
David N. Cooper
The Molecular Genetics of Lung Cancer

David N. Cooper

The Molecular Genetics of Lung Cancer

With 34 Figures, 13 in Colour

 Springer

David N. Cooper
Professor of Human Molecular Genetics
Institute of Medical Genetics
Cardiff University
Heath Park
Cardiff, CF14 4XN, UK

Library of Congress Control Number: 2004111936

ISBN 3-540-22985-X Springer Berlin Heidelberg New York

This work is subject to copyright. All rights are reserved, whether the whole or part of the material is concerned, specifically the rights of translation, reprinting, reuse of illustrations, recitation, broadcasting, reproduction on microfilm or in any other way, and storage in data banks. Duplication of this publication or parts thereof is permitted only under the provisions of the German Copyright Law of September 9, 1965, in its current version, and permission for use must always be obtained from Springer-Verlag. Violations are liable for prosecution under the German Copyright Law.

Springer is a part of Springer Science+Business Media
springeronline.com

© Springer-Verlag Berlin Heidelberg 2005

Printed in Germany

The use of designations, trademarks, etc. in this publication does not imply, even in the absence of a specific statement, that such names are exempt from the relevant protective laws and regulations and therefore free for general use.

Product liability: The publishers can not guarantee the accuracy of any information about dosage and application contained in this book. In every individual case the user must check such information by consulting the relevant literature.

Editor: Dr. Rolf Lange, Heidelberg, Germany

Desk editor: Hiltrud Wilbertz, Heidelberg, Germany

Production editor: Helmut Schwaninger, Heidelberg, Germany

Cover design: Erich Kirchner, Heidelberg, Germany

Typesetting: Mitterweger & Partner, Plankstadt, Germany

Printed on acid-free paper 27/3150 hs - 5 4 3 2 1 0

To Paul, Catrin and Duncan

Preface

Lung cancer, the second most common cancer in men and the third most common in women, is one of the most studied and hence probably one of the best understood of the 'non-heritable' human cancers. Familial aggregation of lung cancer is comparatively rare and, as yet, no gene has been described whose mutation is pathognomonic for, or even largely confined to, lung cancer, as found for example in cancers of the colon, kidney or breast. This should not however be taken as implying that genes are not involved in the etiology of lung cancer. Indeed, a considerable number of inherited genetic variants are now thought to confer susceptibility to the disease. An even larger number of genes have been shown to harbour somatic mutations in lung tumour cells or tissue. The role that these somatic mutations play in lung tumorigenesis can increasingly be understood in terms of their ability to promote cellular growth, to interfere with DNA repair, to confer resistance to apoptosis or to induce cellular transformation, tumour growth, invasiveness, angiogenesis, evasion of host immunity and finally, metastasis.

Perhaps the most characteristic feature of lung cancer is however the very strong association, evidenced by a multitude of epidemiological studies, with cigarette smoking. Although the precise underlying biological mechanisms and pathways responsible for this association are not yet understood, it is nevertheless clear that in lung tumorigenesis we are witnessing a highly complex interplay between genes and environment. Unravelling this complexity promises to be a very substantial undertaking. This is reflected in the fact that the molecular genetics of lung cancer is a burgeoning subject with a very large and widely dispersed literature that is difficult to access. This volume is primarily intended to summarize the current state of knowledge regarding the molecular and genetic mechanisms underlying lung cancer, and focuses specifically on the proximal genetic causes (as evidenced by the different types of somatic and germline mutations in a variety of different genes) and consequences (as adduced by mRNA expression and protein profiling) of this condition.

