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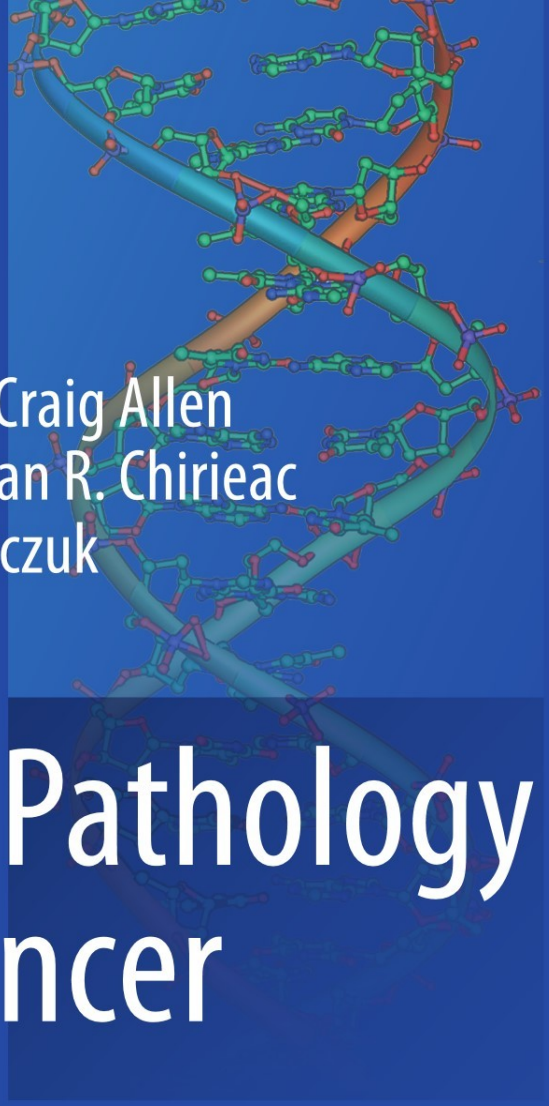

Philip T. Cagle · Timothy Craig Allen
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Sanja Dacic · Alain C. Borczuk
Keith M. Kerr *Editors*

Molecular Pathology of Lung Cancer

Springer

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Molecular Pathology of Lung Cancer

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Series Preface

The past 2 decades have seen an ever-accelerating growth in knowledge about molecular pathology of human diseases which received a large boost with the sequencing of the human genome in 2003. Molecular diagnostics, molecular-targeted therapy, and genetic therapy are now routine in many medical centers. The molecular field now impacts every field in medicine, whether clinical research or routine patient care. There is a great need for basic researchers to understand the potential clinical implications of their research whereas private practice clinicians of all types (general internal medicine and internal medicine specialists, medical oncologists, radiation oncologists, surgeons, pediatricians, family practitioners), clinical investigators, pathologists and medical laboratory directors, and radiologists require a basic understanding of the fundamentals of molecular pathogenesis, diagnosis, and treatment for their patients.

Traditional textbooks in molecular biology deal with basic science and are not readily applicable to the medical setting. Most medical textbooks that include a mention of molecular pathology in the clinical setting are limited in scope and assume that the reader already has a working knowledge of the basic science of molecular biology. Other texts emphasize technology and testing procedures without integrating the clinical perspective. There is an urgent need for a text that fills the gap between basic science books and clinical practice.

In the Molecular Pathology Library series, the basic science and the technology is integrated with the medical perspective and clinical application. Each book in the series is divided according to neoplastic and nonneoplastic diseases for each of the organ systems traditionally associated with medical subspecialties.

Each book in the series is organized to provide specific application of molecular pathology to the pathogenesis, diagnosis, and treatment of neoplastic and nonneoplastic diseases specific to each organ system. These broad section topics are broken down into succinct chapters to cover a very specific disease entity. The chapters are written by established authorities on the specific topic from academic centers around the world. In one book, diverse subjects are included that the reader would have to pursue from multiple sources in order to have a clear understanding of the molecular pathogenesis, diagnosis, and treatment of specific diseases. Attempting to hunt for the full information from basic concept to specific applications for a disease from varied sources is time-consuming and frustrating. By providing this quick

and user-friendly reference, understanding and application of this rapidly growing field is made more accessible to both expert and generalist alike.

