

REVIEW ARTICLE

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Clinical Implications of Genomic Discoveries in Lung Cancer

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N Engl J Med 2016;374:1864-73.

DOI: 10.1056/NEJMra1504688

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LUNG CANCER IS ONE OF THE LEADING CAUSES OF DEATH GLOBALLY.¹ Tobacco smoking causes nearly 90% of lung cancers. The major histologic types of lung cancer include adenocarcinoma, squamous-cell carcinoma, small-cell carcinoma, large-cell neuroendocrine carcinoma, and pulmonary carcinoid tumors.² Although some molecular alterations are shared among various histologic subtypes, the majority of genomic alterations remain distinct. In this review, we discuss recent studies of large-scale genomic analyses of the three most common histologic types of lung cancer — adenocarcinoma, squamous-cell carcinoma, and small-cell carcinoma — and their implications for the management of this disease.

GENOMIC ALTERATIONS

THE GENOMIC LANDSCAPE

Lung cancer related to tobacco smoking is one of the few cancers with a high mutational burden.³ The characteristic cytosine–adenine (C→A) nucleotide transversions (the substitution of a purine with a pyrimidine, or vice versa) that are associated with tobacco exposure are seen predominantly in lung adenocarcinomas from smokers (in whom the rate of transversion is high) rather than from persons who have never smoked (in whom the rate of transversion is low).⁴ Whole-exome sequencing of specimens of malignant tissue from the lungs of tobacco smokers has revealed a mean somatic mutation rate of 8 to 10 mutations per megabase (1 million base pairs), regardless of the histologic subtype. The mutational burden in specimens of adenocarcinomas from the lungs of persons who have never smoked is much lower (0.8 to 1 mutation per megabase).^{4,7} The complexity of the lung-cancer genome is further illustrated by the large number of focal and broad areas of somatic chromosomal copy-number alterations and gene rearrangements.^{4,6,7} This high burden of mutation poses a special challenge for investigators who are trying to discover novel genetic alterations that are present at a lower frequency (<5%). Large numbers of samples (approximately 3000) are required to create a comprehensive catalogue of putative cancer genes that are mutated in 2% or more of the lung cancers found in smokers.⁸

CHROMOSOMAL CHANGES AND ALTERATIONS IN GENE COPY NUMBER

Copy-number analyses of tumor samples from patients with lung cancer have identified some alterations that are common across different histologic subtypes and others that are enriched in tumors belonging to specific histologic subtypes.⁹ For instance, the short arm of chromosome 3 (3p), which contains many tumor suppressors, is commonly deleted early in the development of lung cancer and has been reported in all subtypes of lung cancer. The other most common locus that is deleted in patients with lung cancer involves *CDKN2A*, encoding ARF and p16, which regulate p53 and the CDK4/6/pRB axis, respectively. The selective amplifica-

Table 1. Recurrent Molecular Alterations in Lung Adenocarcinoma, Squamous-Cell Carcinoma, and Small-Cell Carcinoma.*

Type of Alteration	Adenocarcinoma	Squamous-Cell Carcinoma	Small-Cell Carcinoma
Cell-cycle mutations	<i>TP53</i> (46%), <i>CDKN2A</i> (4%)	<i>TP53</i> (91%), <i>CDKN2A</i> (17%), <i>RB1</i> (7%)	<i>TP53</i> (92%), <i>RB1</i> (75%)
	<i>RTK/PI3K-MTOR</i> signaling	<i>RTK/PI3K-MTOR</i> signaling	<i>RTK/PI3K-MTOR</i> signaling: <i>PTEN</i> (5%)
	<i>KRAS</i> (33%), <i>EGFR</i> (14%), <i>BRAF</i> (10%), <i>STK11</i> (17%), <i>MET</i> (8%), <i>NF1</i> (11%), <i>PIK3CA</i> (7%), <i>RIT1</i> (2%)	<i>PIK3CA</i> (16%), <i>PTEN</i> (8%), <i>HRAS</i> (3%)	
Other mutations	Oxidative stress response: <i>KEAP1</i> (17%), <i>MYC</i> pathway; <i>MGA</i> (8%)	Oxidative stress response: <i>CUL3</i> (6%), <i>KEAP1</i> (12%), <i>NFE2L2</i> (15%)	Epigenetic deregulation: <i>EP300</i> (11%), <i>CREBBP</i> (10%)
	Aberrant splicing: <i>U2AF1</i> (3%), <i>RBM10</i> (8%)	Squamous differentiation: <i>NOTCH1</i> (8%), <i>ASCL4</i> (3%), <i>NOTCH2</i> (5%)	Neuroendocrine differentiation: <i>NOTCH1</i> (15%), <i>NOTCH2</i> (5%), and <i>NOTCH3</i> (9%)
Rearrangements	<i>ALK</i> (3–8%), <i>ROS1</i> (2%), <i>RET</i> (1%), <i>NTRK1</i> (3%), <i>NRG1</i> (2%), <i>BRAF</i> (3% in those who never smoked), <i>ERBB4</i> (1%)	<i>FGFRs</i> (rare)	<i>RB1</i> (13%), <i>TP73</i> (7%), <i>CREBBP</i> (4%), <i>PTEN</i> (4%), <i>RBL1</i> (3%)
Amplifications	<i>TTF1</i> (14%), <i>TERT</i> (18%), <i>EGFR</i> (7%), <i>MET</i> (4%), <i>KRAS</i> (6%), <i>ERBB2</i> (3%), <i>MDM2</i> (8%)	Chr3q: <i>SOX2</i> (43%), <i>TP63</i> (29%), <i>PIK3CA</i> (38%), <i>HES1</i> (26%)†	<i>MYC</i> family members (16%): <i>MYC</i> , <i>MYCN</i> , <i>MYCL1</i> , <i>SOX2</i> (27%), <i>FGFR1</i> (8%), <i>IRS2</i> (2%)
Deletions	<i>CDKN2A</i> (20%)	<i>CDKN2A</i> (27%), <i>PTEN</i> (3%)	<i>TP53</i> , <i>RB1</i> , <i>CDKN2A</i> , Chr3p (e.g., <i>FHIT</i> , <i>ROBO1</i>)†
Commonly altered pathways	MAPK and PI3K signaling, oxidative stress response, cell-cycle progression, RNA splicing and processing, nucleosome remodeling	Squamous-cell differentiation, oxidative stress response, MAPK and PI3K signaling	Cell-cycle regulation, PI3K signaling, regulation of nucleosome transcriptional and remodeling, NOTCH signaling and neuroendocrine differentiation