The introductory chapter is intended to provide an overview of the molecular basis of cancer and discusses the different categories of gene known to be involved in tumorigenesis as well as the underlying mechanisms of mutagenesis. Chapter 2 seeks to set the scene, with short sections on the history of lung cancer research, clinical aspects of the disease, disease classification and the major contribution made by both classical cytogenetic and molecular cytogenetic analysis to the identification of the genes involved. In the absence of a familial predisposition to the disease, the molecular genetic analysis of lung cancer (both small cell and non-small

cell) has been largely confined to the study of the tumour cells themselves. Somatic mutations involving some 120 different human genes have so far been characterized in primary lung tumour tissue or in cells derived from lung tumours. Such mutations may be gross and affect entire chromosomal regions or may instead be more subtle and serve to alter the fine structure of specific genes. A growing number of genes have also been shown to be inactivated by promoter methylation ('epimutation') during lung tumorigenesis. Genes compromised by mutation or epimutation include oncogenes and tumour suppressor genes as well as genes encoding proteins that perform functions in DNA repair, telomerase activity, apoptosis, and cell cycle regulation. This topic is reviewed in Chapter 3. Chapter 4 focuses on recent advances in our understanding of signal transduction pathways, apoptosis and cell cycle control mechanisms that are beginning to allow us to account for the emerging mutation data in terms of, for example, either a growth advantage accruing to the cells and/or an escape from apoptosis. The determination of the temporal order in which these lesions occur and the elucidation of the functional consequences of different combinations and permutations of lesions are extremely important. Chapter 4 attempts to address these topics as well as the identification of new prognostic indicators and the potential for early clinical detection.

Without straying into either epidemiology or toxicology, Chapter 5 attempts to review current knowledge about the genetic damage in the lung cells of smokers and smoking lung cancer patients. The cytogenetic, genetic and epigenetic (methylation) changes that have been associated with smoking are discussed, as are reported differences in mutation frequency or lung cell gene expression between smokers and non-smokers. Finally, the ongoing debate and controversy surrounding the potential role of benzo[*a*]pyrene as a mutagen (specifically with respect to the *TP53* gene) is described and discussed in detail.

A variety of inherited variants in genes encoding xenobiotic metabolising enzymes have been described that may influence lung cancer risk by playing a role in determining levels of cellular exposure to potential exogenous mutagens and carcinogens; these are reviewed in Chapter 6. Also covered are inherited variants in genes encoding DNA repair enzymes that can confer inter-individual differences in DNA repair capacity which may, at least in principle, indirectly modulate lung cancer risk.

New approaches to studying the lung tumour cellular phenotype (e.g. cDNA and oligonucleotide microarrays) are explored in Chapter 7. These approaches promise to greatly increase our knowledge of the consequences of mutation at the level of gene expression and are vital not only for obtaining a better understanding of lung cancer pathogenesis, but also for improving existing tumour diagnostics (identification/classificatory system) and for providing the novel prognostic markers and indicators that are a prerequisite for optimal patient management (Chapter 8). Advances in our understanding of the molecular biology of lung cancer have guided the design and application of a variety of new gene therapy approaches that are currently being explored in the search for the next generation of treatments (Chapter 8).

This volume therefore attempts to describe how the new techniques, methods and approaches of molecular genetics have been used in an attempt to unravel the complexities of lung tumorigenesis by analysis at DNA, RNA and protein levels with potentially important implications for diagnosis, prognosis and treatment as

well as providing new insights into how lung tumours arise from precancerous lesions and eventually progress to metastasis. In relation to the prevalence of the disease, research into lung cancer has historically been under-subscribed and poorly resourced by comparison with the common 'heritable cancers'. It is hoped that by stimulating interest in lung cancer research, this volume will help to reverse this trend.

Cardiff, July 2004

David N. Cooper

Acknowledgements

I am most grateful to Anna-Lisa Fisher who originally conceived the idea of a volume on the molecular genetics of lung cancer. Grateful thanks are also due to Karl-Friedrich Baetz, Nadia Chuzhanova, Sunil Dolwani, Michael Krawczak, Paul Lewis, Ann Procter, Nick Thomas, Ray Thornton and Meena Upadhyaya for their help and advice with the manuscript, to Hester Wain and Elspeth Bruford of the *Human Gene Nomenclature Committee* for providing gene symbols, and to Rolf Lange and Julia Heidelmann of Springer-Verlag for their much appreciated practical support during the production process.

