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Lung Cancer

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LUNG CANCER IS THE LEADING CAUSE OF CANCER DEATHS IN THE UNITED States[1] and worldwide. The two major forms of lung cancer are non–small-cell lung cancer (about 85% of all lung cancers) and small-cell lung cancer (about 15%). Despite advances in early detection and standard treatment, non–small-cell lung cancer is often diagnosed at an advanced stage and has a poor prognosis. The treatment and prevention of lung cancer are major unmet needs that can probably be improved by a better understanding of the molecular origins and evolution of the disease.

Non–small-cell lung cancer can be divided into three major histologic subtypes: squamous-cell carcinoma, adenocarcinoma, and large-cell lung cancer. Smoking causes all types of lung cancer but is most strongly linked with small-cell lung cancer and squamous-cell carcinoma; adenocarcinoma is the most common type in patients who have never smoked (Fig. 1[2-8]). This review will focus on major recent advances in the molecular study of the origins and biology of squamous-cell carcinoma and adenocarcinoma, since they constitute the vast majority of diagnosed lung cancers (Table 1[5,8-14]). These advances have been facilitated by the development of molecular techniques and biomarkers for defining cancer risk, prognosis, and optimal therapy aimed at personalized prevention and treatment of lung cancer.

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MOLECULAR ORIGINS

HOST SUSCEPTIBILITY

Epidemiologic studies showing an association between family history and an increased risk of lung cancer provided the first evidence of host susceptibility (Fig. 1). Lung-cancer susceptibility and risk also are increased in inherited cancer syndromes caused by rare germ-line mutations in p53,[15] retinoblastoma,[16] and other genes,[17] as well as a germ-line mutation in the epidermal growth factor receptor (*EGFR*) gene.[18] More recently, three large genomewide association studies identified an association between single-nucleotide polymorphism (SNP) variation at 15q24–15q25.1 and susceptibility to lung cancer. The region of the SNP variation was recently linked to lung carcinogenesis and includes two genes encoding subunits of the nicotinic acetylcholine receptor alpha, which is regulated by nicotine exposure.[19-22]

Lung-cancer susceptibility and risk also increase with reduced DNA repair capacity (particularly when accompanied by exposure to tobacco smoke)[23] that results, for example, from germ-line alterations in nucleotide excision repair genes such as *ERCC1*.[24] Increased expression of DNA synthesis and repair genes, including *RRM1* (the regulatory subunit of ribonucleotide reductase) and *ERCC1*, in non–small-cell lung cancer correlates with a better prognosis overall but no benefit from platinum-based chemotherapy.[25,26] Table 1 presents gene abnormalities involved in the development of different histologic types of lung cancer.

CLONAL EVOLUTION

Changes in certain genes (e.g., proinflammatory interleukin-8 [*IL8*] and some DNA-repair genes) occur in nonmalignant lung tissue of smokers and patients with lung cancer, a finding consistent with diffuse tissue injury.[3,27-29] These changes probably precede epithelial clonal evolution, an important element of the molecular origins of lung and other cancers (Fig. 1). Patches of clonally related cells, or clonal patches containing 40,000 to 360,000 cells, have been mapped in the lung.[30] The size and number of subclones in a clonal patch may contribute to the cancer risk.[31] Early events in the development of non–small-cell lung cancer include loss of heterozygosity at chromosomal region 3p21.3 (site of *RASSF1A*, a member of the Ras association domain family, and *FUS1*), 3p14.2 (*FHIT*, a fragile histidine triad gene), 9p21 (*p16*), and 17p13 (*p53*).[32] All these genes are tumor-suppressor genes. Loss-of-heterozygosity patterns in squamous-cell carcinoma and adenocarcinoma differ (e.g., chromosome 3p deletions are much more extensive in squamous-cell carcinoma). Mutations in the *EGFR* kinase domain occur early in the development of adenocarcinoma that is generally unrelated to smoking, and *KRAS* mutations occur early in the development of smoking-related adenocarcinoma.[33,34] Clonal patches with methylation of promoter regions of genes (epigenetic changes), *p53* mutation, *EGFR* mutation, *c-Myc* amplification, loss of heterozygosity, and microsatellite instability can occur in normal tissue surrounding non–small-cell lung tumors[5,30,32,34,35] and may be associated with a greater risk of recurrence and second primary tumors. These findings suggest that in the future molecular analyses of surgical margins may help identify patients most likely to benefit from adjuvant therapy.

Methylated genes in premalignant squamous-cell lung lesions (e.g., metaplasia and dysplasia) are *p16* and *FHIT* (frequently and very early) and O-6-methylguanine-DNA methyltransferase (*MGMT*), death-associated protein kinase (*DAPK*), and *RASSF1A* (less frequently or rarely and in advanced precancers).[35-41] The early methylation of *p16* in the development of squamous-cell lung cancer (e.g., in normal lung tissue in approximately 50% of smokers)[36] exemplifies differences with that in the development of adenocarcinoma, in which *p16* methylation occurs very rarely and only late in precursors (e.g., high-grade atypical adenomatous hyperplasia).[37] Methylation markers in sputum are associated with the risk of lung cancer (e.g., methylated *p16*)[38] and the recurrence of lung cancer (methylated *ASC-TMS1*,[39] also called *PYCARD*). Recent data show that promoter methylation of various genes, including *p16* in stage I non–small-cell lung cancer, is associated with recurrence after resection.[40,41] Agents that reverse epigenetic changes have shown promise in a mouse model of lung carcinogenesis and are being tested in humans with lung cancer.[42]

Bronchoalveolar stem cells, which may be precursors of lung adenocarcinoma, were identified recently in studies in mice.[43] The KRAS, Pten, phosphoinositide 3-kinase (PI3K), and cyclin-dependent kinase pathways have been implicated in the proliferation of these stem cells.[44,45] The potential role of bronchoalveolar stem cells and other tumorigenic stem-cell populations in the development and prognosis of human lung cancer and its resistance to drugs is an important area of future investigation.