* Percentages represent the prevalence of mutation and were obtained from the cBioPortal for Cancer Genomics (www.cbioportal.org).^{10,11}

† Chromosomes 3q and 3p are cytogenetic bands.

tion of chromosomal regions containing lineage-specific oncogenes — such as *SOX2*, located on the long arm of chromosome 3 (3q) in squamous-cell and small-cell carcinoma, and *NKX2-1*, located on the long arm of chromosome 14 (14q) in adenocarcinoma — tends to be histologically specific. A wide variety of somatic copy-number alterations involving amplifications of oncogenes and deletions of tumor-suppressor genes has been reported in patients with lung cancer (Table 1).^{4,6,7}

DNA SINGLE-NUCLEOTIDE ALTERATIONS AND INSERTIONS AND DELETIONS

The availability of modern, high-throughput sequencing technologies (i.e., next-generation sequencing) has enabled the identification of coding-sequence alterations down to single-base-pair resolution with a fairly high level of precision and accuracy. Investigators from the Cancer Genome Atlas and others have reported genomic alterations in lung adenocarcinoma, squamous-cell carcinoma, and small-cell carcinoma (Table 1).^{4,6,7,12,13} The most commonly mutated onco-

genes in lung adenocarcinoma are *KRAS* (in 33% of tumors), *EGFR* (in 14%), *BRAF* (in 10%), *PIK3CA* (in 7%), and *MET* (in 7%). Mutations involving tumor suppressors include *TP53* (in 46%), *STK11* (in 17%), *KEAP1* (in 17%), *NF1* (in 11%), *RB1* (in 4%), and *CDKN2A* (in 4%). Mutations involving chromatin-modifying genes (*SETD2*, *ARID1A*, and *SMARCA4*) and RNA-splicing genes (*RBM10* and *U2AF1*) are found in approximately 10% of specimens from lung adenocarcinomas. Data from the Cancer Genome Atlas have shown a higher prevalence of *EGFR* mutations than of other mutations in specimens from groups with a low rate of transversion (enriched with those who never smoked), whereas mutations in *TP53*, *KRAS*, *NF1*, *STK11*, and *RBM10* were more common in specimens from groups with a high rate of transversion (enriched with present or past smokers).⁴

Although certain mutations are common in both lung adenocarcinomas and squamous-cell carcinomas (e.g., *TP53* and *CDKN2A*), squamous-cell carcinomas are characterized by fewer mutations in genes encoding receptor tyrosine kinase (RTK) and a greater frequency of loss of tumor-

suppressor functions that affect genes such as *PTEN*, *NOTCH1*, and *RBI*.^{6,14} *CDKN2A* is most commonly lost by means of homozygous deletion (29%), followed by methylation (21%), inactivating mutations (18%), and splicing alterations with the skipping of exon 1 β (4%).

Small-cell lung cancer is characterized by recurrent inactivating mutations in *RBI*, *RBL1*, *RBL2*, *TP53*, and *PTEN*; RNA regulatory genes (*XRNI*); genes encoding G-protein–coupled receptor–signaling molecules (*RGS7* and *FPR1*); and genes with functional roles in centrosome regulation (*ASPM*, *ALMS1*, and *PDE4DIP*).^{7,15} In a large study of somatic genome alterations in small-cell lung cancer, whole-genome sequencing performed on 110 tumor specimens revealed mutations and rearrangements in *TP73* leading to oncogenic activation in 13% of the cases.⁷ A quarter of the tumors harbored *NOTCH*-family inactivating mutations.

