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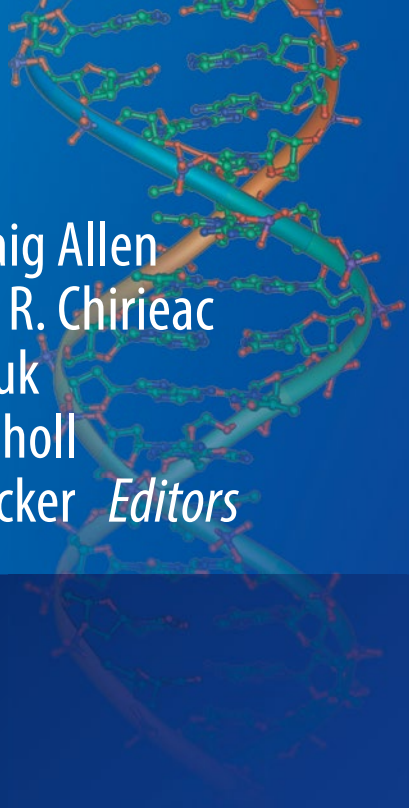

Philip T. Cagle · Timothy Craig Allen
Mary Beth Beasley · Lucian R. Chirieac
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Keith M. Kerr · Lynette M. Sholl
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Precision Molecular Pathology of Lung Cancer

Second Edition

Springer

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Philip T. Cagle
Houston, TX, USA

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Part I
Background

Chapter 1
Precision Medicine in Lung Cancer

Keith M. Kerr and Gavin M. Laing

This textbook discusses in considerable detail the molecular landscape of lung cancer and how the molecular biology of the tumour is involved in the evolution, growth and development of the disease. Huge advances in our knowledge have been made in recent years, thanks in part to technology allowing whole exome and even whole genome sequencing. Apart from elucidating the molecular basis of this most fatal of malignant diseases, the molecular features of lung cancer can also be exploited therapeutically. Pathologists have known for a very long time about how different individual tumours can be from each other; to a large extent, we now understand that this morphological variation is a reflection of molecular heterogeneity. The development of so-called molecularly targeted drugs, and a realization that these drugs do not work for every patient, rapidly led to the need to select patients, often based on their molecular characteristics, to ensure a higher chance of therapy response. This idea of precision or personalized medicine is of course, not new. The concept of selective toxicity was pioneered decades ago [1] and has been a familiar practice in medicine, treating infections with antibiotics based upon sensitivity testing. In oncology, one of the first tumour types to have a precision medicine approach, selecting patients for therapy based upon pathological characteristics, was breast cancer. Oestrogen and progestogen receptor testing for tamoxifen therapy and, later, HER2 testing for trastuzumab therapy are well established in clinical practice. Precision medicine and personalized therapy in lung cancer is a more recent development, but this has developed into an extremely diverse and complex branch of oncology, bringing considerable benefits for groups of patients, multiple choices for oncologists and considerable demands on pathologists.

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Precision Medicine: Impact on Lung Cancer Therapy

In the 1980s cytotoxic agents were used in some patients with small cell lung cancer (SCLC). Systemic chemotherapy, different from that used in small cell carcinoma, was introduced into routine practice as a palliative measure in advanced non-small cell lung cancer (NSCLC) in the early 1990s. Thus, one could argue that the earliest selection of patients with lung cancer was based upon this paradigm of treating advanced small cell lung cancer in one way, and all other lung cancer by other means. Prior to the introduction of such systemic therapy, lung cancer treatment was based upon surgery and radiotherapy for localized disease and best supportive case (basically no active, cancer-directed treatment) for advanced disease. This rather crude discrimination actually spawned the concept of 'non-small cell carcinoma' as a so-called entity, a therapeutic grouping of convenience, which lumped together a group of pathologically and biologically very different diseases because they were all treated in the same way. Multiple cytotoxic chemotherapeutic regimens were developed which improved overall survival in advanced NSCLC to perhaps 6–8 months from diagnosis, but by around 2005, it was felt that a plateau had been reached; various cytotoxic therapy approaches all delivered more or less the same, limited efficacy [2].

Progress in treatment of SCLC has been very limited. Platinum-etoposide-based regimens have been the mainstay of treating this disease for many years. There have been very few signs of success in targeted therapy, and there are none in routine clinical use. The genetics of SCLC are described elsewhere in this book. Genomic studies of SCLC have failed to identify any promising drug targets [3]. One interesting recent development, however, is the exploitation of DLL3 expression on the surface of SCLC cells. An antibody against DLL3 is used as a means to selectively target SCLC cells expressing this marker and deliver an antibody-conjugated cytotoxic agent in patients who express high levels of DLL3 on their tumour, as assessed by a specific anti-DLL3 immunohistochemistry (IHC) assay [4].

Between 2004 and 2008, two therapeutic developments began a significant change towards precision medicine in advanced NSCLC. The anti-angiogenic agent bevacizumab demonstrated an increased risk of fatal haemorrhage in patients with squamous cell carcinoma, but not in adenocarcinoma where survival benefits were demonstrated in combination with platinum-doublet chemotherapy [5, 6]. The drug was approved only for patients with 'non-squamous' NSCLC. A pemetrexed-platinum doublet showed superior outcomes when compared to gemcitabine-platinum in adenocarcinoma and undifferentiated tumours; the drug label for pemetrexed required a diagnosis of adenocarcinoma or large cell carcinoma [7]. These events were the catalyst for the drive towards specific NSCLC subtyping in advanced disease small diagnostic samples and an attempt to eradicate the term NSCLC-NOS (not otherwise specified). This is discussed below and in Chap. 5.

Trials of inhibitors of the epidermal growth factor receptor (*EGFR*) tyrosine kinase (TKI) began to be reported around 2000, with mixed results, but with the observation that certain patients did spectacularly well on these therapies. These

patients tended to be younger, never-smoking females of East Asian ethnicity with advanced adenocarcinoma. It was discovered that these patients who responded particularly well to EGFR TKIs had tumours which bore mutations in the tyrosine kinase domain of the *EGFR* gene [8, 9] and there followed a series of successful trials demonstrating clear clinical benefit for EGFR TKIs in patients with a range of *EGFR* mutations in exons 18–21 [10–13]. This underpinned the need for *EGFR* mutation testing to select patients for EGFR TKI therapy, now a routine practice and standard of care.

