A Translational View of the Molecular Pathogenesis of Lung Cancer

Mitsuo Sato, MD, PhD, David S. Shames, PhD,*§ Adi F. Gazdar, MD,‡ and John D. Minna, MD*†§*

Abstract: Molecular genetic studies of lung cancer have revealed that clinically evident lung cancers have multiple genetic and epigenetic abnormalities, including DNA sequence alterations, copy number changes, and aberrant promoter hypermethylation. Together, these abnormalities result in the activation of oncogenes and inactivation of tumor-suppressor genes. In many cases these abnormalities can be found in premalignant lesions and in histologically normal lung bronchial epithelial cells. Findings suggest that lung cancer develops through a stepwise process from normal lung epithelial cells towards frank malignancy, which usually occurs as a result of cigarette smoking. Lung cancer has a high morbidity because it is difficult to detect early and is frequently resistant to available chemotherapy and radiotherapy. New, rationally designed early detection, chemoprevention, and therapeutic strategies based on the growing understanding of the molecular changes important to lung cancer are under investigation. For example, methylated tumor DNA sequences in sputum or blood are being investigated for early detection screening, and new treatments that specifically target molecules such as vascular endothelial growth factor and the epidermal growth factor receptor are becoming available. Meanwhile, global gene expression signatures from individual tumors are showing potential as prognostic and therapeutic indicators, such that molecular typing of individual tumors for therapy selection is not far away. Finally, the recent development of a model system of immortalized human bronchial epithelial cells, along with a paradigm shift in the conception of cancer stem cells, promises to improve the situation for patients with lung cancer. These advances highlight the translation of molecular discoveries on lung cancer pathogenesis from the laboratory to the clinic.

Key Words: Lung cancer, Molecular pathogenesis, Tyrosine kinase inhibitor, Clinic, Targeted therapy, Early detection, Prevention, Epidermal growth factor receptor.

(*J Thorac Oncol.* 2007;2: 327–343)

Lung cancer is the leading cause of cancer deaths in the United States in both sexes, with an estimated mortality of more than 160,000 in 2006.[1] Current standard therapies include surgical resection, platinum-based doublet chemotherapy, and radiation therapy alone or in combination. Unfortunately, these therapies rarely cure the disease, and the overall 5-year survival rate is still only 15%.[1] To reduce the incidence of lung cancer and to improve the currently poor outcome, several important issues related to the pathogenesis of these diseases need to be addressed by the biomedical community. First, because 85% of lung cancers are caused by tobacco smoke, continued efforts are required to prevent smoking initiation and to aid in smoking cessation.[2] Unfortunately, one outcome of current smoking-prevention efforts is that nearly half of all lung cancer cases are now diagnosed in former smokers. Thus, identifying former smokers at the highest risk of developing lung cancers remains an important priority. Second, because most lung cancers present as advanced cases, which are not amenable to curative surgery or radiotherapy, development of efficient early detection methods are needed. Recent advances in spiral computed tomography scans give some hope of improved early detection, at least for peripheral lung cancers.[3,4] Also, biomarkers based on the common molecular defects found in lung cancers, such as methylated DNA, are being tested for clinical application.[5] Third, more effective drugs that are better suited to the molecular phenotypes of a given cancer are needed because current standard therapy with cytotoxic drugs provides only modest survival benefits.[6]

Extensive molecular genetic studies of lung cancer show that clinically overt lung cancers have multiple genetic and epigenetic alterations (>20 per tumor).[7] In addition, several studies have demonstrated that preneoplastic cells and histologically normal bronchial epithelium harbor many of these abnormalities, suggesting that human lung cancer develops from normal epithelial cells through a multistep process involving successive genetic and epigenetic abnormalities, usually coincident with cigarette smoking.[8] These abnormalities contribute to the initiation, development, and maintenance of lung cancer.

*Hamon Center for Therapeutic Oncology Research Simmons Cancer Center, and Departments of †Internal Medicine, ‡Pathology, and §Pharmacology, The University of Texas Southwestern Medical Center, Dallas, Texas.

Disclosure: JDM has a research grant from Astra Zeneca to study their new drugs preclinically. The authors declare no other conflict of interest.