EPIGENETIC ALTERATIONS

Apart from alterations in DNA sequence, gene transcription may be affected by epigenetic changes that involve histone modifications and DNA methylation. Several tumor suppressors involved in lung cancer are epigenetically silenced. Mutations in chromatin-modifying genes (e.g., *SMARCA4*, *ARID1A*, and *SETD2*) have been reported in lung adenocarcinoma.¹⁶ A subset of lung adenocarcinoma identified as CpG island methylator phenotype–high is enriched for *SETD2* mutations and *CDKN2A* methylation. Mutations in *CREBBP* and *EP300* that affect the activity of histone acetyltransferase have been observed in small-cell lung cancer, as have mutations in *MLL*, a methyltransferase gene.¹³ Integrative analyses of methylation assays that include data on the exome and the transcriptome are necessary to fully understand the implications of epigenetic alterations in lung cancer.

TRANSCRIPTOME ALTERATIONS

Transcriptome analyses of tumor specimens have led to several important observations regarding the effects of DNA-sequence alterations on RNA transcripts, splice-site mutations, and gene fusions.^{4–6} *U2AF1* is an important gene in the regulation of 3' splice-site selection. Not surprisingly, a *U2AF1* mutation (S34F), which is present in 3% of lung adenocarcinomas, is associated with inappropriate splice-site selection in a number of genes, including the site selected for the splicing

of the beta-catenin proto-oncogene *CTNNB1*. Splice-site mutations that result in the skipping of a critical region (exon 14) in the *MET* oncogene lead to a stabilized protein with persistent activation in lung adenocarcinoma. Fusion events and gene rearrangements involving *ALK*, *ROS*, *NTRK1*, *NRG1*, *FGFR4*, *ERBB4*, *BRAF*, and *RET* in lung adenocarcinoma provide opportunities for therapeutic intervention.^{4,17–19} Gene rearrangements involving *FGFR*-family genes that have been reported in squamous-cell cancer in the lung may also be targets for intervention.²⁰ However, most rearrangements reported in small-cell lung cancer involve transcription factors (e.g., *MYCL1*), histone modifiers (e.g., *CREBBP*), or tumor suppressors (e.g., *PTEN*, *RBI*, and *TP73*) that are not readily amenable to targeted therapy at the present time.⁷

PATHWAY ALTERATIONS

Integrated analyses involving the use of whole-exome and transcriptome sequencing have revealed that various components of the RTK–RAS–RAF pathway are almost always affected in lung adenocarcinoma (in 76% of the cases).⁴ Phosphoproteomic studies have suggested that in some *KRAS* wild-type tumors there is substantial activation of the MAPK pathway that may result from alterations in as-yet undiscovered members of the RTK–RAS–RAF pathway. Other pathways affected in lung adenocarcinoma involve cell-cycle regulation (64%), p53 (63%), chromatin and RNA-splicing factors (49%), and the oxidative stress response (22%) (Fig. 1).

A number of genes involved in the pathways related to the oxidative stress response and squamous-cell differentiation are affected in squamous-cell carcinoma as a result of mutations or alterations in copy number.⁶ Mutations in *NFE2L2* or *KEAP1* are found in nearly a third of squamous-cell-carcinoma tumor samples. These two genes play an important role in the cellular response to oxidative damage that is perhaps caused by the continued onslaught of smoking-related cellular injury. Overexpression and amplification of *SOX2* and *TP63*, loss-of-function mutations in *NOTCH1*, *NOTCH2*, and *ASCL4*, and focal deletions in *FOXP1*, all of which are involved in squamous-cell differentiation, are seen in 44% of tumor samples reported in the Cancer Genome Atlas.