MOLECULAR EVOLUTION

EGFR FAMILY

EGFR regulates important tumorigenic processes, including proliferation, apoptosis, angiogenesis, and invasion (Fig. 2), and, along with its ligands, is frequently overexpressed in the development and progression of non–small-cell lung cancer.[2,5,34,46] Clinical trials of the EGFR tyrosine kinase inhibitor erlotinib for second-line or third-line treatment of such tumors and of the monoclonal antibody against EGFR, cetuximab (combined with chemotherapy), for treatment of previously untreated, advanced disease[47,48] validated EGFR as a molecular target for therapy. *EGFR* mutations that were discovered during clinical trials led to extensive studies of the roles of these mutations and *EGFR* amplification in the pathogenesis of the disease and its prognosis and sensitivity to treatment.

Several groups of investigators independently identified somatic mutations in the kinase domain of *EGFR* in lung adenocarcinoma in approximately 10% of specimens from patients in the United States and in 30 to 50% of specimens from patients in Asia[49] (Fig. 3). The mutations occur with increased frequency in women and nonsmokers and are tightly associated with sensitivity to the EGFR tyrosine kinase inhibitors gefitinib and erlotinib and so appear to explain most of the dramatic responses to these agents.[50-52] More than 80% of these mutations in lung cancer involve in-frame deletions within exon 19 or the L858R mutant within exon 21. *EGFR* mutations are associated with an improved prognosis in non–small-cell lung cancer, even when treated with cytotoxic chemotherapy.[53,54] *EGFR* amplification is detected in dysplasia (especially of a high grade), which is associated with lung-cancer risk when detected in the sputum of smokers, and is associated with a poor prognosis but also with sensitivity to EGFR inhibitors.[55,56] The epidemiologic links

highlight three factors — whether the patient is a nonsmoker, Asian, and female — that are associated independently and collectively with an improved response to EGFR tyrosine kinase inhibitors. However, erlotinib appears to prolong survival in virtually all subgroups of patients with non–small-cell lung cancer.[48] There are major differences in clinical, pathological, sex-related, and molecular factors between smokers and lifelong nonsmokers in whom lung cancer develops (Fig. 1).

The vast majority of patients who have an initial response to erlotinib and gefitinib eventually have a relapse.[49,57,58] Recent studies have identified *EGFR* T790M mutations (in exon 20) in tumors before drug treatment[59] and in tumors of patients who had a relapse after therapy with standard reversible EGFR tyrosine kinase inhibitors.[57] The binding kinetics of the mutant *EGFR* appear to be altered by the T790M mutation (Fig. 3). Irreversible EGFR inhibitors suppress T790M-mutant tumor cells in vitro and are promising treatments for T790M-mutant tumors.[60] Amplification of the met proto-oncogene (*MET*), another major mechanism of acquired resistance to EGFR tyrosine kinase inhibitors, marks a poor prognosis.[61-63] Other proposed resistance mechanisms include activation of other receptor tyrosine kinases, such as insulin-like growth factor 1 receptor (which can bypass EGFR to activate critical downstream signaling pathways [Fig. 2]), *KRAS* mutations, and the epithelial-to-mesenchymal transition.[58,64,65] The epithelial-to-mesenchymal transition is a program of cell development involving loss of cell adhesion, repressed E-cadherin expression, and increased cell mobility.

Preclinical and clinical data suggest that *EGFR* mutations are early events in the development of non–small-cell lung cancer.[66-70] *EGFR* mutations, including those involving exons 18, 19, and 20 and L858R, can transform fibroblasts and lung epithelial cells.[67] Furthermore, in transgenic mice with lung-specific expression of exon 19 deletion or the L858R mutation, atypical adenomatous hyperplasia, which is considered to be a precursor lesion of peripheral adenocarcinoma, was followed by lesions resembling bronchioalveolar carcinoma at 5 to 6 weeks of age and invasive adenocarcinomas at 8 to 10 weeks.[68] Deinduction of mutant *EGFR* expression led to regression of tumors, suggesting the need for persistent mutant *EGFR* activity for continued tumor survival. Lung tumors also developed in transgenic mice with lung-specific expression of *EGFR* variant III mutation (in-frame deletion of exons 2–7 from the extracellular domain) (Fig. 3).[13] Mutations of the region that encodes the tyrosine kinase domain of *EGFR* have been detected in specimens of atypical adenomatous hyperplasia from Asian patients with no history of smoking.[71] These mutations also occur in normal epithelium within and adjacent to tumors with *EGFR* tyrosine kinase mutations (a localized-field effect, possibly reflecting stem-cell expansion) and before *EGFR* amplification, a change associated with tumor progression and metastasis.[72]

HER2 mutations and amplification have been identified in patients with lung adenocarcinoma. The frequency of such mutations is less than 5%, and the frequency of such amplification is 5 to 10%. *HER2* kinase domain mutations (in-frame insertions in exon 20) and *EGFR* kinase domain mutations have similar associations with female sex, nonsmoking status, and Asian background in patients with adenocarcinoma.[73] *HER2* amplification is associated with sensitivity to inhibitors of the EGFR tyrosine kinase[74];

HER2 mutations are associated with resistance to such inhibitors but also with sensitivity to HER2-targeted therapy.[75] *HER3* kinase domain mutations have not been detected in patients with non–small-cell lung cancer.[76] Mutations in the *HER4* kinase domain were found in 2 to 3% of Asian patients with this disease, with a possible association with male sex and smoking.[77]