As books that bridge the gap between basic science and clinical understanding and practice, the Molecular Pathology Library series serves the basic scientist, the clinical researcher, the practicing physician, and other health care providers who require more understanding of the application of basic research to patient care, from "bench to bedside." This series is unique and an invaluable resource to those who need to know about molecular pathology from a clinical, disease-oriented perspective. These books are indispensable to physicians and health care providers in multiple disciplines as noted above, to residents and fellows in these multiple disciplines as well as their teaching institutions, and to researchers who increasingly must justify the clinical implications of their research.

Houston, TX, USA Philip T. Cagle, MD

Preface

The past few years have seen a revolution in the molecular pathology of lung cancer, including exciting advances in predictive biomarker testing and molecular targeted therapy. Clinical trials in 2009 demonstrated the superiority of tyrosine kinase inhibitor therapy to conventional chemotherapy in patients with advanced lung cancers with activating epidermal growth factor receptor (EGFR) mutations. Response to anaplastic lymphoma kinase (ALK) inhibitor was demonstrated in patients whose lung cancers contained ALK fusion genes in 2010. These and other advances have led to a proposed new classification of adenocarcinoma of the lung by the International Association for the Study of Lung Cancer in February 2011 and Lung Cancer Predictive Biomarker Guidelines to be published by the College of American Pathologists, the International Association for the Study of Lung Cancer and the Association for Molecular Pathology in 2012. This breathtaking chain of events is the impetus for the publication of this book, *Molecular Pathology of Lung Cancer*, in the Molecular Pathology Library series. The editors have been involved in both original research on these topics and in the expert panel for biomarker guidelines referred to above. Our objective is to provide the reader with a basis for understanding current concepts in the molecular pathology of lung cancer in keeping with the aspirations of this book series.

Houston, TX, USA — Philip T. Cagle, MD

Contents

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Part I

Molecular Pathology of Lung Cancer: General Principles

Approach to Personalized Care of the Lung Cancer Patient

1

Philip T. Cagle

As part of the personalized health care of lung cancer patients, identification of predictive biomarkers for targets of molecular therapy is the most reliable basis for selecting patients for targeted therapies. Currently "established" predictive biomarker tests for lung cancer are EGFR mutation analysis and FISH for ALK fusion gene that are the primary subject of the new College of American Pathologists (CAP)/International Association for the Study of Lung Cancer (IASLC)/Association for Molecular Pathology (AMP) Lung Cancer Biomarker Guidelines. Multiple other predictive biomarkers, particularly K-Ras, are mentioned in the guidelines as forthcoming [1].

There is an association between the cell type of a lung cancer, and even cell subtype, and the presence of specific predictive biomarkers [1–9]. For example, EGFR mutations and ALK fusion genes that are likely to respond to currently available tyrosine kinase inhibitors are both strongly associated with adenocarcinoma cell type. Both also have associations with subtypes of adenocarcinoma (lepidic, papillary, and micropapillary patterns are associated with EGFR mutations and solid and acinar patterns are associated with ALK fusion genes) [1–9]. Since exclusion based on clinical criteria, such as gender, ethnicity, and smoking status are likely to omit patients who might benefit from targeted therapy, current evidence indicates that tumor histology is the most reliable criteria for selecting patients for biomarker testing [1]. Therefore, the pathologist has a crucial role in the selection of which lung cancers receive testing for a particular predictive biomarker. This is a major development after decades in which the primary role of the pathologist in patient therapy was to diagnose small cell carcinoma versus non-small cell carcinoma [3, 4].

Either the pathologist or oncologist may order the biomarker test on an individual case basis in some institutions, but automatic reflex testing of all lung cancers meeting selection criteria may be required in other institutions. Pathologists who supervise molecular diagnostic laboratories may be directly involved in the performance of predictive biomarker tests for molecular targeted therapies, but currently a greater number of pathologists are involved in diagnosing, processing, and selecting tissues for these tests. In the pre-analytic phase of testing, a pathologist must review a representative tissue section to determine the cellularity and purity of the tumor sample being submitted for biomarker testing. The pathologist must differentiate cancer from noncancer, viable tissue from nonviable tissue, adequate sample size from inadequate sample size, etc., when selecting tissue samples to send for biomarker testing. Although histology does not trump molecular analysis in predicting which lung cancers are likely to respond to a targeted therapy,

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pathologists have a crucial new role in suggesting which molecular tests are most likely to yield positive results for a given cancer based on the association of specific mutations with specific histologic types and subtypes [1, 3–5].