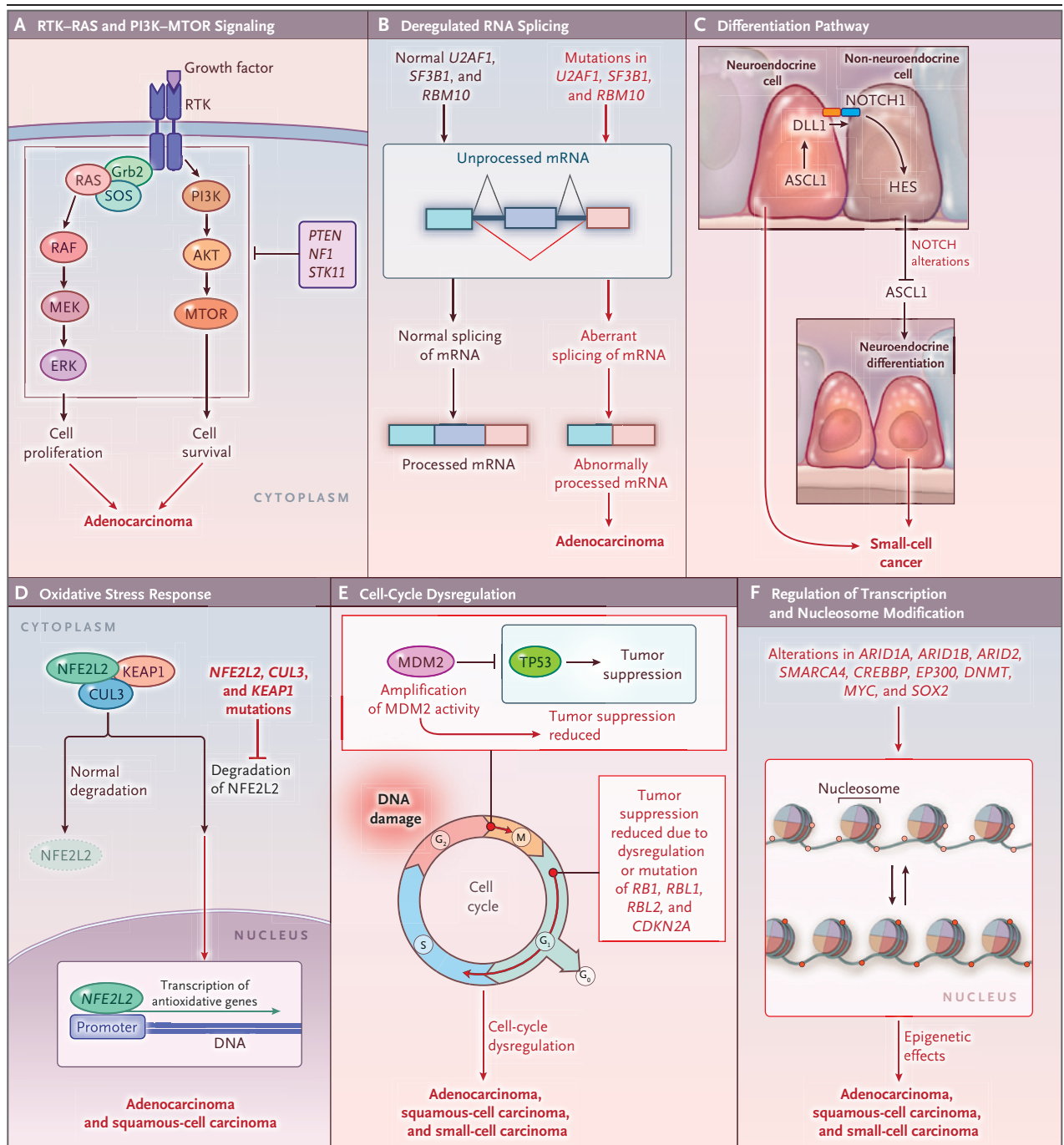


Figure 1. Recurrent Molecular Themes in Lung Cancer.

The figure shows a few common recurrently altered pathways in lung cancer. Approximately 76% of lung adenocarcinoma samples show driver alterations in the receptor tyrosine kinase (RTK)–RAS–RAF signaling pathway (Panel A). Genes that play a crucial role in alternative splicing, such as *U2AF1*, *RBM10*, and *SF3B1*, are altered in lung adenocarcinoma and result in aberrant splicing of oncogenes (Panel B). Notch signaling plays an important role in neuroendocrine differentiation, and alterations in this pathway are frequently seen in small-cell lung cancers (Panel C). Activation of the oxidative stress pathway (Panel D) has been implicated in xenobiotic metabolism and treatment resistance and is seen in non-small-cell lung cancers. Alterations in *PI3K* signaling, cell-cycle regulation (Panel E), and nucleosome modification (Panel F) are seen in all subtypes of lung cancer.

Small-cell lung cancer is characterized predominantly by alterations in cell-cycle regulation, Notch signaling, and neuroendocrine differentiation. Genes involved in receptor kinase–PI3K signaling and transcriptional regulation are affected in a minority of samples of tumors with small-cell lung cancer.⁷ Low-grade neuroendocrine tumors of the lung, particularly well-differentiated carcinoids, seldom show the alterations in *TP53* and *RB1* that are characteristic of small-cell lung cancer.²¹

CELL OF ORIGIN

The airway epithelium is fairly heterogeneous, being composed of multiple cell types. Both the cell type and the proportion of each cell type vary in accordance with a proximal–distal axis. Whereas in proximal airways, basal, club, ciliated, neuroendocrine, and goblet cells predominate, alveoli are made up of type I and type II pneumocytes.²² The final histologic content of lung cancer seems to depend on specific molecular characteristics of the cell of origin, alterations that deregulate differentiation pathways in these cells, and the cellular context in which this process occurs (Fig. 2).²³ Data from studies in mice suggest that the loss of *TP53* and *RB1* in airway neuroendocrine cells is sufficient to give rise to small-cell lung cancer.²⁴ Type II pneumocytes, junction cells, and club cells of the bronchoalveolar duct have been shown to serve as the

cells of origin for adenocarcinoma of the lung in mice. Type II pneumocytes play a role in the renewal of both type I and type II pneumocytes — a process that can be induced by dying type I cells and that depends on signaling by epidermal growth factor receptor (EGFR), RAS, and transforming growth factor β (TGF- β).^{25,26} Although conclusive functional evidence is still lacking, basal cells in the proximal airway are hypothesized to serve as the cell of origin for squamous-cell lung cancer. Studies in mice allow lineage tracing that cannot be performed in humans. A comprehensive molecular analysis of tumor-initiating cells at various points during tumor evolution in animals would substantially improve our understanding of the molecular processes involved in cancer initiation and progression.