Ras–Raf–Mek—The Ras–Raf–Mek pathway is involved in signaling downstream from EGFR and in other pathways leading to the growth of cancer cells and tumor progression (Fig. 2). Activating *KRAS* mutations are limited to non–small-cell lung cancer (predominantly adenocarcinomas), virtually mutually exclusive of mutations in the *EGFR* and *HER2* kinase domains, and associated with resistance to EGFR inhibitors (tyrosine kinase inhibitors and cetuximab) and chemotherapy.[58,78,79] Most *KRAS* mutations in lung adenocarcinoma are smoking-related G→T transversions (substitutions of a purine for a pyrimidine) and affect exon 12 (in 90% of patients) or exon 13.[2,79] A distinct *KRAS* mutational profile consisting of G→A transition mutations was recently detected in patients with adenocarcinoma who had never smoked; its functional significance is unclear.[79] Transversions (smokers) and transitions (non-smokers) also have been reported for *p53* mutations in lung adenocarcinoma.[2] *KRAS* mutations appear to be an early event (e.g., detectable in the preinvasive lesions of atypical adenomatous hyperplasia and bronchoalveolar carcinoma[33]) that precedes smoking-related lung adenocarcinoma. They generally mark a poor prognosis. Further evidence supporting this gene's role in the pathogenesis of lung cancer comes from transgenic mice bearing a mutated *KRAS* and in which multifocal atypical adenomatous hyperplasia and adenocarcinoma develop.[80] *MET* activation occurs early in KRAS-induced carcinogenesis in this model.[81] *BRAF* mutations have also been detected in non–small-cell lung cancer[9] and may be an early event in lung tumorigenesis.[82]

PI3K–Akt–mTOR—The pathway consisting of PI3K, Akt, and mammalian target of rapamycin (mTOR), which is downstream of EGFR, is activated early in lung carcinogenesis.[83] Akt is also overexpressed in bronchial dysplasia. Inhibition of Akt can induce apoptosis of human premalignant and malignant lung cells and prevent lung carcinogenesis in an animal model. An mTOR inhibitor can block malignant progression of atypical adenomatous hyperplasia lesions in the *KRas* mouse model.[84] Since mTOR drives tumorigenesis in part through macrophages, a prominent component of the tumor microenvironment, the antitumor effect of mTOR inhibition requires the tumor microenvironment. There is mutation or amplification of *PIK3CA*, which encodes the PI3K catalytic subunit, in a subgroup of non–small-cell lung tumors, especially squamous-cell carcinoma, in association with increased PI3K activity and Akt expression.[85]

LKB1—*LKB1* (also called *STK11*) is frequently mutated in non–small-cell lung tumors and is thought to act as a tumor-suppressor gene through interactions with *p53* and *CDC42*, modulating the activity of AMPK (a multifunctional protein kinase) and other possible mechanisms that are just beginning to be studied.[86,87] *LKB1* is thought to function in early tumorigenesis, subsequent differentiation, and the development of metastases.[88] Results in transgenic *KRas*-mutant mice in which *LKB1* was inactivated suggest that the gene plays

a role in the differentiation and invasive behavior of such tumors.[87] The presence of *LKB1* mutations alone (i.e., without *KRas* mutations) was not associated with the development of lung cancer in mice. Low levels of LKB1 protein were associated with high grades of dysplasia in atypical adenomatous hyperplasia lesions, suggesting that LKB1 has an early role in the development of premalignant lesions in the lung.[89] *LKB1* mutations (including point mutations and deletions) were found in 34% of adenocarcinomas and 19% of squamous-cell carcinomas from 144 human specimens of non–small-cell lung cancer.[87] However, much lower rates of *LKB1* mutation (<5%) were found in adenocarcinomas from Asian patients.[90,91] *LKB1* mutations are associated with smoking and with *KRAS* mutations and are virtually exclusive of *EGFR* mutations.[91]

TITF1—Amplification of thyroid transcription factor 1 (*TITF1*, also called *NKX2-1*) in the 14q13.3 region was the most common focal event in a high-resolution analysis of gene copy numbers in human lung adenocarcinoma.[92] This study used an array with the capacity to genotype many SNPs. As a result, the investigators also identified amplification in regions containing *KRAS, Myc*, vascular endothelial growth factor (*VEGF*), and several cell-cycle genes in the tumor specimens. *TITF1* encodes a lineage-specific transcription factor that is essential for the formation of cells lining lung alveoli (type II pneumocytes). In vitro, transfection of immortalized normal human lung epithelial cells with at least two of the three genes *TITF1, NKX2-8*, and *PAX-9* in the 14q13.3 region caused increased growth of the cells,[93] suggesting that these three genes may work cooperatively in the pathogenesis of lung cancer in which there is amplification at 14q13.3 (detected by high-resolution comparative genomic hybridization array). Recent data indicate that squamous-cell carcinoma also exhibits *TITF1* amplification, as detected on fluorescence in situ hybridization, but not TITF1 protein, in contrast to adenocarcinoma.[94]

ANGIOGENESIS

VEGF levels in bronchial epithelial cells of smokers increase in association with the progression of bronchial dysplasia from low grade to high grade.[95] Bronchial hyperplasia, metaplasia, and carcinoma in situ are associated with increased microvessel density, and a distinctive pattern known as angiogenic squamous dysplasia can occur.[96] Factors associated with increased tumor angiogenesis correlate with the development and prognosis of lung cancer.[97-99] Circulating VEGF levels may predict the clinical benefit of VEGF inhibitors in patients with this disease. Many angiogenic factors are regulated at least in part through the hypoxia-regulated pathways, such as hypoxia-induced factor (HIF) 1α and 2α.[100,101] In addition to hypoxia, VEGF and other angiogenic factors are also regulated by EGFR through HIF-dependent and independent mechanisms[102] and by oncogenes such as *KRAS* and *p53*. VEGF has recently been validated as a therapeutic target on the basis of the results of a phase 3 trial, which led the Food and Drug Administration (FDA) to approve the VEGF monoclonal antibody bevacizumab in combination with standard chemotherapy for previously untreated, advanced non–small-cell lung cancer.[103]

Interactions between the VEGF and EGFR pathways and an association between acquired resistance to EGFR blockade and increased VEGF expression in preclinical models[104] led to the hypothesis that dual blockade of VEGF and EGFR might be more effective than

either approach alone. Randomized phase 2 trials of dual inhibition with bevacizumab plus erlotinib[105] or the VEGF receptor–EGFR tyrosine kinase inhibitor vandetanib (combined with chemotherapy)[106,107] had promising results. Phase 3 testing of both approaches in patients with platinum-resistant disease is ongoing.