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Etiology of Lung Cancer

2

Philip T. Cagle

Lung cancer is the number one cause of cancer deaths in the US population [1] and in the global population [2]. An estimated 156,940 deaths from lung cancer are expected in the United States in 2011, more than the combined total of deaths from the next three most common causes of cancer deaths (colon, breast, and prostate cancers) [1]. In 2011, about 28% of all cancer deaths among men and 26% of all cancer deaths among women in the United States will be due to lung cancer. An estimated 1,378,400 deaths from lung cancer are expected globally in 2011 [2]. Globally, 5-year survival for lung cancer patients ranges from 6 to 14% for men and 7 to 18% for women [3, 4].

Approximately 85% of the lung cancers in men and 47% of the lung cancers in women are caused by tobacco smoking globally [3]. In the United States and Europe, tobacco smoking causes over 90% of the lung cancers in men and 75–85% of the lung cancers in women [5]. Globally, the number of lung cancers has increased by about 51% since 1985, due to tobacco smoking, increased population and aging [2–4], although there is a slight decrease in the rate of lung cancer among men and women in the United States in recent years related to a decrease in tobacco smoking [1].

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Although tobacco smoking is, by far, the major risk factor for lung cancer, only about 10% of tobacco smokers develop lung cancer, indicating that other factors also play a role in the development of lung cancer in tobacco-exposed individuals. Among tobacco smokers, the development of chronic obstructive pulmonary disease (COPD) is by far the greatest risk factor for lung cancer. This increased risk of lung cancer among smokers with COPD is probably related to activation of common signaling pathways for both diseases by tobacco smoke as well as smoking-induced chronic inflammation which is a risk factor for both diseases [6]. Latency period and total exposure (pack years or number of packs smoked per day × number of years smoked) as well as differences in enzymes that metabolize carcinogens in tobacco smoke and differences in enzymes that repair DNA, discussed in Chap. 3 Genetic Susceptibility to Lung Cancer, are also important factors specific to each smoker [5, 7, 8]. The risk of developing lung cancer remains elevated in a former smoker for decades after cessation of smoking, although it does gradually decline compared to those smokers who do not quit [5, 6, 9–13].

Although the majority of lung cancers are caused by tobacco smoking, a significant number of lung cancers develop in never smokers [14–21], representing 15% of lung cancers in men and 53% of lung cancers in women globally [3]. To put this in perspective, among US patients, death rates from lung cancer among female never smokers are comparable to death rates from leukemia and endometrial cancer and among male never

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smokers comparable to death rates from cancers of the esophagus, kidney, and liver [14, 18, 20]. A demographic group overrepresented among lung cancer patients are Chinese and other Asian women who are never smokers and have a high incidence of adenocarcinoma of the lung often associated with epidermal growth factor receptor mutations or anaplastic lymphoma kinase fusion genes which are targets of new molecular therapies as discussed in Chaps. 9 and 10 [3, 20]. Risk factors for lung cancer in never smokers may also play a role in smokers and former smokers, in addition to direct tobacco exposure in the latter two groups. These risk factors include family history/heritable factors, environmental tobacco smoke (secondhand smoke), exposure to certain cooking fumes (particularly among Chinese women), occupational and environmental exposures (radon, asbestos, arsenic, pollutants, etc.), hormonal factors, preexisting lung disease (including pneumonia and tuberculosis), dietary factors (including vitamin D levels), human immunodeficiency virus infection, human papilloma virus infection, and exposure to ionizing radiation [15, 18–24].

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Genetic Susceptibility to Lung Cancer

3

Timothy Craig Allen

Tobacco smoke, with its many carcinogens, procarcinogens, and suspected carcinogens, such as nitrosamines, aromatic amines, polycyclic aromatic hydrocarbons (PAHs), and free radical species, is strongly linked to lung cancer risk. Indeed, compared to tobacco smoke, other environmental exposures implicated in lung cancer have little overall impact on lung cancer risk [1]. Notwithstanding the predominance of smoking in lung cancer causation, only 10–20% of smokers develop lung cancer; and approximately 25% of lung cancers occur in never-smokers. Although never-smokers may have been exposed to environmental carcinogens or procarcinogens, their lung cancers are typically considered to be idiopathic. Nonetheless, many people have similar environmental exposures without developing lung cancer. Further, the histologic types occurring in never-smokers differs from those occurring in smokers [2].