Table of Contents

CHAPTER 1

An Introduction to the Molecular Basis of Cancer	1
Cancer genes	3
Oncogenes	3
Tumour suppressor genes	4
Mutator genes and genetic instability.	5
Cell cycle control genes	6
Apoptosis regulatory genes	7
Cancer as a disease of differentiation?	9
Cancer, signaling and acquired capabilities	9
Mutational mechanisms in cancer.	13
Pathological mutations in inherited disease and cancer.	14
Mutational spectra in cancer	15
Mutation in lung cancer	17

CHAPTER 2

Lung Cancer: Setting the Scene.	19
Introduction	19
Human lung development.	20
A short history of lung cancer research.	21
Lung cancer classification, staging, treatment and prognosis	28
Familial aggregation of lung cancer.	32
Clues to candidate genes from cytogenetic abnormalities and loss of heterozygosity studies	35

CHAPTER 3

Genes Involved in Sporadic Forms of Lung Cancer.	45
Oncogenes.	46
Tumour suppressor genes.	53
Apoptosis regulatory genes.	64
Cell cycle control and DNA damage checkpoint genes	66

Mutator (DNA mismatch repair) genes and microsatellite instability	68
DNA methylation and lung cancer	70
The potential significance of tumour suppressor gene location in lung cancer	78
The potential significance of oncogene location for chromosomal amplification and gene over-expression in lung cancer	79
Telomere length and telomerase activity	81

CHAPTER 4

Somatic Mutation in Lung Cancer	85
Functional consequences of somatic mutation in lung cancer	85
Ras/MAP kinase pathway	85
Rb/E2F pathway	88
p53 pathway	88
APC/ β -catenin pathway	89
TGF β signaling pathway	91
Protein phosphatases	91
Retinoic acid-mediated growth inhibition	92
DNA repair	93
Cell cycle control and DNA damage checkpoint genes	96
Apoptosis signaling pathways	98
Autocrine and paracrine growth factors	100
Angiogenesis	100
Evasion of host immunity	101
Metastasis	102
Other miscellaneous genes	104
Order and timing of mutations and changes in gene expression in lung cancer	107
Detection of mutations or aberrant methylation in sputum or plasma/serum from lung cancer patients	111
Early diagnosis and identification of prognostic factors	113
Molecular genetics of chemotherapy and chemoresistance	116

CHAPTER 5

Genetic Approaches to Studying the Association Between Smoking and Lung Cancer	119
Genetic changes associated with smoking	120
Loss of heterozygosity and smoking	120
Aberrant DNA methylation in lung cancers of smokers	121
Telomerase activity and smoking	122
Gene expression studies of lung tumour tissue from smokers and non-smokers	122

Differences in mutation frequency/mutational spectra between smokers and non-smokers	124
p53 mutations, benzo[a]pyrene and lung cancer: the controversy	125
The BPDE-induced mutagenesis model	126
Endogenous <i>versus</i> exogenous causes of mutation	128
A re-examination of the BPDE-induced mutagenesis model	129
Have some <i>TP53</i> mutations occurred during cell culture rather than in the tumour?	133
Other problems for the BPDE-induced mutagenesis model	134
The key importance of the quality of the IARC database.	137
Putting the p53/BPDE-induced mutagenesis controversy in its proper context.	139
The genetics of nicotine addiction	140

CHAPTER 6

Evidence for Genetic Susceptibility to Lung Cancer Derived from Polymorphism-disease Association Studies 143

Polymorphisms and polymorphism-disease association studies	143
Polymorphism-disease association studies in lung cancer.	145
Interpreting the role of xenobiotic metabolizing enzyme polymorphisms in lung cancer	154
Interpreting the role of DNA repair enzyme polymorphisms in lung cancer	159
DNA repair activity for oxidative damage; the contribution of OGG1	161
<i>TP53</i> gene polymorphisms and lung cancer risk	166

CHAPTER 7

Gene Expression Studies in Lung Cancer 167

Studies of the expression of individual genes.	167
Studies of the expression of multiple genes by microarrays and similar techniques.	170
Lung tumour cell ontogeny may be determined by gene expression pathways that recapitulate lung development	185

CHAPTER 8

Lung Cancer Pathogenesis and Future Prospects for Treatment and Prevention 187

Towards an understanding of the pathogenesis of lung cancer at the molecular level.	187
Prevention.	189
Novel treatment modalities offered by gene therapy.	190
Future prospects	193

Relevant Websites 197

Glossary 203

Tables 213

References 279

Subject Index 367