MOLECULAR PROFILING

TECHNICAL ADVANCES

Molecular profiling, including the profiling of genes and proteins, to guide treatment may improve the clinical outcome in patients with non–small-cell lung cancer (Fig. 1). Progress in the identification of markers, mutations, and genomic signatures far outstrips the modest improvement in treatments that are based on these molecular advances. Formidable obstacles to developing effective markers include tumor heterogeneity, the highly complex interplay between the environment and host and the complexity, multiplicity, and redundancy of tumor-cell signaling networks involving genetic, epigenetic, and microenvironmental effects. Emerging high-throughput techniques for assessing genomic DNA, messenger RNA (mRNA), microRNA, methylation, and protein or phosphoprotein signaling networks should help address these obstacles (Fig. 4). The Cancer Genome Atlas is a large-scale project designed to provide a comprehensive profile of human tumors according to their gene mutations, alterations in gene copy number, and epigenetic changes. Squamous-cell carcinoma of the lung will be one of the first tumors profiled by this atlas.

GENE PROFILING

Tumor molecular heterogeneity is a major reason that patients with non–small-cell lung cancer with a similar clinical stage and tumor histology can have dramatically different clinical outcomes and responses to treatment. Microarray techniques that profile the expressions of tens of thousands of genes simultaneously can measure this tumor heterogeneity at a global level. Gene-expression profiles that are associated with subtypes of non–small-cell lung cancer[108,109] and with reduced recurrence-free or overall survival of patients have been identified.[110-113] Combined clinical and molecular information provides better indications of cancer risk[114] and prognosis.[115]

In a recent analysis of 672 invasion-associated genes from 125 frozen specimens of early-stage tumors,[111] microarray and reverse-transcriptase–polymerase-chain-reaction (RT-PCR) analyses identified a molecular signature of five genes as an independent predictor of relapse-free and overall survival. In two validation cohorts, another recently developed gene-expression profile (metagene) predicted clinical outcome with an accuracy of 72% and 79% (greater than that for tumor stage, tumor diameter, nodal status, or other clinical measures) and predicted the outcome in patients with stage IA tumors.[112] Randomized, controlled trials will need to validate these signatures and establish whether the patients with stage IA tumors who were identified as being at high risk will benefit from adjuvant therapy. One such phase 3 trial, coordinated by the Cancer and Leukemia Group B, is approved and under final review. It will evaluate a large predictive set of metagenes (or subgroups of gene-expression profiles consisting of 25 to 200 genes) in patients with stage IA tumors who are undergoing adjuvant chemotherapy.

For the majority of patients with advanced or metastatic non–small-cell lung cancer, the most important potential effect of molecular markers is likely to be in predicting the response to specific therapies with the goal of "personalizing" treatment (Fig. 4). Many exciting potential predictive markers have been developed in vitro and need validation in tumor samples and clinical trials.[113] For example, gene-expression signatures have been developed for cisplatin and pemetrexed on the basis of in vitro sensitivity; the cisplatin in vitro signature predicted the likelihood of response. Recently developed in vitro profiles predicting the sensitivity of tumors to EGFR inhibitors and other therapies have yet to be assessed clinically.[113]

MicroRNA has recently emerged as an important regulator of gene expression. High-throughput analyses have shown that microRNA expression is commonly deregulated in lung and other cancers.[116,117] Using real-time RT-PCR, investigators recently identified a five-microRNA signature that is associated with treatment outcome.[116] Loss of microRNA-128b, a putative regulator of EGFR that is located on chromosome 3p, has been shown to correlate with the response to EGFR inhibition in patients with lung cancer.[117]

Studies suggest that information about tumor-specific genetic and epigenetic changes also may be obtained from the blood of patients with lung cancer. Circulating DNA can be detected in the plasma and serum of such patients, and levels of this DNA are associated with a poor prognosis.[118,119] Tumor-specific DNA alterations (such as loss of heterozygosity), promoter methylation, and *KRAS* and *EGFR* mutations have also been detected in the blood of patients with lung cancer.[120-122] New techniques for capturing circulating tumor cells allow the detection of EGFR-activating mutations and the drug-resistance allele T790M. Such techniques appear to be more sensitive than those for capturing circulating DNA. Furthermore, a decline in the number of circulating tumor cells was associated with tumor response on radiography.[123] These studies suggest that blood profiling may provide useful information about genetic changes in tumors that could ultimately help detect lung cancer and guide therapy.

PROTEIN PROFILING

Profiling of genomic and mRNA expression provides an incomplete picture of the heterogeneity of non–small-cell lung cancer. Levels of mRNA do not always correlate with protein levels and do not provide information on protein–protein interactions or post-translational modifications such as phosphorylation that may be critical for regulating protein activity.[124] Furthermore, most targeted therapeutic agents are designed to inhibit the activity of proteins such as tyrosine kinases. Therefore, protein-based profiling is likely to be essential in understanding the complexity of protein signaling networks and developing molecular signatures that predict a response to therapy.