People are thought to have different susceptibilities to cancer risk factors, including lung cancer risk factors [3–33]. A genetic basis for differing cancer risk factor susceptibilities has been proposed based on to the observation that different susceptibilities appear to be inherited based on aggregation of cancers within families [34–61]. Inherited susceptibilities would help explain why some people develop lung cancer, such as individuals with minimal or no tobacco smoke exposure [25, 31, 62–70], frequently in association with family histories positive for cancer [26, 70–74], or those who develop lung cancer from exposure at a significantly earlier-than-average age [75–79].

These features support the propositions that genetic differences in susceptibility to tobacco smoke carcinogens exist; that there are complex gene–environment interactions that occur; and that lung cancers in smokers and never-smokers might have both common and distinct risk factors and gene–environment interactions [2]. It is not fully understood whether the specific host susceptibility contributes to lung cancer carcinogenesis alone or in synergy with smoking and other environmental factors [80]; nonetheless, these genetic differences, gene–environment interactions, and risk factors could help explain why some people develop lung cancer with little or no smoke exposure, or at younger ages; why some heavy smokers do not develop lung cancer; and why some lung cancer patients have strong family histories of cancer. Clearly, genetic susceptibility to lung cancer plays some role in lung cancer susceptibility; it is being actively studied [81–83]. Although genetic influences on lung cancer development have been studied since the 1980s [2, 84–87], the recent availability of the human genome database, better genotyping technology, and a better understanding of the role genetics plays in smoking behavior and nicotine addiction have led to a renewed interest in determining the genetic role of lung cancer development [29, 83, 88, 89].

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It is important to keep in mind when considering genetic susceptibility to lung cancer that, although difficult to obtain, large numbers of cases and control subjects are required for studies to produce result that are robust. Such increased numbers typically requires the use of data pooled from many cooperating centers [2]. Also, it is important for studies to consider smoking history, tumor histologic type, patient ethnicity, and patient gender, as lung cancers in smokers and nonsmokers are different diseases, and ethnic differences in lung cancer genetics have been shown [2]. The completion of the Human Genome Project in 2003 and subsequent International Haploid Mapping Project has allowed for a large number of risk association studies that catalogue single-nucleotide polymorphisms (SNPs) in the human genome [2].

Familial Clustering of Lung Cancer and Genetic Predisposition to Smoking

Familial clusters of lung cancer have also been identified; however, it is controversial as to whether these familial clusters lung cancer merely represent familial clusters of smokers or not [55, 90–92]. Nonetheless, some studies, controlling for confounding factors, such as shared smoking habits, secondhand smoke, occupational exposures, etc., have identified an increased risk of lung cancer in relatives of lung cancer patients [40, 44, 49, 54, 59]. Inherited polymorphisms in DNA repair genes and xenobiotic-metabolizing enzyme genes could account for such increased risk. Unfortunately, "progress in identifying specific susceptibility loci and genes has been slow, mainly due to inadequate study designs, underpowered sample sized and preferential reporting of false-positive findings" [93]. Another potential reason for familial clustering of lung cancer is the genetic influence of substance dependence, including nicotine dependence, has been shown [94]. Studies have identified hundreds of potential addiction genes that may contribute to nicotine dependence [95–99]. Multiple genetic loci may be involved in nicotine dependence, including the promoter region of CHRNA5, a locus on chromosome 15 [100–102]. Ongoing studies may provide a better understanding of the genetics of addictive behavior in general, and of nicotine addiction in particular; and what relationship it has with familial clustering of lung cancer. An increased incidence of lung and other cancers in lung cancer patient's family members has been reported [26, 34–38, 40, 43–55, 57–60, 70].