The EGFR story highlighted the importance of identifying cancers driven by so-called addictive oncogenic changes [14]. Addictive oncogenes make excellent drug targets and provide biomarkers which are highly predictive of therapy response. The next to be discovered in NSCLC was a group of rearrangements involving the anaplastic lymphoma kinase (*ALK*) gene and a number of gene partners, leading to activation of the *ALK* gene tyrosine kinase [15]. The ALK TKI crizotinib rapidly proved its worth in treating patients with adenocarcinomas bearing *ALK* rearrangements [16–18]. There are now several other ALK TKIs at various stages in the trial regulatory process. Several other apparently addictive oncogenic changes have been discovered in lung adenocarcinomas which are variably associated with, but not exclusive to, the same patient demographic as for EGFR-mutated tumours. Essentially, this reflects an adenocarcinoma phenotype whose genesis is unknown; other than that tobacco carcinogens are not involved. ROS proto-oncogene 1 (*ROS1*) gene rearrangements are also associated with this adenocarcinoma phenotype, and crizotinib is now approved in many countries for the treatment of patients with such rearrangements [19–22]. RET proto-oncogene (*RET*) and neurotrophic receptor tyrosine kinase 1 (*NTRK1*) gene rearrangements and B-raf proto-oncogene (*BRAF V600E*) and erb-b2 receptor tyrosine kinase 2 (*HER2*) mutations account for small groups of adenocarcinoma patients with drugs undergoing trials [19–22]. *BRAF* inhibitors will probably be the first in this latter group to gain regulatory approval. MET proto-oncogene (*MET*) exon 14 skipping mutations are a promising target found in a number of NSCLC tumour types [23, 24].

The benefits to patients, of discovering therapeutically targetable molecular drivers in their tumours, and delivering the appropriate therapy, have been demonstrated [25]. The benefit is a real, treatment-related effect, rather than a prognostic effect related to the molecular alteration. The use of EGFR and ALK TKIs is now regarded as standard of care in those molecularly defined groups of patients, treatment for *ROS1* rearrangements is similarly regarded in many countries and as more drugs gain regulatory approval, so practice will change as newly introduced therapies are incorporated into treatment guidelines for advance stage NSCLC [26, 27]. Immunotherapy, specifically through the use of anti-PD1 and anti-PD-L1 immune checkpoint inhibitors, is rapidly becoming established in the treatment of advanced NSCLC, and with some of these drugs comes the need for biomarker-based patient selection. This matter is discussed in some detail in Chap. 20. All of these developments reflect the remarkable success or personalized, precision medicine for patients with lung cancer. Almost all of the recently approved treatments, which are largely responsible for overall survival for advanced NSCLC extending out to

beyond 12 months, are prescribed on the basis of a biomarker test. These advances have also transformed the diagnostic pathways for lung cancer, presenting exciting new opportunities and challenges in equal measure, for pathologists dealing with these cases.

Precision Medicine: Impact on Lung Cancer Pathology

Diagnostic Complexity

The development of lung cancer therapies specifically targeting pathologically and/or molecularly defined subsets of patients, as described above, has had an enormous impact on the diagnostic process required for cases of lung cancer, especially in the setting of advanced disease [28–31].

The diagnostic journey begins with the identification of carcinoma in the submitted sample. As discussed below, and in Chap. 5, most diagnostic samples from lung cancer patients provide only very limited amounts of tumour. Through dialogue with colleagues, discussion at the tumour board or multidisciplinary team (MDT) meeting and what should be regarded as mandatory clinical information provided with the samples sent for diagnosis, the pathologist should be aware of the likelihood of a diagnosis of primary lung cancer or any possibility of metastases to the lung. Separation of SCLC from other tumour types is followed by the subtyping of NSCLC cases as accurately as possible. IHC now plays a pivotal role in this process, as discussed in Chap. 5. It is imperative that IHC is carried out only when required. If the diagnosis of adenocarcinoma or squamous cell carcinoma can be made by morphology alone, usually so in 60–75% of cases in small sample diagnosis, IHC should not be carried out to confirm tumour subtype. IHC should only be used in those cases which the pathologist would morphologically classify as NSCLC-NOS. In a case of adenocarcinoma, clinical details should drive any IHC-based investigation of possible primary sites other than lung. There is evidence that pathologists overuse IHC in the small sample diagnosis of lung cancer and thus waste precious tissue, compromising the subsequent molecular testing in appropriate cases [32].

Current guidelines recommend that cases of possible, probable or definite adenocarcinoma should be submitted for molecular testing [33, 34], as these are the samples most likely to bear a targetable molecular alteration. Rare cases of squamous cell or small cell carcinoma in never or long-time ex-smokers should also be tested. As more molecular targets are defined, with approved drugs being made available, it may be justifiable to test all patients with NSCLC, using multiplex testing approaches such as next-generation sequencing (NGS) [35–37]. Currently, however, this broad approach is not financially justifiable, based on the limited number of drugs available in most health systems, and the very strong bias of current targets to an adenocarcinoma phenotype. In an academic setting, a more broad approach, such as testing for multiple targets to select

patients for clinical trials, is rapidly becoming common practice [36]; drug availability is the most important driver of testing practice, and this is highly variable from a global perspective.

Tissue Handling

Lung cancer patients mostly present with advanced, metastatic disease and are suitable for only palliative systemic therapy, if any treatment at all. A significant proportion of lung cancer patients are too unwell, either for investigation and tissue confirmation of their disease or systemic therapy. Practice varies, but 15–25% of patients with a clinical diagnosis of lung cancer are unfit for, or refuse, further investigation. If patients do have a tissue diagnosis of their tumour, this will usually be based upon a small tissue biopsy or a cytology sample taken from a site or sites deemed most easily accessible. As a consequence of limited accessibility due to disease location and patient comorbidities, lung cancer samples are almost universally small and contain relatively little tumour [38]. It is therefore essential that these samples are handled with great care, without waste and in ways that facilitate the possible extensive biomarker investigation that may be required once the complete histological diagnosis is achieved.