Immunohistochemical analysis remains the most widely applied method for assessing individual proteins and may be useful for estimating prognosis and predicting the response to therapy.[25,26] Emerging high-throughput proteomic techniques, such as mass spectrometry and protein microarrays, have the potential to view signal transduction networks more globally than is possible with immunohistochemical analysis. Such techniques are feasible in small amounts of tumor tissue.[125] Proteomic signatures for prognosis and predicting

the response to chemotherapy or EGFR inhibitors have been developed in tumors and cell lines.[126,127]

Proteomic profiling from blood is also under study, allowing repeated measurements during treatment without the need for tumor tissue. Serum mass spectrometry profiles can distinguish patients with non–small-cell lung cancer from normal controls[124,128] and patients with better outcomes from those with worse outcomes after treatment with EGFR tyrosine kinase inhibitors.[129] New techniques also permit the multiplex analysis of dozens of cytokines and angiogenic factors in small amounts of serum or plasma. This approach is being used in developing predictive markers in non–small-cell lung cancer. Although promising, blood- and tissue-based proteomic approaches remain investigational and await prospective testing and validation in large, randomized trials before they can be applied clinically.

CONCLUSIONS

The molecular origins of lung cancer lie in complex interactions between the environment and host genetic susceptibility. Lung cancer then evolves through genetic and epigenetic changes, including deregulated signaling pathways, which are potential targets for chemoprevention and therapy. Emerging techniques for genomic, gene-expression, epigenetic, and proteomic profiling[92,114,125,130,131] could revolutionize clinical approaches across the spectrum of lung-cancer types and subtypes by identifying practical molecular markers of risk (in precancer), early detection and prognosis (in early-stage cancer), and treatment sensitivity (in early-stage and advanced-stage cancer). Genomewide and other molecular assessments are helping elucidate germ-line variations that may contribute to lung cancer risk,[19-21] prognosis,[132] and treatment sensitivity[133,134] and somatic genetic alterations that occur in lung adenocarcinomas[14,50-52] and in high-risk lung tissue associated with tumors or in smokers.[3,28,29] Molecular targeted research has produced the recently FDA-approved EGFR and VEGF inhibitors erlotinib and bevacizumab, which have modestly improved the outcome in patients with non–small-cell lung cancer.[48,103] Molecular profiling of the type described in this review has begun in clinical trials[112,135-137] and promises to select patients who are most likely to benefit from therapy and to guide the development of more effective agents that will personalize standard medicine for lung cancer.[138]

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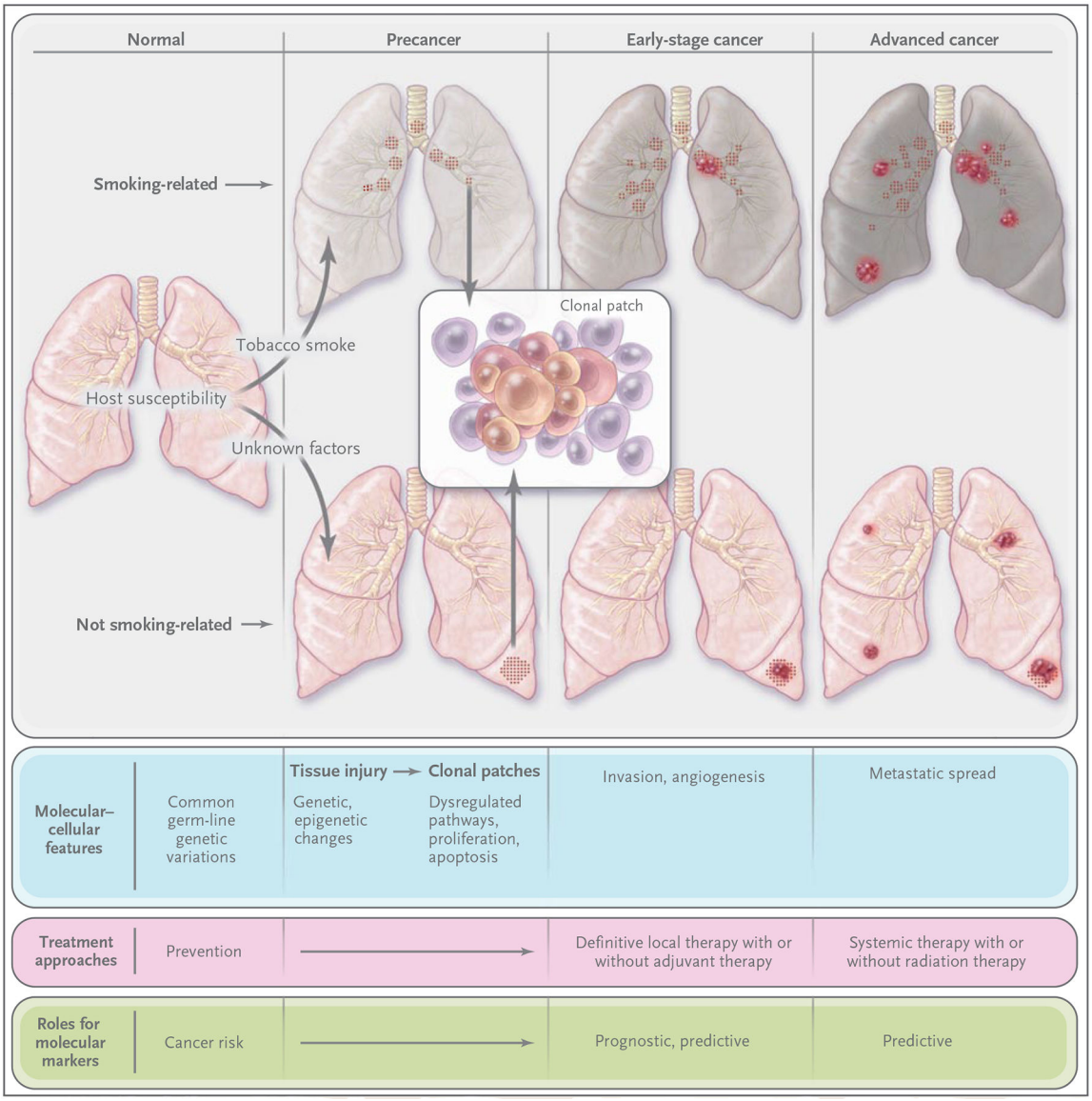

Figure 1. Molecular Evolution of Lung Cancer.