CLONAL EVOLUTION AND INTRATUMOR HETEROGENEITY

Genomic analyses of solid tumors are increasingly revealing evidence of branched evolution, wherein tumors consist of multiple distinct subclones that share a common ancestor but differ in terms of subtle or profound genomic alterations that occur later in the evolution of the tumor. Such subclones may be intermixed within one tumor sample or regionally separated within

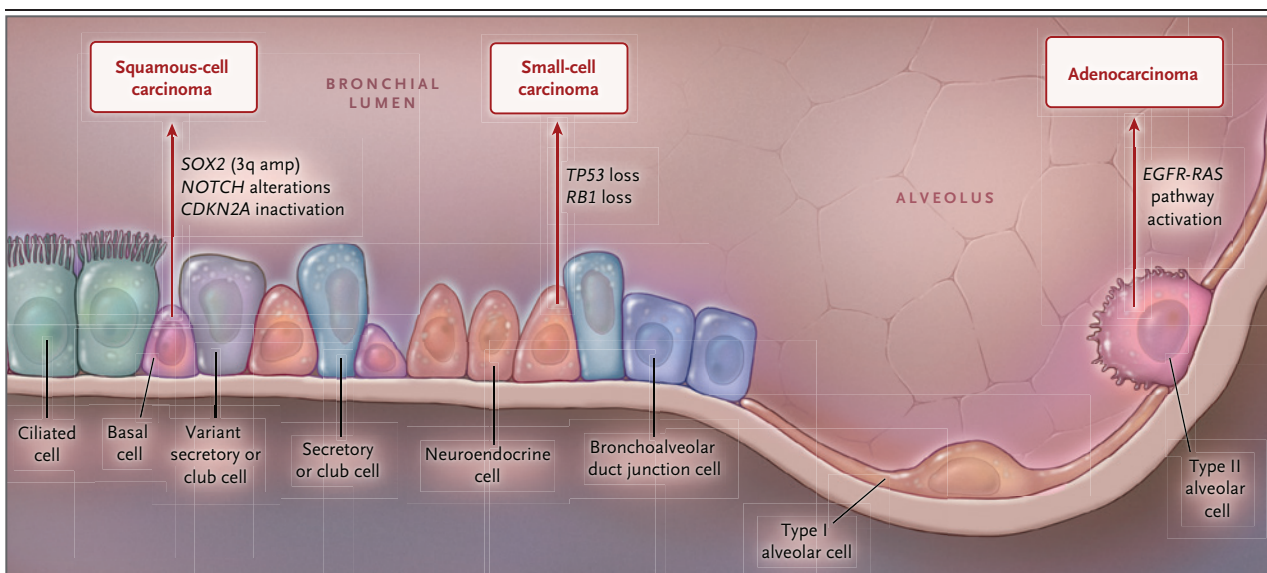


Figure 2. Cells of Origin and Characteristic Alterations According to Histologic Subtype.

The likely cells of origin for the three common histologic subtypes of lung cancer — adenocarcinoma, squamous-cell carcinoma, and small-cell carcinoma — are depicted.