Address for correspondence: John D. Minna, Hamon Center for Therapeutic Oncology Research NB8.206, The University of Texas Southwestern Medical Center at Dallas, 6000 Harry Hines Blvd., Dallas, TX 75390. E-mail: John.Minna@UTsouthwestern.edu

ISSN: 1556-0864/07/0204-0327

TABLE 1. Genetic Alterations Found in Lung Cancer and Drugs or Therapeutics Targeting These Alterations

Gene	Type of Alteration	Drug or Therapeutics Targeting Abnormalities
EGFR	Mutation and amplification	Tyrosine kinase inhibitors (gefitinib, elrotinib)
		Chimeric IgG monoclonal antibody (cetuximab)
HER2	Mutation and amplification	Pan-ERBB tyrosine kinase inhibitor (CI-1033)
		Humanized monoclonal antibody (trastuzumab)
c-KIT	Overexpressed	Tyrosine kinase inhibitor (imatinib)
SRC	Constitutively activated	Src inhibitor (dasatinib)
BRAF	Mutation	Raf kinase inhibitor (sorafenib)
RAS	Mutation	Farnesyl transferase inhibitors (tipifarnib, lonafarnib)
MEK	Constitutively activated	Inhibitors of MEK (CI-1040, PD325901)
PI3K/AKT/mTOR	Constitutively activated	PI3K inhibitor (LY294002)
		mTOR inhibitor (rapamycin) and its derivatives (CCI-779, RAD001, AP23576)
BCL2	Overexpressed	Antisense oligonucleotide (oblimersen sodium)
		Inhibitor of BCL2 (ABT-737)
p53	Mutation and deletion	*p53* adenoviral vector (Advexin)
FUS1	Loss of protein expression	*FUS1* nanoparticles (DOTAP:Chol-*FUS1*)
VEGF	Overexpressed	Humanized monoclonal antibody (bevacizumab)
		VEGFR-2 and EGFR inhibitor (ZD6474)
Telomerase	Overexpressed	Telomerase template antagonist (GRN163L)

EGFR, epidermal growth factor receptor; VEGF, vascular endothelial growth factor.

Large-scale molecular genetic studies have led to the discovery of several potential molecular targets for therapeutic design, such as vascular endothelial growth factor (VEGF) and epidermal growth factor receptor (EGFR). Various drugs targeted against these molecular changes have been developed and are being tested for clinical use in lung cancer therapy (Table 1 and Figure 1). The promise of these drugs is that they are specific for particular—often aberrant—molecules that are altered in cancer cells but not in normal cells; thus, they have a higher therapeutic ratio for cancer cells compared with normal cells. Some of these drugs, such as the monoclonal anti-VEGF antibody bevacizumab (Avastin), have shown a significant impact on patient survival.[9] In addition, the recent discovery of tyrosine kinase (TK) domain

