwang
Research Instructor
Georgetown University School of Medicine

Peter Inskip
Investigator
National Cancer Institute

Erick Ishii
Health Statistician
Department of Veterans Affairs

Kumiko Iwamoto
Program Director
National Cancer Institute

John Jackson
Einstein Fellow
National Science Foundation
Division of Graduate Education

Scott Jenkins
Reporter
FDC Reports

Libin Jia
Research Fellow
National Institutes of Health

Alicia Johnson
Physicians for Social Responsibility

David Johnson
Chief
Bureau of Environmental Epidemiology
Florida Department of Health

Wanda K. Jones
Deputy Assistant Secretary for Health (Women's Health)
U.S. Department of Health and Human Services

Thea Kalebic
Special Expert
National Cancer Institute
National Institutes of Health
Division of Cancer Prevention

Sarah Keim
Study Coordinator
National Institute of Environmental Health Sciences

Michele Kendus
Attorney
Venable, Baetijer and Howard, LLP

Richard Kerber
Associate Professor
University of Utah

Judith Klotz
Program Manager
Cancer Surveillance
New Jersey Department of Health and Senior Services

Ann Kolker
Executive Director
Ovarian Cancer National Alliance

Amy Kostant
Program Director
Environmental Media Services

Naomi Kulakow
Coordinator
Education and Outreach
Food and Drug Administration

Elaine Lanza
Senior Investigator
National Cancer Institute
National Institutes of Health

Barbara Larcom
Lieutenant Colonel, United States Air Force
Deputy for Public Health, Deputy Assistant Secretary Air Force
Environment, Safety, and Occupational Health

Beth L. Law
Vice President for Law and Environmental Affairs
American Trucking Associations, Inc.

Deirdre Lawrence
Epidemiologist
National Cancer Institute
National Institutes of Health

Paul Levine
Clinical Professor
George Washington University

Anna Levy
Deputy Director
Office of Women's Health
National Cancer Institute

Wenchi Liang
Assistant Professor
Georgetown University

Geoffrey Lomax
University of California, Berkeley

Raya Mandler
Research Fellow
Metabolism Branch
National Cancer Institute

Saralyn Mark
Senior Medical Advisor
Office on Women's Health
U.S. Department of Health and Human Services

Cheryl L. Marks
National Cancer Institute

Edward Master
Environmental Protection Specialist
U.S. Environmental Protection Agency, Region 5

Caitlin McAleese
Miami University, Ohio

Catherine E. McDemott
President and CEO
National Committee for Quality Health Care

Hugh McKinnon
Associate Director for Health
National Risk Management Research Laboratory
U.S. Environmental Protection Agency

Chad McMullin
Project Director
Intercet, Ltd.

Sandra Melnick
Chief
Analytic Epidemiology Research Branch
National Cancer Institute

E. Jeffrey Metter
Medical Officer
National Institute on Aging

Katherine Meyer
Associate Professor
University of Massachusetts, Darmouth

Nancy E. Miller
Senior Science Policy Analyst
Office of the Director
National Institutes of Health

Linda Min
Nutritions Programs Coordinator
Egg Nutrition Center

Daniel Mullins
Accenture

Marjan Najafi
Research Associate
Institute of Medicine

Patricia Newman
Science Writer
National Cancer Institute

Angela Ng
Center for Scientific Review
National Institutes of Health

Jing Nie
Graduate Student
State University of New York at Buffalo

Jim O'Hara
Executive Director
Health-Track

Domyung Paek
Associate Professor
Seoul National University

Rebecca Parkin
Associate Research Professor
The George Washington University

A. R. Patel
Program Director for Diet–Nutrition
National Cancer Institute

Brenda Perkovich
Toxicologist
Office of Children's Health Protection

June Peters
Genetic Counselor
National Cancer Institute
National Institutes of Health

Heather Pierce
Genetic Counselor, Research Coordinator
Memorial Sloan-Kettering Cancer Center
Clinical Genetics Service

Warre Pleger
Professor and Associate,
Director for Basic Research
H. Lee Moffitt Cancer Center and Research Institute

Susan Polan
Director of Governmental Relations
Trust for America's Health

Carolyn Poppel
Staff Scientist
Physicians for Social Responsibility

Christopher Portier
Acting Director
Environmental Toxicology Program
National Institute of Environmental Health Sciences

Edwin Pratt, Jr.
Director of Liaison and Governmental Relations
National Association of Local Boards of Health

Claudio Ramirez
Consultant
Pan American Health Organization

Kathie Reed
Branch Chief, Science Planning
National Cancer Institute

David Reese
U.S. Environmental Protection Agency

Martin Root
BioSignia, Inc.

Tina Rouse
Program Officer
National Academy of Sciences

Deborah Ruggles

Jim Ruttenberg
Associate Professor
University of Colorado School of Medicine

Linda Sabatini
Assistant Professor
University of Wisconsin-Milwaukee

Keeran Sampat
Student
National Cancer Institute

Prem Sarin
Research Professor
George Washington University School of Medicine

Anne Sassaman
National Institute of Environmental Health Sciences

Samir Sauma
Scientific Projects Manager
National Cancer Institute
National Institutes of Health

Heather Schlecht
Legislative Assistant
American Chemical Society

Dina Schreinemachers
Health Scientist
U.S. Environmental Protection Agency

P. Francisco Semião
Prevention Policy Analyst
Association of State and Territorial Health Officials

Caroucha Senilla
Intern Labor Advisor
North American Agreement on Labor Cooperation

Kathryn Sessions
Consultant
Health and Environmental Funders Network

Cinzia Sevignani
Research Instructor
Thomas Jefferson University, Kimmel Cancer Center

Youn Shim
Epidemiologist
Agency for Toxic Substances and Disease Registry

Cheryl Siegel Scott
Epidemiologist
U.S. Environmental Protection Agency

Paul Silverman
Chief, Disease Prevention and Control
Delaware Division of Public Health

Mary Jane Slesinski
Scientific Review Administrator
Division of Extramural Activities
National Cancer Institute

John T. Soler
Epidemiologist
Minnesota Cancer Surveillance System

Patricia Stanco
Researcher
Bennett, Turner and Coleman, LLP

Linda Stapleton
Research Technician
National Cancer Institute
National Institutes of Health

Susan Steck-Scott
Fellow
National Cancer Institute

Karen Steinberg
Chief
Molecular Biology Branch
Centers for Disease Control and Prevention

Victoria Stevens
Visiting Scientist
National Center for Environmental Health
Centers for Disease Control and Prevention

David Sundwall
President
American Clinical Laboratory Association

Richard Thomas
Managing Director
Intercet, Ltd.

Sterling Thomas
BioResearch Scientist
Intercet, Ltd.

William Toscano
Professor and Head
University of Minnesota
Department of Environmental Health

Leslie Tucker
Center for Children's Health and the Environment

Carmella Veneroso
Researcher
George Washington University

Regina Vidaver
Program Manager
Society for Women's Health Research

Jennifer Weber
Associate
N. Chapman Associates, Inc

Charles Wells
Director
Environmental Justice and Public Health Activities
National Institute of Environmental Health Sciences

Amina Wilkins
Senior Environmental Scientist
U.S. Environmental Protection Agency

Maureen Wilson
Executive Secretary
President's Cancer Panel

Lee-Jun C. Wong
Associate Professor
Georgetown University Medical Center

Keith Zachman
Biological Lab Technician
National Institute of Child Health and Human Development
National Institutes of Health

Peter Zentai
Chief Correspondent
Hungarian Radio