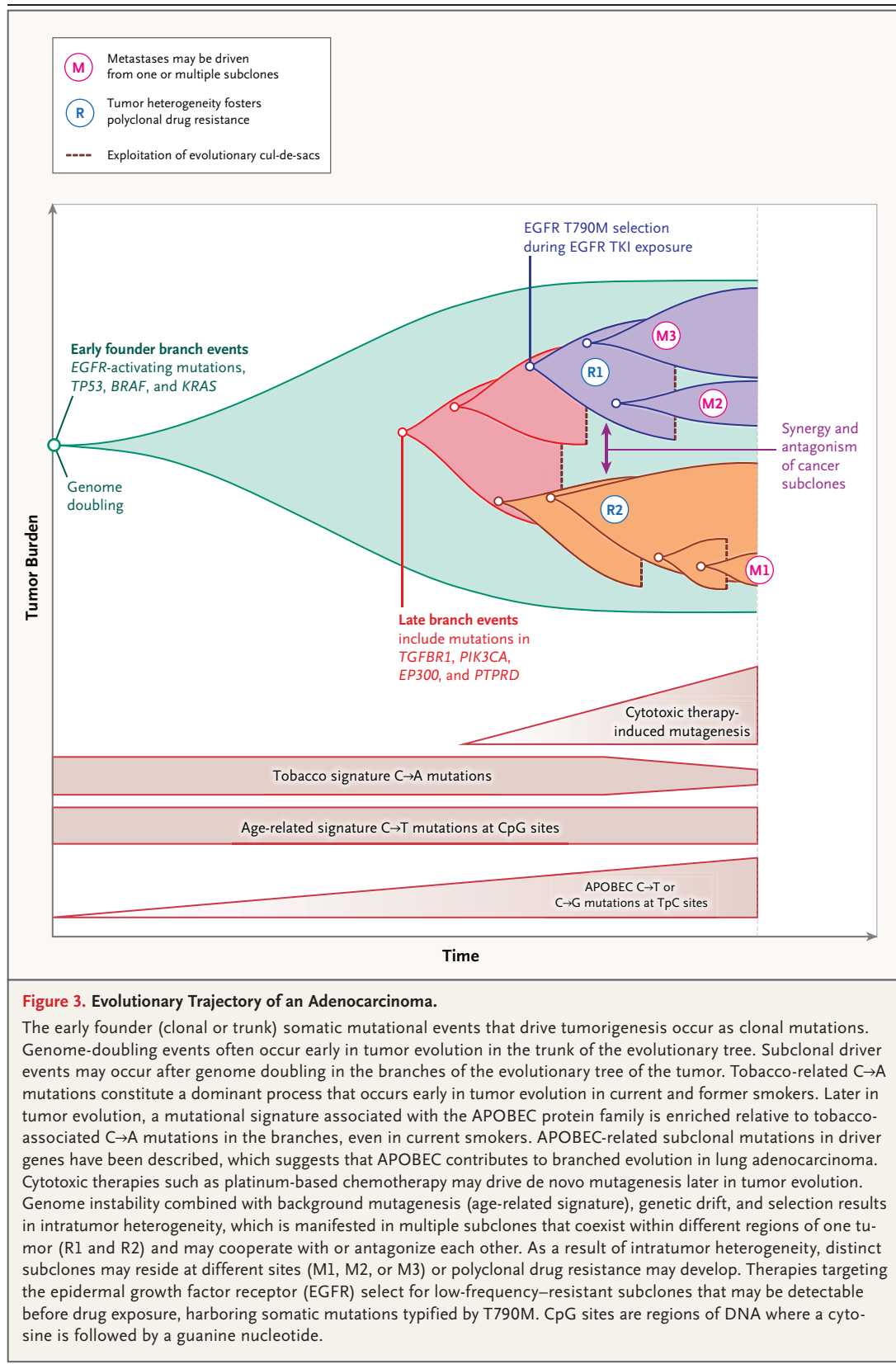
a primary tumor, between primary and metastatic sites, or between metastatic sites — providing a substrate for tumor adaptation, selection, and evolutionary fitness (Fig. 3)^{27,28}

Zhang and colleagues applied multiregion, whole-exome sequencing to specimens from 11 patients with early-stage lung adenocarcinomas (8 of whom had stage I disease).²⁹ Clear evidence of intratumor heterogeneity and branched evolution was found in every case. Among the 21 mutations known to be related to the disease, 20 were present in every region, suggesting that single sampling approaches in early-stage non-small-cell lung cancer are sufficient to depict the driver of the mutational landscape in this disease. The authors studied the associations of the number of subclones in a tumor with relapse and found a larger fraction of subclonal mutations in patients who had a relapse within 21 months after surgery than in those who did not have recurrence of disease. Larger prospective studies are needed to confirm these observations. De Bruin and colleagues investigated 7 non-small-cell lung cancers, including 5 tumors in stages II through IIIB, by means of whole-exome multiregional sequencing.³⁰ There was evidence of both somatic mutational and copy-number heterogeneity and of genome-doubling events that in many cases occurred early in the evolution of the tumor and were present in each subclone analyzed within each tumor region — events that represent the trunk of the tumor's evolutionary tree (Fig. 3). The median proportion of heterogeneous mutations found in one region but not another, termed branched events, was 30%. There was a 42% probability that a category 1 “high-confidence” driver mutation (defined as a disrupting mutation in tumor-suppressor genes or an activating amino acid substitution in oncogenes) would be missed in this higher-stage cohort when a single biopsy specimen was profiled. These data suggest that the risk of missing a high-confidence-driver event from a single biopsy specimen may increase with tumor stage. However, larger studies, such as the Tracking Cancer Evolution through Therapy (Rx) (TRACERx) program (ClinicalTrials.gov number, NCT01888601) in the United Kingdom, will be required to formally examine this hypothesis.