Environmental factors, such as tobacco smoke, and genetic susceptibility interact to influence carcinogenesis. Factors that are unrelated to smoking — including genetic, hormonal, and viral (e.g., human papillomavirus) factors — have been suggested.[2] Tissue injury (e.g., from tobacco smoke, reflected in the discolored smoking-related lungs) initially occurs in the form of genetic and epigenetic changes (e.g., mutations, loss of heterozygosity, and promoter methylation) and global transcriptome changes (e.g., inflammation and apoptosis pathways). These changes can persist long term[3,4] and eventually lead to aberrant pathway activation and cellular function (e.g., dysregulated proliferation and apoptosis) to produce premalignant changes, including dysplasia and clonal patches. Additional changes can result in angiogenesis, invasion and early-stage cancer, and advanced cancer and metastasis.[5] Many molecular changes in earliest-stage cancer also occur in advanced disease.[6,7] Premalignant patches contain clones and subclones (inset), which can involve

loss of heterozygosity, microsatellite instability, and mutations (e.g., in *p53* and epidermal growth factor receptor [*EGFR*]). Lung cancers unrelated and related to smoking have strikingly different molecular profiles, including those of mutations in *p53*, *KRAS, EGFR*, and *HER2*. Smoking-related patches and primary cancers (usually squamous-cell carcinoma and small-cell lung cancer) most often develop in the central airway.[4,8] Most tumors that are not related to smoking are adenocarcinomas and develop in the peripheral airways. Molecular markers can signify risk (in people without cancer), prognosis (outcome independent of treatment), and sensitivity to treatment through predictive markers. Such stage-specific markers can span the course of disease from its early stages through its late stages. They also can help define mechanisms of resistance to therapy.

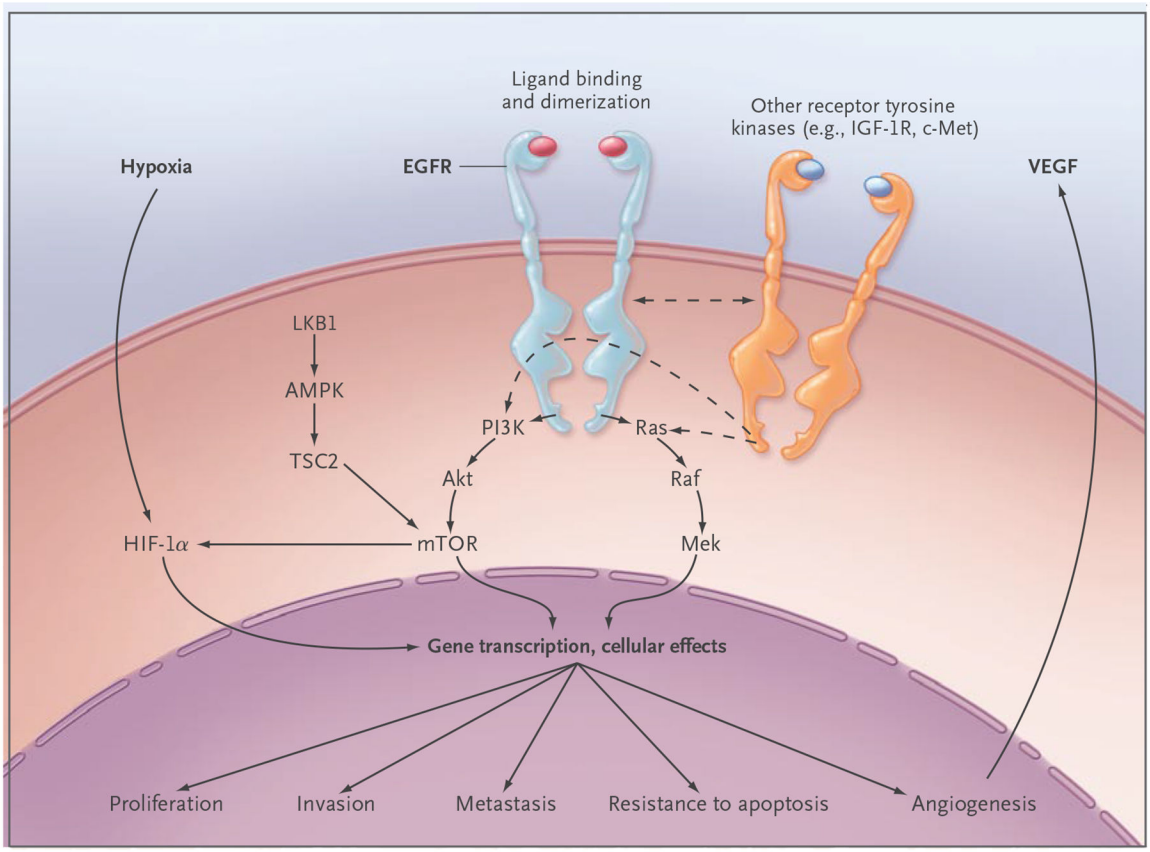

Figure 2. Epidermal Growth Factor Receptor (EGFR) Cell-Signaling Pathways.

EGFR activates several major downstream signaling pathways, including Ras–Raf–Mek and the pathway consisting of phosphoinositide 3-kinase (PI3K), Akt, and mammalian target of rapamycin (mTOR), which in turn may have an effect on proliferation, survival, invasiveness, metastatic spread, and tumor angiogenesis through pathways that are either dependent on or independent of the hypoxia inducible factor (HIF). These pathways also may be modulated by other receptor tyrosine kinases, such as insulin-like growth factor 1 receptor (IGF-1R) and cMET, and by the LKB1–amp-activated protein kinase (AMPK) pathway, which is involved in energy sensing and cellular stress. Most of these functions depend on signaling through the kinase domain. However, kinase-independent functions, such as maintaining glucose transport, have been reported.[46] TSC2 denotes tuberous sclerosis complex 2, and VEGF vascular endothelial growth factor.