Tissue samples have to be fixed and processed before sections can be made for staining and examination. Although certain types of biomarker testing may be favoured by different fixation and processing methods, a sample can only be fixed and processed once, and that has to be suitable for all of the possible testing approaches that may be required. Thus, standard fixation using 10% neutral buffered formalin is recommended, and tissue should be fixed for between 6 and 72 h. Outside this window, DNA damage and protein epitope alterations may occur [39]. We have also learned that some IHC epitopes are not well preserved by alcohol fixation, and very short fixation times, which may help preserve DNA, can lead to poor IHC performance.

A conservative approach to the use of IHC in the initial diagnostic phase has already been emphasised. Biomarker testing in lung cancer is now pursued along two separate methodological lines. Some tissue from the formalin-fixed, paraffin-embedded tissue block is used for DNA and, perhaps, RNA extraction. Tissue sections are also required for morphology-based tests such as predictive IHC-based biomarker testing or biomarkers based upon in situ hybridization. For the latter, fluorescence methods (FISH) are more often used than bright field approaches such as chromogenic or silver-precipitant (CISH or SISH) methodology.

For samples where a possible lung cancer diagnosis is likely (various thoracic samples in a relevant clinical context), block cutting strategies can be employed to limit the number of times a block is (re)cut, as this wastes tissue on each occasion. Extra tissue sections taken up front, in anticipation of need, can be used as required for deeper sections, IHC, FISH, etc. It would be rare for laboratories to be able to

take sections for molecular analysis in this way, as those sections are normally made separately on a molecularly sterile instrument. These strategies certainly help maximize the use of very limited tissue resources [40].

Genes Versus Proteins

A central tenant in molecular biology is the transcription of DNA to produce mRNA message that is translated into protein. In cells, proteins are the active, effector molecules, encoded by genes. Proteins drive oncogenic events and are also the targets of drugs. Depending on the molecular abnormality being targeted by a drug, it may make more sense to use DNA, for example, for mutation testing, whilst some biomarker tests, for example, PD-L1, directly target the protein itself. Testing lung cancer samples for *ALK* gene rearrangements has been approached in many different ways [40]. The change in the DNA sequence signalling the rearrangement can be sought at the DNA level by FISH or next-generation sequencing approaches; unique mRNA transcripts can be looked for or elevated levels of ALK protein demonstrated by IHC. Each approach appears to predict for therapeutic response. It is likely that *ROS1* rearrangement testing will also develop in a similar way. Diagnostic practice is driven partly by evidence for the most efficacious approach, but also by perceptions about which is the easiest, quickest and cheapest method. Simple, low-cost, rapid testing is always attractive, but it may not necessarily provide the best answers, and pathologists need to be careful not to move their testing approaches too far away from what was validated in clinical drug trials; otherwise, patient selection may become inaccurate and inefficient.

Diagnostics and Quality Assurance

The rapid expansion in the number of possible biomarker tests required on a small lung cancer tissue sample poses the pathology community with some interesting challenges [28–31]. Some drugs are approved with a so-called companion diagnostic test. This is a specific test, carried out in a particular way, and is often the exact test used in clinical trials which provided the evidence of drug (and test) efficacy which underpins drug approval. An example would be the anti-PD-L1 IHC assay using the 22C3 clone marketed by Dako (Carpenteria, CA, USA). Prescription of pembrolizumab requires demonstration of PD-L1 expression in the tumour (see Chap. 20). Other commercially produced anti-PD-L1 IHC assays, based on clones 28–8 (Dako) or SP142 (Ventana, Tucson, AZ, USA), are marketed as complementary diagnostics for immunotherapy agents nivolumab and atezolizumab, respectively. Complementary diagnostics are not mandated for drug prescription but are regarded as an optional, though potentially informative, test [41]. This distinction is a relatively new concept in biomarker diagnostics. It remains to be seen how pathologists and oncologists navigate this matter.

Commercially manufactured kits for biomarker testing are generally high-quality, reliable products which have been manufactured using stringent quality controls. As mentioned above, they are frequently the tests that were used and validated in clinical drug trials. They are, however, generally relatively expensive and usually require specific equipment for their use. These latter factors often limit the adoption of commercial kit tests in some laboratories, where instead, pathologists prefer (or are obliged) to develop their own assays, so-called laboratory-developed tests (LDTs). Test cost is especially a factor when screening large numbers of patients for rare alterations [42]. Whilst these LDTs may be cheaper, they require rigorous validation. For some biomarker tests, it can be argued that the test methodology used is less critical, provided its characteristics are of adequate sensitivity and specificity for the biomarker assessed, in the clinical samples in use. How one demonstrates the presence of an EGFR mutation is less important provided test performance is adequate, and its use is regularly validated by external quality assurance. For IHC-based biomarker tests, the issue is less clear-cut. Subjective assessments, variable primary antibody performance and assay dependence on the detection system all introduce potential variation. This makes the use of LDTs, whose characteristics could vary markedly from a trial-validated 'gold standard', and for which, almost by definition, there are unlikely to be any clinical validating data, open to question. The College of American Pathologists has produced guidelines for LDT validation, but these are designed largely for diagnostic IHC tests, rather than predictive biomarker tests [43, 44].

The importance of biomarker tests in delivering precision medicine for lung cancer patients is clear. It is vital that the test performs in the required way, to guarantee the correct patient selection in order to ensure the predicted likelihood of therapy response. Following best laboratory practices and procedures, and an awareness of the pre-analytical factors that may influence test outcome, is complemented by participation and adequate performance in external quality assurance (EQA) schemes. These schemes generally drive up testing quality and help highlight issues, for example, the potential risks of using some LDTs [45].