The Zhang and de Bruin studies also shed light on the mechanistic basis of branched evolution in these tumors. In lung adenocarcinomas

from former and current smokers, the proportion of C→A transversions within the clonal mutations was reduced in the branches (depicting subclonal mutations, which are present in some cells but not others), suggesting that alternative mutational processes might dominate the carcinogenic effect of smoking exposure later in tumor evolution. In findings consistent with this observation, subclonal mutations were enriched with C→T and C→G mutations at TpC sites, typified by a mutational process attributed to the APOBEC (apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like) cytidine deaminase family of proteins (these enzymes convert cytosine to uracil during RNA editing) (Fig. 3). The APOBEC family of antiviral enzymes provides an important innate immune defense and serves to engender mutations in viral DNA or RNA, rendering them replication-incompetent. APOBEC3B is emerging as a major mutagenic source in cancer. Subclonal mutations in driver genes have occurred within an APOBEC context, fostering tumor diversification and branched evolution³¹ — a finding that is consistent with the importance of the APOBEC mutational process. Evidence also suggests that cytotoxic agents such as platinum drugs that are used to treat non-small-cell lung cancer may also leave a subclonal mutagenic footprint (Fig. 3). Statistical power to detect novel environmental and endogenous mutational processes across the genome will increase as the number of sequenced genomes that are available increases, revealing mutagenic processes that drive the acquisition of mutations and branched evolution. In vitro genotoxic screening and cancer genome analysis, as exemplified by efforts such as those of the COMSIG (Causes of Mutational Signatures) consortium, are likely to enable a broader understanding of the potential effects of exogenous mutagens on the genome and inform efforts to limit harmful exposures.

Studies in mice are also shedding light on the complexities of the metastatic spread of small-cell lung cancer driven by intratumoral heterogeneity, with evidence of polyclonal seeding of metastases from a primary tumor, linear sequential spread of one metastasis to another,³² and subclonal dependencies for metastatic colonization (Fig. 3).³³ In rare instances, lung adenocarcinoma driven by mutations in *EGFR* undergoes transformation to small-cell cancer, with loss of the *RB1* tumor suppressor.³⁴



THERAPEUTIC IMPLICATIONS

IDENTIFICATION OF MOLECULAR TARGETS FOR THERAPY

Substantial progress has been made in the treatment of specific molecular subsets of lung adenocarcinoma.³⁵ Patients with metastatic disease who have activating mutations in the *EGFR* tyrosine kinase domain have been shown to have significantly higher rates of response and of progression-free survival with the administration of specific *EGFR* kinase inhibitors than with cytotoxic platinum-based chemotherapy. Similar results have been observed with the use of *ALK* inhibitors in patients with metastatic non-small-cell lung cancer whose tumor cells have *ALK* rearrangements. The use of specific kinase inhibitors are also being studied for additional molecular targets involving *ROS1*, *RET*, and *BRAF*. The Lung Cancer Mutation Consortium has reported better outcomes in patients with clearly defined molecular alterations who are treated with specific, molecularly targeted therapies than in patients without such targets in the tumor tissues, which highlights the need to identify and target pathogenic alterations in tumor cells.³⁶ Targeted, next-generation sequencing of a limited number of cancer genes in a single platform is being increasingly used in the clinic.³⁷ It is important to obtain an adequate amount of tissue by means of core biopsies or, even better, excisional biopsies when feasible. Fine-needle aspiration is not a suitable method for making a molecular diagnosis. In Lung-MAP (ClinicalTrials.gov number, NCT02154490), a large, ongoing study funded by the National Cancer Institute, researchers are exploring the usefulness of molecularly targeted therapy in patients with squamous-cell carcinoma of the lung by using one such multi-gene assay that involves next-generation sequencing technologies. The National Lung Matrix Trial (ClinicalTrials.gov number, NCT02664935), which is being conducted in the United Kingdom, involves the use of targeted therapy in patients with non-small-cell lung cancer. The trial has 21 groups that are categorized according to individual or combinations of genetic biomarkers.

IMPLICATIONS FOR CLONAL EVOLUTION IN LUNG CANCER

Evidence from studies of lung adenocarcinoma and other solid tumors implicates Darwinian selection of either de novo or preexisting sub-

clones during therapy that harbor somatic events that make cells more fit in the presence of drug-selection pressure. Neutral evolution may also occur in non-small-cell lung cancer that may further increase the substrate of diversity on which selection can act. Most large-scale genomic studies of lung cancer have been performed with the use of samples procured at the time of surgery from patients who had not received any systemic therapy. It will be important to obtain tissue specimens at the time of disease progression to fully understand clonal evolution after systemic therapy. As sequencing and tumor sampling methods improve, investigators are documenting multiple mechanisms of resistance to targeted therapies in lung adenocarcinoma.^{38,39} These data suggest that clonal evolution and the intratumoral heterogeneity of somatic events should be considered in future drug-development strategies.

PREDICTORS OF RESPONSE TO IMMUNOTHERAPY

Efforts are ongoing to identify biomarkers of response to drugs that target the programmed death 1 (PD-1) receptor in advanced non-small-cell lung cancer. Rizvi and colleagues showed that the response to anti-PD-1 therapy correlated with a smoking signature and the nonsynonymous (coding) mutation burden in the tumor.⁴⁰ Moreover, tumor regression was correlated with a neoantigen-specific response by CD8+ T cells, which points to the potential for selecting and customizing immunotherapy on the basis of the genomic characteristics of a tumor.