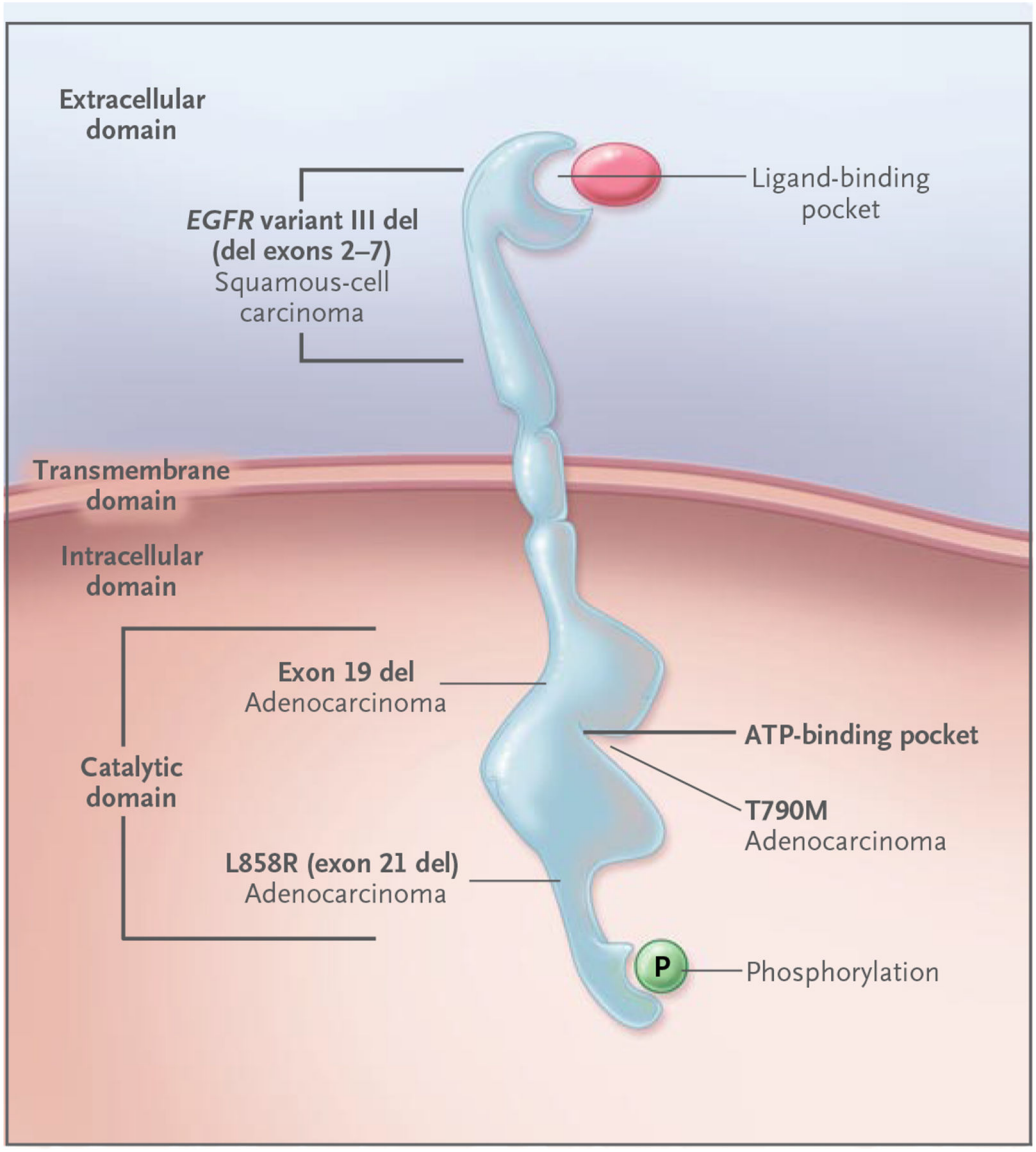

Figure 3. Effect of Deletions and Mutations in the Epidermal Growth Factor Receptor Gene (*EGFR*) on Disease Development and Drug Targeting.

Ligand binding to the EGFR extracellular domain results in receptor homodimerization and tyrosine phosphorylation, with the phosphate derived from ATP bound within the kinase catalytic domain. *EGFR* mutations have transforming potential in preclinical lung models and can occur early in human lung carcinogenesis. *EGFR* point mutations (e.g., L858R) and exon 19 deletions, which occur predominantly in adenocarcinoma of the lung, are located within the catalytic domain and result in constitutive EGFR activation. These mutations are associated with increased sensitivity to EGFR tyrosine kinase inhibitors, such as erlotinib and gefitinib. In contrast, mutations in T790M (an amino acid located within the ATP binding site of the *EGFR* kinase domain) are associated with acquired resistance to these drugs. *EGFR* variant III mutant deletions occur in the extracellular domain and are associated with squamous-cell cancer.