Making Sense of It All

Biomarker testing is now a standard of care for patients with advanced NSCLC. This has made the pathological diagnostic process extremely complex, and this will only increase as more drugs, with their own biomarkers, are approved for use. The introduction of massively parallel sequencing technology (NGS) [35–37] allows the simultaneous screening of large panels of genes for mutations, rearrangements and, in some circumstances, gene copy number. Whilst these techniques are very powerful and allow assessment of many genes, in samples that might be insufficient to support multiple 'standalone' tests, they also generate a huge amount of additional data, on a large range of genes that may not be clinically useful. As mentioned above, in an academic setting, these data may be useful if they allow patients access to more drugs through clinical trials [36]. For routine practice, however, depending

on the health system environment involved, this data output can be extremely valuable or may be mostly un-actionable and can cause confusion [37]. Virtually all trials of targeted agents have involved selection using a single biomarker. Very little is known about the influence of coexisting mutations or other genetic changes that might alter treatment response to the primary target. Trials addressing this are underway, but they pose many challenges [46].

Molecular biology is a topic relatively unfamiliar to many tissue pathologists, but the growth of precision, personalized lung cancer medicine is requiring those working on lung cancer to learn fast! Collaboration with molecular pathologists is essential, to ensure that adequate and appropriate material is submitted to the molecular laboratory, consistent with the assay technology in use. The results of the assays need to be interpreted in the context of the samples used, and of any known issues with content, quality, processing and so on, as well as the actual tumour pathology. Much of this collaboration and dialogue is increasingly conducted in a molecular MDT or tumour board meeting, where experts in the significance of molecular findings can combine with tissue pathologists, oncologists and others to determine the best management plan for the patient.

Pathologists are increasingly using IHC as part of the biomarker testing plan for NSCLC samples. The interpretation of IHC-based biomarkers is often more complex than for IHC used as an adjunct to morphological diagnosis. For some assays, there may be a particular quantitative, as well as qualitative element to reading the IHC slides and signing out an opinion upon which clinical action should be taken. Training in reading some assays is often needed. An increased awareness of the assay details, how the dynamic range of staining looks and how that influences test results, and the staining artefacts and other characteristics that may lead to a false positive read [47], all take on great importance.

There is great interest in alternatives to tumour tissue samples, as sources of biomaterial for biomarker testing. The so-called liquid biopsy, using blood-borne, circulating cell-free DNA or tumour cells, or DNA from urine, appears to be a potentially effective alternative to tissue, especially for the detection of the *T790M EGFR* exon 20 resistance mutation [48, 49], but this approach is still evolving. Biomarker testing, in general, in the setting of the almost inevitable relapses suffered by patients after responses to many targeted TKI therapies, is also rapidly emerging as a significant problem, for patients, oncologists and laboratories. Resistance mechanisms are highly varied, in tumour regrowing after EGFR or ALK TKI therapy [50–54], and this creates another menu of biomarker tests that may have to be pursued, presuming new tumour samples can be accessed at the time of disease relapse, and that there are therapeutic interventions available, determined by those tests.

Conclusion

Precision, personalized medicine for patients with advanced NSCLC is now well established in routine clinical practice. Patients who have a targetable factor in their tumour, and who receive the targeted treatment, generally benefit, and to a greater

extent when compared to 'standard of care' unselected chemotherapy. Response rates to some targeted agents, when given to biomarker 'positive' patients, can be over 70%, when compared to response rates to chemotherapy of around 30%. The need for biomarker testing is clear and will increase as more drugs are approved. This continually poses challenges for pathology laboratories, dealing with limited sample resources. Diverse and increasingly sophisticated technology is needed to generate a wide range of biomarker data. We are still very early in the process of understanding the significance of interrelationships between various genetic changes which may be found in a patient's tumour. The molecular landscape in lung cancer is extremely complicated but is offering more and more opportunity for new treatments for our patients. In the following chapters are detailed discussions of the molecular pathology of lung cancer and its importance, not only in terms of tumour biology but also with regard to more effectively treating patients with these diseases.

References

1. Albert A. Selective toxicity. 5th ed. London: Chapman and Hall; 1973.
2. Schiller JH, Harrington D, Belani CP, et al. Comparison of Four chemotherapy regimens for advanced non-small-cell lung cancer. N Engl J Med. 2002;346:92–8.
3. Peifer M, Fernandez-Cuesta L, Sos ML. Integrative genome analyses identify key somatic driver mutations in small cell lung cancer. Nature. 2012;44:1104–10.
4. Rudin CM, Pietanza MC, Bauer TM, et al. Rovalpituzumab tesirine, a DLL3 targeted drug-antibody conjugate, in recurrent small cell lung cancer. A first-in-human, first-in-class, open label study. Lancet Oncol. 2017;18:42–51.
5. Johnson DH, Fehrenbacher L, Novotny WF, et al. Randomized phase II trial comparing bevacizumab plus carboplatin and paclitaxel with carboplatin and paclitaxel alone in previously untreated locally advanced or metastatic non-small cell lung cancer. J Clin Oncol. 2004;22:2184–91.
6. Sandler A, Gray R, Perry MC, et al. Paclitaxel-carboplatin alone or with bevacizumab for non-small cell lung cancer. N Engl J Med. 2006;355:2542–50.
7. Scagliotti GV, Parikh P, Pawel JV, et al. Phase III study comparing cisplatin plus gemcitabine with cisplatin plus pemetrexed in chemotherapy-naïve patients with advanced-stage non-small cell lung cancer. J Clin Oncol. 2008;26:1–10.
8. Paez JG, Janne PA, Lee JC, et al. EGRF mutations in lung cancer: correlation with clinical response to gefitinib therapy. Science. 2004;304:1497–500.
9. Lynch TJ, Bell DW, Sordella R, et al. Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small cell lung cancer to gefitinib. N Engl J Med. 2004;350:2129–39.
10. Mok TS, Wu YL, Thongprasert S, et al. Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. N Engl J Med. 2009;361:947–57.
11. Maemondo M, Inoue A, Kobayashi K, et al. Gefitinib or chemotherapy for non-small cell lung cancer with mutated EGFR. N Engl J Med. 2010;362:2380–8.
12. Mitsudomi T, Morita S, Yatabe Y, et al. Gefitinib versus cisplatin plus dicetaxel in patients with non small-cell lung cancer harbouring mutations of the epidermal growth factor receptor (WJTOG3405): an open label, randomized phase 3 trial. Lancet Oncol. 2010;11:121–8.
13. Rosell R, Moran T, Queralt C, et al. Screening for epidermal growth factor receptor mutations in lung cancer. N Engl J Med. 2009;361:958–67.
14. Weinstein IB. Cancer. Addiction to oncogenes—the Achilles heel of cancer. Science. 2002;297:63–4.