POTENTIAL USES OF CIRCULATING TUMOR BIOMARKERS

Sensitive sequencing techniques can now be used in early- and late-stage cancer to detect somatic mutations in tumors and copy-number aberrations in cell-free circulating tumor DNA obtained with the use of a “liquid biopsy” (i.e., a blood test).⁴¹⁻⁴⁴ Higher plasma levels of cell-free DNA were found in patients with resectable non-small-cell carcinoma than in healthy persons or persons with chronic respiratory inflammation.⁴⁵ This technology has the potential to be used in tracking the genomic evolution of tumors over time and may have therapeutic implications in terms of its ability to detect actionable events or resistant subclonal populations while avoiding the need to conduct repeated biopsies. The analysis and propagation of circulating tumor cells in studies in mice have shown promise in helping to

support the development of new drug combinations, particularly in small-cell lung cancer, in which access to tumor material is often limited.⁴⁶

DIRECTIONS OF FUTURE RESEARCH

Progress over the past 5 years has shed light on the early somatic events of tumorigenesis in lung cancer. As sequencing methods become increasingly sensitive, it is likely that the capacity to detect early-stage lung adenocarcinoma and squamous-cell carcinoma by means of cell-free DNA analysis will improve and that this technique will be used as a complement to radiologic-based screening approaches. Although we have made substantial progress in identifying biomarkers in order to select patients for molecularly targeted therapies, less progress has been made in our ability to identify patients who are likely to have relapse after surgical resection. Moreover, relatively little is known about the metastatic process and biologic characteristics of late-stage disease, a situation that should mandate the expedited performance of autopsy studies to investigate these processes further. Evidence suggesting the mutual dependencies of cancer subclones for tumor growth and metastatic colonization may lead to new therapeutic approaches.

As is the case with ecologic evolution, the evolution of cancer is a constrained process whose progress is probably limited by the host genome, antecedent steps in tumor evolution, and the microenvironment of the tumor. A deeper understanding of the spatial and temporal dynamics of the evolution of lung cancer may lead to new therapeutic approaches that will forestall the next evolutionary move of cancer and exploit evolutionary dead ends. An understanding of the evolution of lung cancer through space and time after surgery and during the onslaught of environmental, therapeutic, and immune-based selection pressures will be required to address these challenges. Efforts toward understanding these issues are being made in studies such as TRACERx, for which researchers are harnessing assiduous approaches to spatial and temporal tumor sampling linked to autopsy programs and developing their understanding of circulating biomarkers.

As deep-sequencing approaches become more common in clinical care, developments in informatics and the analysis of genomic data for clinical use will be essential. Such developments must provide real-time feedback that prioritizes

actionable genomic information that is based on up-to-date knowledge from resources available at an affordable cost to clinicians and patients. The consent forms used in genomic studies should inform patients about the risks associated with genomic testing, including loss of privacy, and about the implications of tests that may detect deleterious alterations in the germline that are associated with the future development of certain diseases. Patient preferences with regard to being informed of the incidental findings should be incorporated into the consent forms related to genomic studies.

Therapeutically, there is still much progress to be made. For example, the development of strategies to target *KRAS* mutations, the most common driver oncogene in lung adenocarcinoma, should be a top priority in research.⁴⁷ There is much interest in developing strategies to address synthetic lethality, an occurrence in which the disruption of two or more gene products leads to cell death, but the inhibition of either one alone does not. The concept of synthetic lethality is particularly relevant to relatively intractable therapeutic targets, such as *KRAS*.⁴⁷ As our knowledge of genetic dependencies in lung cancer improves, the vulnerabilities of the disease may reveal new therapeutic avenues.

The mutational burden of non-small-cell lung cancer (both squamous-cell carcinoma and adenocarcinoma) may be the Achilles' heel of immunotherapy. The iatrogenic effect of mutagenic therapy may need to be considered when determining the order of therapeutic regimens, since some cytotoxic chemotherapies and radiation may increase the acquisition of new subclonal mutations that might affect the response to immunotherapy. Furthermore, current approaches to the prediction of the development of tumor neoantigens are relatively rudimentary and are restricted to class I rather than class II major-histocompatibility-complex (MHC) antigens. Efforts to better delineate the development of both MHC class I- and class II-restricted neoantigens may improve our understanding of immune surveillance during tumor evolution. Finally, understanding the ways in which the immune microenvironment edits the cancer genome during the disease course and the ways in which the host immune system might be leveraged to target tumor neoantigens within the context of a heterogeneous genomic landscape may provide promising avenues to improve survival outcomes in patients with lung cancer.

Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

We thank Siddhartha Devarakonda, M.D., and Subramanian

Venkatesan, Ph.D., for their assistance with the figures and David Ornitz, Ph.D., for his helpful suggestions.

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