Because family members have common genetics but also often live in common environments, a familial increase in cancer risk could be due to exposures common to the family members sharing the same environment or common lifestyles; and confounding factors, such as common occupational exposures, secondhand smoke, and similar smoking habits must be considered before a familial cluster of cancer can be legitimately considered to arise from a genetic cause. Studies taking into account these confounding factors have shown a statistically significant increased lung cancer risk in relatives of lung cancer patients [26, 35, 39–41, 43, 45–47, 51, 53, 56, 58, 59]. Further, studies of lung cancer patients' families who were nonsmokers or significantly younger than average have shown an increased familial risk of lung cancer, supporting the premise that genetic susceptibility is a factor in lung cancer development [28, 29, 33, 42, 44, 54, 62–66, 71, 72, 75–77, 79, 92, 103]. Inherited polymorphisms in the DNA repair genes, as noted above, and xenobiotic-metabolizing enzyme genes, discussed below cause some degree of familial lung cancer risk. Potential chromosomal loci for lung cancer susceptibility in families have been studied; for example, in a study of multigenerational families with lung, throat, and laryngeal cancer, a lung cancer susceptibility locus was mapped to chromosome 6q23-25 [52].

Gender and Lung Cancer Risk

Potential gender differences in lung cancer risk have been studied [104–112]. Research is conflicting as to whether women smokers have an increased risk of developing cancer relative to men smokers with similar smoking histories

[104–117]. Some studies suggest that women smokers have an increased risk of developing lung cancer relative to men with the same smoking histories [111, 112, 118] while others have not identified any differences [113, 114]. The International Early Lung Cancer Action Program Investigators, studying 7,498 women and 9,427 men, found an increased susceptibility to tobacco carcinogens in women [117]; however, neither the Nurses' Health Study, examining smoking and lung cancers in more than 60,000 women, nor the Health Professionals Follow-Up Study, studying more than 25,000 men, identified an increased lung cancer risk in women [114]. Environmental factors and hormonal influences are reasons that have been proposed for reported differences in gender-associated lung cancer susceptibility, as have differences in xenobiotic-metabolizing enzymes between men and women [119, 120].

Xenobiotic-Metabolizing Enzymes

Xenobiotics, chemicals within the body, such as drugs, toxins, solvents, and poisons, are metabolized or altered by xenobiotic-metabolizing enzymes; and xenobiotics often induce xenobiotic-metabolizing enzymes by various methods, including by acting as substrate-ligands that bind receptors, by activating the xenobiotic enzymes by transcription, or by stabilizing the protein product. Phase I xenobiotic-metabolizing enzymes metabolize the xenobiotic chemicals into other compounds; but paradoxically can metabolically bioactivate xenobiotic substrates, transforming them into active or more potent toxins or carcinogens, the so-called reactive intermediates. The cytochrome P450s or CYPs are important phase I xenobiotic-metabolizing enzymes. Phase II enzymes detoxify reactive intermediates and transform them into compounds that can be removed from the body; the glutathione-*S*-transferases (GSTs) are an important class of phase II enzymes. Phase III transporters, including P-glycoprotein (P-gp), multidrug resistance-associated proteins (MRPs), and organic anion transporting polypeptide 2 (OATP2) are associated with xenobiotic transport and excretion [121–135].

Phase I enzymes P450s or CYPs primarily catalyze xenobiotic oxidation; however, they also catalyze reduction reactions. Also, CYPs are involved in other processes, such as biosynthesis of steroid hormones and prostaglandins [132–145]. These reactions generally occur in the liver but can occur in other tissues, including lung tissue [146–149]. CYP-dependent metabolism often produces intermediate compounds called reactive intermediates that may be more potent carcinogens than their parent compounds, and that could covalently bind to DNA and form adducts. DNA adduct formation is an important step in carcinogenesis. These intermediate compounds are also converted to more soluble, inactive products that may be excreted or compartmentalized by phase II enzyme-dependent conjugation reactions. CYP metabolism therefore may be a double-edged sword, leading to production of reactive intermediates that are more carcinogenic than the original compounds, but also more readily detoxified and removed than the original compounds. Nearly, 60 active human P450 genes, mostly polymorphic, have been identified. CYP enzymes and genes are designated by family number (an Arabic number), subfamily letter (A, B, C, etc.), and individual members of a subfamily (also an Arabic number). Class I polymorphic CYP enzymes, which include CYP1A1, CYP1A2, CYP1B1, CYP2A6, CYP2E1, and CYP3A4, metabolize procarcinogens. CYP1A1 and CYP1B1 are particularly important for the metabolism of PAHs from tobacco smoke, and CYP2A6 and CYP2E1 are involved in the metabolism of nitrosamines from tobacco smoke [122, 132–136, 139–142, 144, 145].