15. Soda M, Choi YL, Enomoto M, et al. Identification of the transforming EML4-ALK fusion gene in non-small-cell lung cancer. Nature. 2007;448:561–6.
16. Camidge DR, Bang YJ, Kwak EL, et al. Activity and safety of crizotinib in patients with ALK-positive non-small-cell lung cancer: updated results from a phase 1 study. Lancet Oncol. 2012;13:1011–9.
17. Ou SH, Bartlett CH, Mino-Kenudson M, et al. Crizotinib for the treatment of ALK-rearranged non-small cell lung cancer: a success story to usher in the second decade of molecular targeted therapy in oncology. Oncologist. 2012;17:1351–75.
18. Shaw AT, Kim DW, Nakagawa K, et al. Crizotinib versus chemotherapy in advanced ALK-positive lung cancer. N Engl J Med. 2013;368:2385–94.
19. Pao W, Iafrate AJ, Su Z. Genetically informed lung cancer medicine. J Pathol. 2011;223:230–40.
20. Pao W, Girard N. New driver mutations in non-small-cell lung cancer. Lancet Oncol. 2011;12:175–80.
21. Tsao AS, Scagliotti GV, Bunn PA, et al. Scientific advances in lung cancer 2015. J Thorac Oncol. 2016;11:613–38.
22. Tan W-L, Jain A, Takano A, et al. Novel therapeutic targets on the horizon for lung cancer. Lancet Oncol. 2016;17:e347–62.
23. Frampton GM, Ali SM, Rozenzweig M, et al. Activation of MET by diverse exon 14 splicing alterations occurs in multiple tumor types and confers clinical sensitivity to MET inhibitors. Cancer Discov. 2015;5:850–9.
24. Paik PK, Drilon A, Fan P-D, et al. Response to MET inhibitors in patients with stage 4 lung adenocarcinomas harbouring MET mutations causing exon 14 skipping. Cancer Discov. 2015;5:842–9.
25. Kris MG, Johnson BE, Berry LD, et al. Using multiplexed assays of oncogenic drivers in lung cancers to select targeted drugs. JAMA. 2014;311:1998–2006.
26. Reck M, Popat S, Reinmuth N, et al. Metastatic non-small-cell lung cancer (NSCLC): ESMO clinical practice guidelines for diagnosis, treatment and follow-up. Ann Oncol. 2014;25(Suppl 3):iii27–39.
27. Ettinger DS, Wood DE, Akerley W, et al. NCCN guidelines insights: non-small cell lung cancer, version 4.2016. J Natl Compr Cancer Netw. 2016;14:255–64.
28. Cagle PT, Allen TC, Dacic S, et al. Revolution in lung cancer. New challenges for the surgical pathologist. Arch Pathol Lab Med. 2011;135:110–6.
29. Kerr KM. Personalized medicine in lung cancer: new challenges for pathologists. Histopathology. 2012;60:531–46.
30. Thunnissen E, Kerr KM, Herth FJ, et al. The challenge of NSCLC diagnosis and predictive analysis on small samples. Practical approach of a working group. Lung Cancer. 2012;76:1–18.
31. Davidson MR, Gazdar AF, Clarke BE. The pivotal role of pathology in the management of lung cancer. J Thorac Dis. 2013;5:s463–78.
32. Cane P, Linklater KM, Nicholson AG, et al. Morphologic and genetic classification of lung cancer: variations in practice and implications for tailored treatment. Histopathology. 2015;67:216–24.
33. Lindeman NI, Cagle PT, Beasley MB, et al. Molecular testing guideline for selection of lung cancer patients for EGFR and ALK tyrosine kinase inhibitors: guideline from the College of American Pathologists, International Association for the Study of Lung Cancer, and Association for Molecular Pathology. J Thorac Oncol. 2013;8:823–59.
34. Kerr KM, Bubendorf L, Edelman MJ, et al. Second ESMO consensus conference on lung cancer: pathology and molecular biomarkers for non-small-cell lung cancer. Ann Oncol. 2014;25:1681–90.
35. Ross JS, Cronin M. Whole cancer genome sequencing by next generation methods. Am J Clin Pathol. 2011;136:527–39.
36. Hyman DM, Solit DB, Arcila ME, et al. Precision medicine at Memorial Sloan Kettering Cancer Center: clinical next generation sequencing enabling next generation targeted therapy trials. Drug Discov Today. 2015;20:1422–8.