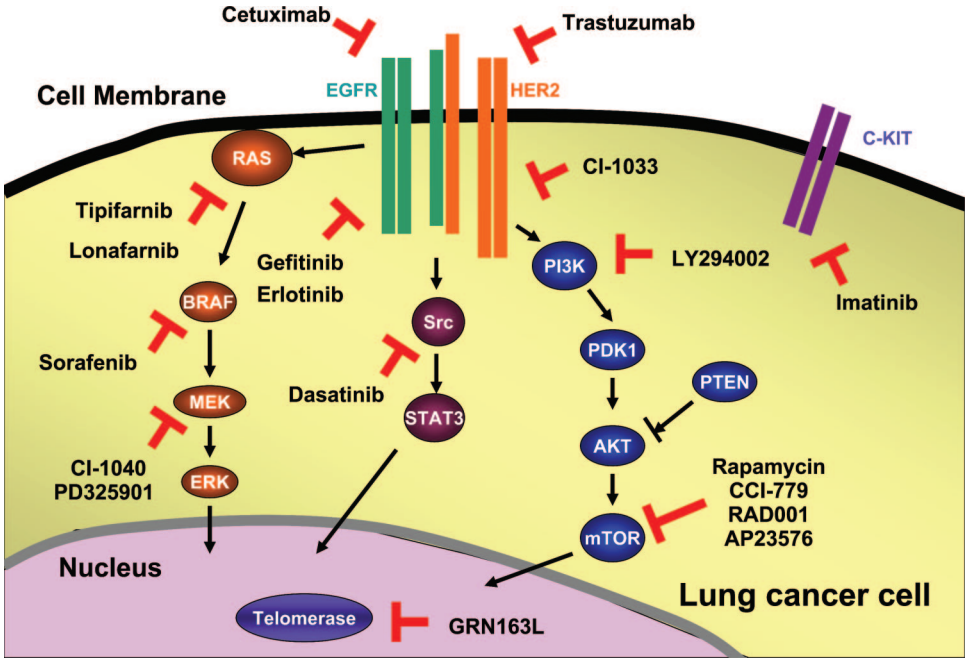

FIGURE 1. Major growth transduction pathways involved in lung cancer pathogenesis and drugs targeting altered molecules in the pathways.

mutations in the *EGFR* of non-small cell lung cancers (NSCLCs), and the finding that such tumors are particularly sensitive to EGFR TK inhibitor (TKI) therapy, indicate the possibility of molecular typing of tumors to aid in therapy selection.[10,11]

The main aims of this review are to provide a comprehensive summary of the latest discoveries in the pathogenesis of lung cancer and to discuss how some of these findings are the subject of ongoing translational efforts to bring novel and effective drugs to the clinic. We summarize important aspects of lung cancer pathogenesis that include (1) the causes of lung cancer, (2) genomic instability in lung cancer, (3) abnormalities in growth-stimulatory signaling pathways (protooncogenes), (4) abnormalities in growth-inhibitory tumor-suppressor pathways (tumor-suppressor genes [TSGs]), (5) abnormalities leading to evading apoptosis, (6) cell immortalization and the activation of telomerase, (7) sustained angiogenesis, and (8) abnormalities in immune response in lung cancer and in immunotherapy for lung cancer. Finally, we will focus on recently developed technologies and novel concepts in lung cancer research that include (9) genome-wide approaches for identifying regions of genetic changes, (10) gene expression profiling by microarray technology, (11) transgenic mouse models of lung cancer, (12) immortalized human bronchial epithelial cell (HBEC) models, and (13) the concept of lung cancer stem cells.

CAUSES OF LUNG CANCER

Tobacco Smoke and Lung Cancer

Tobacco smoke contains more than 60 carcinogens, and among these, more than 20 carcinogens are strongly associated with lung cancer development.[12] The most notorious of these compounds include the polycyclic aromatic hydrocarbons and the tobacco-specific nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone, both of which lead to genetic mutations through DNA adduct formation.[13] There are two groups of enzymes that are involved in DNA adduct formation: P450 enzymes, encoded by CYP family genes; and glutathione S-transferases (GSTs). The carcinogenes are metabolically activated by P450 enzymes and are either secreted or can bind to DNA, leading to DNA adduct formation. By contrast, GSTs detoxify the intermediates of carcinogens, thus protecting against adduct formation. In most cases, these adducts are repaired, but sometimes the damage is severe enough to cause apoptosis. Chronic exposure to these compounds often leads to mutations in critical genes such as *p53* or *RAS*, which lead to the initiation or progression of the disease. Tobacco smoke also induces oxidative DNA lesions. 8-oxoguanine is a major oxidative lesion that causes G-to-T transversion, possibly leading to mutations in critical genes involved in lung cancer pathogenesis. 8-oxoguanine is repaired by 8-oxoguanine DNA N-glycosylase 1 (OGG1) and, thus, polymorphisms in *OGG1*, with reduced enzymatic activity of OGG1 are possibly associated with increased risk for lung cancer.