Many *CYP*s are induced by the aryl hydrocarbon receptor (AhR), which acts by dimerizing with the AhR nuclear translocator (Arnt) and inducing expression of *CYP1A1* and *CYP1B1*. *CYP1A1* and *CYP1B1* encode aryl hydrocarbon hydroxylases as well as *CYP1A2*. Ligands for AhR include PAHs and other xenobiotics which are also substrates for the activated CYP enzymes. AhR shows either low affinity or high affinity for its ligands, producing low or high inducibility of

CYP1 enzymes. AhR, after binding its ligand, translocates into the nucleus and dimerizes with Arnt protein. The AhR/Arnt dimer then binds to xenobiotic responsive elements (XREs) of the *CYP1A1* gene and activates its transcription [150–156].

Benzo(a)pyrene is an extensively studied PAH found in tobacco smoke. It binds to AhR in the lungs, causing the induction of *CYP1A1* and *CYP1B1*. CYP enzymes metabolically activate benzo(a)pyrene to benzo[*a*]pyrene-7,8-diol-9,10-epoxide (BPDE). BPDE is a carcinogen that damages DNA by covalently bonding to the DNA, forming bulky chemical adducts, for example by binding to guanine nucleobases in codons 157, 248, and 273 of *p53*—mutational "hotspots" in smoking-related lung cancers [157–164]. Along with PAHs, tobacco smoke contains *N*-nitrosamines, including 4-(methylnitrosoamino)-1-(3-pyridyl)-1-butanone (NNK), *N*-dimethylnitrosoamine (NDMA), *N*-diethylnitrosoamine (NDEA), *N*-nitrosopheny lmethyl-amine (NMPhA), and *N*-nitrosonornicotine (NNN). These *N*-nitrosamines are metabolically activated by CYP2A6 and CYP2E1 to compounds that form chemical adducts with DNA [165–169].

The phase II enzymes GSTs act mainly to catalyze the conjugation of glutathione (GSH) to xenobiotics containing an electrophilic center, forming more soluble, nontoxic peptides that are excreted or compartmentalized by other enzymes, the phase III enzymes. The GST superfamily is made up of enzymes that catalyze the conjunction of glutathione to xenobiotics; and is divided into three subfamilies, each composed of multigene families—soluble or cytosolic (canonical) GSTs, microsomal, or membrane-associated proteins involved in eicosanoid and glutathione metabolism (MAPEG) GST, and the plasmid-encoded bacterial fosfomycin-resistance GSTs. The cytosolic GSTs are polymorphic and make up seven classes—alpha, mu, and pi are regarded as specific, and sigma, omega, theta, and zeta as common. Importantly, the cytosolic GSTs assist in the metabolism of tobacco-derived carcinogens are GSTM1, GSTM3, and GSTP1 that detoxify reactive intermediates of PAHs, such as benzo(a)pyrene, and GSTT1 that detoxifies reactive oxidants, such as ethylene oxide [170–176].

There are other phase II enzymes, including *N*-acetyltransferases (NAT), sulfotransferases (ST), UDP-glucuronosyltransferases (UGT), and NAD(P)H:quinone oxidoreductase (NQO1). Microsomal epoxide hydrolase (mEH) is a phase II enzyme which also acts as a phase I enzyme; it catalyzes the *trans*-addition of water to xenobiotics, such as the PAH benzo(a)pyrene, producing dihydrodiol reactive intermediates involved in PAH-initiated carcinogenesis [177–182].

DNA Adducts and Lung Cancer

DNA adducts from metabolically activated intermediates of compounds found in tobacco smoke are mutagenic and carcinogenic [183–187]. Bulky DNA adducts can be identified with 32P-postlabeling of tumor tissues, peripheral blood lymphocytes and other tissues, immunoassays and immunohistochemistry, mass spectrometry, fluorescence, HPLC-electrochemical detection, and phosphorescence spectroscopy [188]. PAH-DNA adducts can be identified by BPDE-DNA immunoassays, such as the BPDE-DNA chemiluminescence immunoassay (BPDE-DNA CIA) [189].