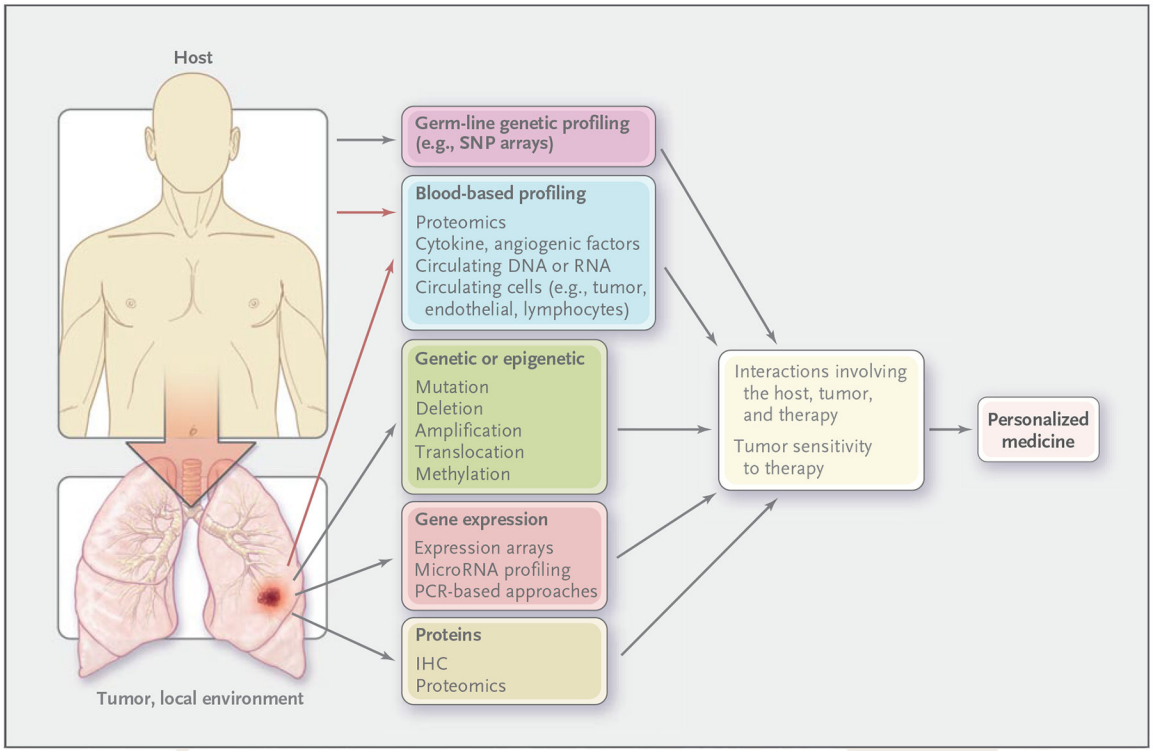

Figure 4. Molecular-Profiling Approaches to the Development of Personalized Therapy.

Host profiling involves innate characteristics of the cancer patient. All markers that are involved in profiling lung cancer can apply to the tumor or its local environment. Predictive markers identify groups of patients who are likely to have increased sensitivity or resistance to a given therapy, a critical step in personalizing treatment. It has been traditional to assess individual genetic or protein prognostic or predictive markers (e.g., HER2 for breast cancer), but emerging techniques permit global analyses of the genomic, gene-expression, epigenetic, and protein profiles of the host (innate), including markers in blood and in tumor or nonmalignant lung tissue. These methods include single-nucleotide polymorphism (SNP) arrays to assess genomic alterations, bisulfite sequencing, and methylation-specific polymerase chain reaction (PCR) to assess epigenetic changes, microarrays for assessing gene expression or microRNA levels, and proteomic methods (such as mass spectroscopy, reverse-phase protein arrays, and multiplex beads) to assess intracellular signaling in tumor tissue and cytokines and angiogenic factors in blood. Blood-based profiling includes markers derived from the host (e.g., lymphocytes) and the tumor and local environment (e.g., circulating tumor cells and tumor-derived cytokines) (red arrows). IHC denotes immunohistochemical analysis.

Table 1.

Genetic Abnormalities Specific in the Lung to Non–Small-Cell Lung Cancer and Small-Cell Lung Cancer.[*]

Abnormality	Non–Small-Cell Lung Cancer		Small-Cell Lung Cancer
	Squamous-Cell Carcinoma	Adenocarcinoma	
Precursor			
Lesion	Known (dysplasia)	Probable (atypical adenomatous hyperplasia)	Possible (neuroendocrine field)[†]
Genetic change	*p53* mutation	*KRAS* mutation (atypical adenomatous hyperplasia in smokers), *EGFR* kinase domain mutation (in nonsmokers)	Overexpression of c-MET
Cancer			
KRAS mutation	Very rare	10 to 30%[‡]	Very rare
BRAF mutation	3%	2%	Very rare
EGFR			
Kinase domain mutation	Very rare	10 to 40%[‡]	Very rare
Amplification[§]	30%	15%	Very rare
Variant III mutation	5%[¶]	Very rare	Very rare
HER2			
Kinase domain mutation	Very rare	4%	Very rare
Amplification	2%	6%	Not known
ALK fusion[‖]	Very rare	7%	Not known
MET			
Mutation	12%	14%	13%
Amplification	21%	20%	Not known
TITF-1 amplification	15%	15%	Very rare
p53 mutation	60 to 70%	50 to 70%[‡]	75%
LKB1 mutation	19%	34%	Very rare
PIK3CA			
Mutation	2%	2%	Very rare
Amplification	33%	6%	4%

[*] Non–small-cell lung cancer includes squamous-cell carcinoma and adenocarcinoma.

† Neuroendocrine fields have been detected only in tissue surrounding tumors and have been characterized by extremely high rates of allelic loss and by c-MET overexpression (Salgia R: personal communication).

‡ Variations are based in part on smoking profiles.

§ The percentages include increased gene copy numbers from amplification or polysomy and represent percentages from resected cancers. The percentages are higher in primary tumors from patients with metastatic disease. Increased copy numbers have been reported in squamous dysplastic lesions but not in adenocarcinoma precursors.

¶ Genomic *EGFR* variant III mutations have been detected only in lung squamous-cell carcinoma, and these tumors are sensitive preclinically to irreversible EGFR tyrosine kinase inhibitors. The incidence of 5% is substantially lower than that of 30 to 40% for the detection in squamous-cell carcinoma or adenocarcinoma by immunohistochemical analysis or other techniques.

‖ The anaplastic lymphoma kinase (*ALK*) fusion gene (involving chromosome 2p), consisting of parts of *EML4* and *ALK*, is transforming in fibroblasts and occurs in adenocarcinoma but not in other types of non–small-cell lung cancer or other nonlung cancers.