Although it is generally accepted that tobacco smoke causes lung cancer, not everyone who smokes develops lung cancer. Epidemiologic studies have shown that smokers are 14 times more likely to develop lung cancer than nonsmokers, but only about 11% of heavy smokers develop lung cancer in their lifetime.[14] As a result, some have suggested that genetic factors may predispose people to lung cancer development. Many studies have examined the relationship between polymorphic variants of the genes involved in tobacco smoke metabolism and DNA repair pathways, including *P450* and *GST* family genes and *OGG1*, and the risk for lung cancer, but the results of these studies have been inconclusive.[15] Nevertheless, a case control study has shown that low activity of OGG1 correlates with an increased risk of lung cancer, suggesting that people with low OGG1 activity could be good candidates for smoking-cessation programs.[16]

Inherited Susceptibility to Lung Cancer

Epidemiologic studies show a 2.5-fold increased risk attributable to a family history of lung cancer after controlling for tobacco smoke, suggesting that genetic factors other than those related to metabolizing carcinogens from tobacco smoke may influence a person's susceptibility to lung cancer.[14] A recent large-scale linkage analysis (52 pedigrees) by the Genetic Epidemiology of Lung Cancer Consortium suggests that a major autosomal susceptibility locus for inherited lung cancer exists on 6q23-25.[17] This region contains many potential genes of interest, including *SASH1, LATS1, IGF2R, PARK2,* and *TCF21*.[17,18] If a common polymorphism is found in one of the genes in this region that predisposes these families to lung cancer, it could be used to screen the broader population to identify people with the prediposing allele. These people would be candidates for early detection and prevention programs.

GENOMIC INSTABILITY IN LUNG CANCER

Most solid tumors are genetically unstable at two distinct levels: large-scale chromosomal instability (CIN) and microsatellite instability (MSI).[19] CIN refers to losses or gains of whole or large portions of chromosomes. The underlying mechanisms of CIN have not been fully elucidated; mutations in mitotic checkpoint gene, such as *BUB1*, are clearly associated with the CIN phenotype in colon cancer, but they rarely occur in lung cancer.[20,21] Mice heterozygous for *mad2*, another mitotic checkpoint gene, develop lung cancer at a higher rate than wild-type mice, suggesting that CIN may be important to lung cancer pathogenesis.[22]

MSI is defined as a DNA sequence change of any length attributable to insertion or deletion of the microsatellite one- to four-base DNA repeating units within a tumor.[23] The most widely used method for MSI study is a polymerase chain reaction–based method, where DNA sequence changes are detected by comparing the electrophoresis patterns of polymerase chain reaction products targeting microsatellite loci between tumor and normal samples from the same individuals. Studies have reported frequencies of MSI ranging from approximately 2% to approximately 70% in both small cell lung cancers (SCLCs) and NSCLCs.[24–27] The wide variation in the studies is probably attributable to studies of different microsatellites and different methods of analysis. Sozzi et al.[28] evaluated the usefulness of detecting microsatellite alterations in plasma or serum DNA from patients with

NSCLC as a noninvasive strategy for early detection. They found that altered DNA sequences (either MSI or loss of heterozygosity) could be found in 43% of blood samples from patients with stage I disease, with no alterations found in control cases, suggesting that detecting these altered DNA sequences may be useful as a tool for diagnosis and early detection screening.

ABNORMALITIES IN GROWTH-STIMULATORY SIGNALING PATHWAYS: PROTOONCOGENES

Although there are multiple components to each of the growth signaling pathways involved in lung cancer, we will focus the discussion on those proteins that are frequently affected by genetic abnormalities in cancer. It has become clear that these mutated proteins, while driving affected cells toward transformation, also "addict" the cells to their abnormal function. This concept is referred to as "oncogene addiction" and represents a cellular physiologic state in which the continued presence of the abnormal function, although oncogenic, also becomes required for the tumor to survive.[29] This means that if the function is removed or inhibited (e.g., by a targeted drug), the tumor cells die. By contrast, bystander normal cells, which are not addicted to the mutant protein, are much less sensitive to the drug; thus, the targeted drugs have great tumor cell specificity. The most important example of this concept for lung cancer is *EGFR* TK mutation. Tumors with mutations in *EGFR* are dependent on survival signals transduced by mutant *EGFR* and, thus, are particularly sensitive to TKIs.[30] These findings have led to massive genome-wide sequencing efforts targeting thousands of genes to find additional mutated oncogene targets for rational therapeutics design. Whether this approach will be similarly useful for targeting genes frequently overexpressed but not mutated in lung cancers, such as *MYC*, remains to be determined.