Elevated DNA adduct levels have been found in smokers' lung and other tissues; and more DNA adducts are found in patients with smoking-related cancers than in patients without cancer [190–199]. Veglia et al. performed a meta-analysis that included data on 691 cancer patients and 632 controls from six studies (five studies involved lung cancer, one study oral cancer and one study bladder cancer) and found that smokers with smoking-related cancers had a statistically significant (83% higher) level of DNA adducts than controls [194]. Gyorffy et al. studied 85 lung cancer patients—47 smokers, 23 long-term former smokers, 15 never-smokers—and identified increased levels of DNA adducts in smokers' lungs relative to non- and never-smokers' lungs [195]. Along with studies demonstrating the carcinogenicity of DNA adducts from tobacco smoke, these studies support a link between DNA adduct number and lung cancer development. However, it must be remembered that in retrospective case–control studies

the possibility that the levels of DNA adducts are the result of, rather than the cause of, the disease cannot be completely excluded. Nonetheless, that DNA adducts are causative is strongly supported by prospective studies where DNA adducts were measured in blood samples collected years before cancer onset. Tang et al., comparing blood samples from 89 subjects enrolled in the prospective Physicians' Health Study who developed primary lung cancers with 173 controls, found that disease-free current smokers with elevated levels of DNA adducts in blood leukocytes were three times more likely to be diagnosed with lung cancer 1–13 years later than current smokers with lower DNA adduct levels [191].

Peluso et al. studied patients enrolled in the European Prospective Investigation into Cancer and Nutrition (EPIC) investigation and found that the levels of leukocyte DNA adducts in blood samples collected several years before the onset of cancer were associated with the subsequent risk of lung cancer [196]. The association with lung cancer was stronger in never-smokers—whose sources would be environmental, such as secondhand tobacco smoke and air pollution—and in younger patients. These prospective studies strongly support a relationship between DNA adduct levels and lung cancer risk. The studies also suggest that individual patients have differing susceptibilities to carcinogen exposures, highlighted by the risks observed in those with fewer years of exposure—younger patients—and those with lesser levels of exposure—never-smokers.

Polymorphisms and DNA Adduct Levels

DNA adduct levels differ not only due to exposure levels, but also due to the activity levels of xenobiotic enzymes [199–207]. Some specific xenobiotic enzymes alleles are more active than other alleles; and a variant of a phase I enzyme that is highly active (extensive metabolizer) may produce a greater number of reactive intermediates and, therefore, more DNA-adducts than a less active variant of the same phase I enzyme (poor metabolizer). A less active phase II enzyme variant may detoxify reactive intermediates more slowly than a more active variant, resulting in a greater accumulation of reactive intermediates and, therefore potentially creating more DNA adducts. As such, polymorphisms of xenobiotic enzymes may contribute to differing DNA adduct levels in patients, which could cause patients to exhibit different susceptibilities to lung cancer. This is also true when less active variants of DNA repair genes repair damage from DNA adducts, or other sources, at a reduced rate.

Differing levels of DNA adducts may occur in association with different variants of xenobiotic enzymes [199–207]. Patients without the GSTM1 enzyme have higher DNA adduct levels compared to *GSTM1*-positive patients. GPX1 is a phase II enzyme that conjugates PAH-diols to glutathione; and in *GPX1*, the *Pro198Leu* allelic variant has lower enzyme activity, resulting in less detoxification, and consequently higher DNA adduct levels compared to wild-type patients. mEH is a phase II enzyme; and the slow allelic variant *mEH*2* yields increased epoxide intermediates, and consequently higher DNA adduct levels [206, 207].

Specific Polymorphisms Associated with Lung Cancer Susceptibility

Studies of polymorphisms of xenobiotic-metabolizing genes and DNA repair genes, described below, have found potential allelic variants associated with greater or lesser risk of lung cancer [208–213]. The concept of polymorphisms of xenobiotic-metabolizing enzymes and DNA repair enzymes is appealing; however, studies correlating single-locus alleles with lung cancer risk have generally produced conflicting results, probably due to a number of factors. In some studies, the number of cases might be too few to reliably gauge the effects on lung cancer risk. Also, the polymorphisms studied might vary. Further, different ethnic groups exhibit widely differing frequencies of some polymorphisms, effecting results according to the ethnic group studied. Finally, as the metabolism,