37. Deeb KK, Hohman CM, Risch NF, et al. Routine clinical mutation profiling of non small cell lung cancer using next generation sequencing. Arch Pathol Lab Med. 2015;139:913–21.
38. Coghlin CL, Smith LJ, Bakar S, et al. Quantitative analysis of tumor in bronchial biopsy specimens. J Thorac Oncol. 2010;5:448–52.
39. Dietel M, Bubendorf L, Dingemans AM, et al. Diagnostic procedures for non-small-cell lung cancer (NSCLC): recommendations of the European Expert Group. Thorax. 2016;71:177–84.
40. Kerr KM, Lopez-Rios F. Precision medicine in NSCLC and pathology: how does ALK fit in the pathway? Ann Oncol. 2016;27(Suppl 3):iii16–24.
41. Milne C-P, Bryan C, Garafalo S, et al. Complementary versus companion diagnostics: apples and oranges? Biomark Med. 2015;9:25–34.
42. Atherly AJ, Camidge DR. The cost-effectiveness of screening lung cancer patients for targeted drug sensitivity markers. Br J Cancer. 2012;106:1100–6.
43. Fitzgibbons PL, Bradley LA, Fatheree LA, et al. Principles of analytic validation of immunohistochemical assays. Guidelines from the College of American Pathologists pathology and laboratory quality center. Arch Pathol Lab Med. 2014;138:1432–43.
44. Lin F, Chen Z. Standardization of diagnostic immunohistochemistry. Literature review and Geisinger experience. Arch Pathol Lab Med. 2014;138:1564–77.
45. Ibrahim M, Parry S, Wilkinson D, et al. ALK immunohistochemistry in non-small cell lung cancer (NSCLC). Discordant staining can impact patient treatment regimen. J Thorac Oncol. 2016;11:2241–7.
46. Hiley CT, Le Quesne J, Santis G, et al. Challenges in molecular testing in non-small-cell lung cancer patients with advanced disease. Lancet. 2016;388:1002–11.
47. Bussolati G, Leonardo E. Technical pitfalls potentially affecting diagnoses in immunohistochemistry. J Clin Pathol. 2008;61:1184–92.
48. Mok T, Wu Y-L, Lee JS, et al. Detection and dynamic changes of EGFR mutations from circulating tumour DNA as a predictor of survival outcomes in NSCLC patients treated with first line intercalated erlotinib and chemotherapy. Clin Cancer Res. 2015;21:3196–203.
49. Karlovich C, Goldman JW, Sun J-M, et al. Assessment of EGFR mutation status in matched plasma and tumour tissue in NSCLC patients from a phase 1 study of Rociletinib (CO-1686). Clin Cancer Res. 2016;22:2386–95.
50. Katayama R, Shaw AT, Khan TM, et al. Mechanisms of acquired crizotinib resistance in ALK rearranged lung cancers. Sci Transl Med. 2012;4:120ra17.
51. Sasaki T, Janne PA. New strategies for the treatment of ALK rearranged non small cell lung cancers. Clin Cancer Res. 2011;17:7213–8.
52. Dong X, Fernandez-Salas E, Li E, et al. Elucidation of resistance mechanisms to second-generation ALK inhibitors alectinib and ceritinib in non-small cell lung cancer cells. Neoplasia. 2016;18:162–71.
53. Cortot AB, Janne PA. Molecular mechanisms of resistance in epidermal growth factor receptor-mutant lung adenocarcinomas. Eur Respir Rev. 2014;23:356–66.
54. Piotrowska Z, Seiquist L. Epidermal growth factor receptor-mutant lung cancer. New drugs, new resistance mechanisms, and future treatment options. Cancer J. 2015;21:371–7.

Chapter 2
Lung Cancer Epidemiology and Demographics

Ross A. Miller and Philip T. Cagle

The leading overall cause of cancer-related death in the United States [1] and the global population [2] continues to be lung cancer. Globally, it is the leading cause of cancer death in men and the second leading cause in women (second to breast cancer). However, in developed countries (including the United States), lung cancer mortality has surpassed breast cancer. Worldwide, nearly 1.6 million deaths were attributed to lung cancer in 2012 (1.1 million in men and nearly 500,000 in women) [2]. In the United States, 27% of cancer deaths in men and 26% in women are attributed to lung cancer [1], with 158,080 estimated deaths expected in 2016. This number surpasses the combined total estimate of cancer mortality for the next three most common causes of cancer death in men and women residing in the United States (men: prostate, colon and rectum, pancreas; women: breast, colon and rectum, pancreas) [1]. Lung cancer continues to be one of the most lethal forms of cancer with global 5-year survival rates ranging from around 10 to 20% [3] despite improvements in therapy and surgical technique. The net 5-year survival rate in the United States is around 19%, with only liver cancer having a worse 5-year survival [3].

Lung cancer incidence is tightly linked to tobacco use trends in a given region [4]. The differences in lung cancer rates seen between men and women correlate with historical differences in onset and cessation of tobacco use between the sexes. In relative terms, countries where tobacco use peaked early (such as the United States, the United Kingdom, and Denmark) have seen decreasing lung cancer rates in men; rates in women have leveled off [5, 6]. Countries where tobacco use peaked

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later (e.g., Spain and Hungary) are now seeing a decrease in lung cancer among men but an increase in women [5]. In countries where tobacco use is currently on the rise or is at its peak (China, Indonesia, some African countries), lung cancer rates are expected to continue to rise [5, 7, 8].

Undoubtedly, the largest risk factor for lung cancer development is cigarette smoking [9]. Cigar and pipe tobacco increases risk as well. However, only around 10% of tobacco smokers develop lung cancer, implying other factors exist with regard to cancer development. In tobacco smokers, chronic obstructive pulmonary disease (COPD) is the greatest risk factor for lung cancer development; this may indicate activation of common signaling pathways by tobacco smoke for both diseases [10]. The latency period, total amount of exposure or "pack years" (pack years equals the number of packs smoked per day X number of years smoked), and enzymatic differences in the metabolism of tobacco smoke carcinogens and DNA repair are factors in cancer pathogenesis as well [11, 12]. The risk of developing lung cancer remains elevated in a former smoker for decades after smoking cessation. The risk does gradually decline compared to those smokers who do not quit [12–15].

Exposure to other agents can also increase one's risk for developing lung cancer. For example, exposure to radon gas (from soil and/or building materials) is thought to account for 8–15% of lung cancer cases in North America and Europe. Radon gas is the leading cause of lung cancer in certain regions after cigarette smoking [16]. Indoor air pollution, particularly from cooking fumes produced by burning solid fuels (like coal, which is fairly common in low-middle socioeconomic countries), is thought to account for 2% of lung cancer deaths in these particular regions [17]. A wide array of other agents and compounds increases one's risk; some of these include secondhand smoke, asbestos, various metals, organic chemicals, radiation, pollutants, dietary factors, and exposure to various other occupational-related compounds (particularly rubber manufacturing, paving, roofing, painting, and chimney sweeping) [18, 19]. Certain infections, for example, human immunodeficiency virus infection [20], human papillomavirus infection, and those with a history of tuberculosis [18], are also thought to be at increased risk for lung cancer as well.

An overrepresented demographic afflicted by lung cancer includes never-smoking young women, often of Chinese/Asian descent. This demographic has a particularly high incidence of lung adenocarcinoma that is often associated with particular molecular aberrations (e.g., epidermal growth factor receptor mutations, anaplastic lymphoma kinase fusion genes discussed in more detail in subsequent chapters) [7, 21].

References

1. Siegal RL, Miller KD, Jemal A. Cancer statistics, 2016. CA Cancer J Clin. 2016;66(1):7–30.
2. Bray F, Ren JS, Masuyer E, Ferlay J. Estimates of global cancer prevalence for 27 sites in the adult population in 2008. Int J Cancer. 2013;132(5):1133–45.

3. Allemani C, Weir HK, Carreira H, et al. Global surveillance of cancer survival 1995–2009: analysis of individual data for 25,676,887 patients from 279 population based registries in 67 countries (CONCORD-2). Lancet. 2015;385(9972):977–101.
4. Colditz GA, Sellers TA, Trapido E. Epidemiology—identifying the causes and preventability of cancer? Nat Rev Cancer. 2006;6(1):75–83.
5. Torre LA, Siegel RL, Ward EM, Jemal A. International variation in lung cancer mortality rates and trends among women. Cancer Epidemiol Biomark Prev. 2014;23(6):1025–36.
6. Bosetti C, Malvezzi M, Rosso T, et al. Lung cancer mortality in European women: trends and predictions. Lung Cancer. 2012;78(3):171–8.
7. Youlden DR, Cramb SM, Baade PD. The international epidemiology of lung cancer: geographical distribution and secular trends. J Thorac Oncol. 2008;3(8):819–31.
8. Lam WK, White NW, Chan-Yeung MM. Lung cancer epidemiology and risk factors in Asia and Africa. Int J Tuberc Lung Dis. 2004;8(9):1045–57.
9. Eriksen M, Mackay J, Ross H. The Tobacco Atlas. 4th ed. Atlanta: American Cancer Society; 2012.
10. Adcock IM, Caramori G, Barnes PJ. Chronic obstructive pulmonary disease and lung cancer: new molecular insights. Respiration. 2011;81(4):265–84.
11. Alberg AJ, Semet JM. Epidemiology of lung cancer. Chest. 2003;123(supplement):21S–49S.
12. Cagle PT, Allen TC. Genetic susceptibility to lung cancer. In: Zander D, Popper HH, Jaigrdar J, Haque A, Cagle PT, Barrios R, editors. Molecular pathology of lung diseases. New York: Springer; 2008.
13. Cagle PT. Carcinoma of the lung. In: Churg AM, Myers JL, Tazelaar HD, Wright JL, editors. Pathology of the lung. 3rd ed. New York: Thieme Medical Publishers; 2005. p. 413–79.
14. Hrubec Z, McLaughlin JK. Former cigarette smoking and mortality among US veterans: a 26-year follow-up, 1954–1980. In: Burns DM, Garfinkel L, Samet JM, editors. Changes in cigarette-related disease risks and their implications for prevention and control. Bethesda: US Government Printing Office; 1997. p. 501–30.
15. US Department of Health and Human Services: The health benefits of smoking cessation. US Department of Health and Human Services, Public Health Service, Centers for Disease Control, Center for Chronic Disease Prevention and Health Promotion, Office on Smoking and Health. DHHS Publication No (CDC) 89–8416, 1990.
16. El Ghissassi F, Baan R, Straif K, et al. A review of human carcinogens–part D: radiation. Lancet Oncol. 2009;10(8):751–2.
17. Danaei G, Vander Hoorn S, Lopez AD, Murray CJ, Ezzati M. Causes of cancer in the world: comparative risk assessment of nine behavioural and environmental risk factors. Lancet. 2005;366(9499):1784–93.
18. American Cancer Society. Global cancer facts and figures. 3rd ed. Atlanta: American Cancer Society; 2015.
19. Herr C, Greulich T, Koczulla RA, Meyer S, Zakharkina T, Branscheidt M, Eschmann R, Bals R. The role of vitamin D in pulmonary disease: COPD, asthma, infection, and cancer. Respir Res. 2011;12:31.
20. Kirk GD, Merlo CA. Lung HIV study. HIV infection in the etiology of lung cancer: confounding, causality, and consequences. Proc Am Thorac Soc. 2011;8(3):326–32.
21. Sholl LM, Nishino M. Adenocarcinoma. In: Cagle PR, Allen TC, editors. Lung and pleural pathology. China: McGraw-Hill Education; 2016. p. 41–72.

Chapter 3
Genetic Susceptibility to Lung Cancer

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Lung Cancer in Nonsmokers

Most lung cancers are attributable to tobacco smoking, which exposes airways and lung parenchyma to numerous carcinogens and procarcinogens including free radical species, aromatic amines, polycyclic aromatic hydrocarbons, and nitrosamines. Compared to cigarette smoking, other exposures implicated in lung cancer impact lung cancer risk much less [1, 2]. Approximately 15% of lung cancers in men, and 50% of lung cancers in women, are not related to tobacco smoking [3, 4]. Overall, approximately 25% of lung cancer patients are never smokers [3, 4]. Although passive inhalation of tobacco smoke, also termed environmental tobacco smoke, is believed to play a role in some percentage of cases of lung cancer in never smokers [3], these tumors are usually designated idiopathic; and their histologic types differ from the types found in cigarette smokers [5]. Many of these never smokers who develop lung cancer are young women who develop adenocarcinoma and who show an overall better prognosis than patients with smoking-related lung cancers [6, 7]. Nonsmoking-related lung cancers are being increasingly recognized; and the disease likely represents a disease process unrelated to smoking-related lung cancer. People are thought to have variable susceptibilities to cancer risk factors, including lung cancer risk factors [8–38]. A genetic basis for differing cancer risk factor susceptibilities has been proposed based on the observation that different susceptibilities appear to be inherited based on aggregation of cancers within families [39–66]. Inherited susceptibilities would help explain why some people develop lung cancer, such as individuals with minimal or no tobacco smoke exposure [30, 36, 67–75], frequently in association with family histories positive for

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cancer [31, 75–79], or those who develop lung cancer from exposure at a significantly earlier-than-average age [80–87].

Complex gene-environment interactions occur, and genetic differences in susceptibility to tobacco smoke carcinogens exist; and lung cancers in both smokers and never smokers may have mutually common and distinct risk factors and gene-environment interactions [5]. Host susceptibility to lung cancer, individually or in synergy with smoking, is uncertain [88]; however, gene-environment interactions and genetic differences likely have a significant role in the development of lung cancer in never smokers [3, 86]. They also likely help explain why some heavy smokers do not develop lung cancer [3, 86, 89–92] and why some lung cancer patients have strong family histories of cancer [5, 93–96]. The human genome database and improving genotyping technology have substantially aided researchers in their search for and understanding of the genetic role of lung cancer development [3, 34, 86, 87, 91, 92, 97, 98].

Familial Clustering

Epidemiological studies suggest familial clustering of lung cancer occurs [3, 86, 87, 92]; the literature is in fact robust [60, 99–101]. Studies for which smoking exposure and occupational exposure were controlled have shown an increased risk of lung cancer in relatives of lung cancer patients [45, 49, 54, 59, 64]. Inherited polymorphisms in DNA repair genes and xenobiotic-metabolizing enzyme genes might account for the elevated risk, as might the genetic influence of substance dependence, including nicotine dependence [87, 102–107]. Multiple genetic loci may relate to nicotine dependence, including the promoter region of CHRNA5, a locus on chromosome 15 [108–110]. 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 [33, 34, 38, 47, 49, 59, 67–71, 76, 77, 80–82, 84, 87, 92, 101, 111].

A 2012 study identified a 1.25-fold risk increase for family history of lung cancer in nonsmokers who developed lung cancer [3, 112]. It is important to understand, however, that familial aggregation of lung cancer alone does not in and of itself prove inheritance of genetic risk variants [3]. Clustering of close relatives with similar exposures to environmental risk factors may exhibit itself as a familial aggregation. However, there is evidence that some situations exhibiting familial aggregation are the result of genetic variants [3].

Gender

Gender affects lung cancer incidence [85]. Female lung cancer patients who are never smokers are influenced by familial history than by radon gas or environmental tobacco smoke exposure [85]. Research is conflicting as to whether women smokers

have an increased risk of developing cancer relative to men; however, some studies suggest women have an increased risk [113]. Some studies suggest that women smokers have an increased risk of developing lung cancer relative to men with the same smoking histories; however, other studies show women's risk to be equivalent to men's. Environmental factors, hormonal influences, and gender differences in xenobiotic-metabolizing enzymes are proposed reasons for reported differences in gender-associated lung cancer susceptibility.

Driver Genes

Epidermal growth factor receptor (EGFR), echinoderm microtubule-associated protein-like 4-anaplastic lymphoma kinase (EML4-ALK), proto-oncogene B-Raf (BRAF), Kirsten rat sarcoma viral oncogene (KRAS), and phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha (PIK3CA) are driver genes that may be mutated in pulmonary adenocarcinomas [114]. Gefitinib and erlotinib are EGFR tyrosine kinase inhibitors (EGFR-TKIs) used for molecular therapy of pulmonary adenocarcinomas [115]. EGFR-TKI-responsive EFGR mutations are identified more frequently in pulmonary adenocarcinomas of nonsmoking Asian women than in other groups of patients and are thought to arise early [116]. For example, one study of lung cancer patients showed EGFR mutations in 44% of pulmonary adenocarcinomas, with 47.5% occurring in women and 15% occurring in men and 42% occurring in nonsmokers and 14% occurring in smokers [117]. Nonsmoking Asian women whose pulmonary adenocarcinoma contains the EFFR mutation have been shown to have improved survival, particularly when the tumor demonstrates a significant lepidic pattern, compared to men with lung cancers who have a smoking history and whose pulmonary adenocarcinomas do not contain a significant lepidic pattern [2, 87, 118]. Pulmonary adenocarcinoma is identified relatively more frequently in nonsmoking women, and a relationship between EGFR mutation and membranous ERα expression has been identified as an independent prognostic factor in patients with pulmonary adenocarcinoma [116, 119]. HER2, a proto-oncogene of the receptor tyrosine kinase superfamily, binds other members of the EGFR family in the activation of EGFR signaling; and HER2 gene polymorphisms have been shown to increase susceptibility to pulmonary adenocarcinoma in nonsmoking Korean women [120].

Genome-Wide Association Studies

Genome-wide association studies (GWASs) are population-level studies to identify genetic alleles associated with disease status or clinical phenotypes within the genome rather than in relation to a specific gene [85]. Lung cancer GWASs have shown several factors that correlate with lung cancer occurrence and progression [3, 85]. Several single-nucleotide polymorphisms (SNPs) in various genetic loci related

to lung cancer susceptibility have been found; however, the three major susceptible loci associated with lung cancer risk are loci 15q24–25, 5p15, and 6p21 [121–124]. Locus 15p24–25 has been associated with lung cancer risk in Caucasian populations only, while loci 5p15 and 6p21 have been associated not only with lung cancer in Caucasian populations but also with lung cancer in East Asian (Korean, Japanese, Chinese) populations [123]. Other less well-established loci, including 3q28–29, 13q12.12, 22q12.2, and 18p11.22, have been identified as being associated with lung cancer in Asian populations [125].

Specific Polymorphisms Associated with Lung Cancer Susceptibility

Polymorphisms of xenobiotic-metabolizing genes and DNA repair genes have found potential allelic variants associated with lung cancer risk [126, 127]. 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, detoxification, and repair processes involved with DNA adducts are complex, one single polymorphism most likely does not account for differences in DNA adduct levels. Studies examining several or many polymorphisms simultaneously in a single population are more likely to yield more comprehensive and consistent results; and newer technologies, permitting the study of SNPs and haplotypes, increase statistical sensitivity [85].

Xenobiotic-Metabolizing Enzymes

Xenobiotics are drugs, toxins, solvents, and poisons, which are metabolized xenobiotic-metabolizing enzymes. 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, 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 [128–131].

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 [129, 132, 133]. These reactions generally occur in the liver but can occur in other tissues, including lung tissue. 237–240 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 polycyclic aromatic hydrocarbons (PAHs) from tobacco smoke, and CYP2A6 and CYP2E1 are involved in the metabolism of nitrosamines from tobacco smoke [129, 131, 133, 134].

Many CYPs 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 [135, 136].

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 [137, 138]. Along with PAHs, tobacco smoke contains *N*-nitrosamines including 4-(methylnitrosoamino)-1-(3-pyridyl)-1-butanone (NNK), *N*-dimethylnitrosoamine (NDMA), *N*-diethylnitrosoamine (NDEA), *N*-nitrosophenylmethyl-amine (NMPhA), and *N*-nitrosonornicotine (NNN).