Receptor TKs

The EGFR Family

The *EGFR* family of receptors are transmembrane TK receptors and are composed of *EGFR* (*HER1* or *ERBB1*), *HER2* (*EGFR2* or *ERBB2/NEU*), *HER3* (*EGFR3* or *ERBB3*), and *HER4* (*EGFR4* or *ERBB4*).[31] Although these four *EGFR* family receptors are homologous in the TK domains (59%–81% identity), each has unique properties: *HER2* lacks a functional ligand-binding domain, and *HER3* lacks kinase activity.[31] On ligand binding, these EGFR family members form active homo- and heterodimers, leading to autophosphorylation and activation of intracellular signaling cascades. EGFR and HER2 are overexpressed in approximately 70% and 30% of NSCLCs, respectively, but they are rarely expressed in SCLCs.[7,32] There are several currently available drugs targeting EGFR or HER2, including the small-molecule TKIs gefitinib (Iressa, targeting EGFR) and erlotinib (Tarceva, targeting EGFR), and the monoclonal antibodies cetuximab (Erbitux, targeting EGFR) and trastuzumab (Herceptin, targeting HER2).

Recently, several mutations in the TK domain of *EGFR* have been described.[10,11] A review of nine published studies suggests that *EGFR* mutations are common (24%; 477/2000) in NSCLC.[33] These mutations are limited to the first four exons of the TK domain and are categorized into three different types (deletions, insertions, and missense point mutations). In-frame deletions in exon 19 (44% of all mutations) and missense mutations in exon 21 (41% of all mutations) are the most frequent, together accounting for more than 80% of all mutations.[33] Importantly, the presence of mutations in the TK domain correlates with tumor drug sensitivity to TKIs.[10,11] An intriguing characteristic of *EGFR* mutations is that they tend to occur in a highly selected subpopulation: adenocarcinoma histology, never-smoker, East Asian, and female sex.[34] Notably, all of these clinicopathological factors are associated with response to TKIs.[35–38]

Although several studies have confirmed the close relationship between the presence of mutant *EGFR* and the response to TKIs, it is becoming evident that a subset of NSCLC patients with mutant *EGFRs* do not respond to TKIs.[10,11,39] Shortly after the discovery of the *EGFR* mutations, a second TK domain mutation (T790M) was reported in the same tumors that have the *EGFR* TK domain mutations.[40,41] This mutation was found in four out of seven patients who relapsed after TKI treatment, suggesting its contribution to acquired resistance to TKIs. Nevertheless, it should be noted that several examples of the T790M mutations have occurred in lung tumors not treated with EGFR TKIs, and often the mutation has only been in a small subset of the tumor cells.[42] This contrasts with the other *EGFR* TK domain mutations that are in all tumor cells. In addition, a germline *EGFR* T790M mutation has been reported to be associated with familial NSCLC, suggesting that this mutation could predispose people to lung cancer.[43] There are EGFR-targeted TKIs that inhibit EGFR with the T790M mutation, and several derivatives are being tested clinically.[44]

Also, some patients without *EGFR* mutations respond to TKIs. Insufficient sensitivity for detecting mutant *EGFR* might be one possible explanation for this. Nevertheless, several predictive markers other than *EGFR* mutation have been reported to correlate with TKI response, including *EGFR* amplification, elevated EGFR protein, *HER2* amplification, and activation of AKT protein kinase B (Vtakt murine thymoma viral oncogene homolog 1).[38,45–48] In addition, the presence of *KRAS* mutation is shown to be a negative predictor for TKI response.[49] These studies suggest that other biological features besides *EGFR* mutation status determine TKI response. Clinicopathological and biological factors reported to be associated with TKI response are summarized in Table 2. Among biological predictors, *EGFR* mutation and amplification by fluorescence in situ hybridization are invariably correlated with TKI response, whereas the results of EGFR protein expression are conflicting.[10,11,39,45,50] Also, whether *EGFR* mutation predicts patient survival remains unclear.[38,51–53] Because of these conflicting data, there is still no standard method for selecting patients with NSCLC for TKI therapy. Nevertheless, in practice, patients whose tumors have *EGFR* mutations or who are never-smokers, particularly those with adenocarcinoma histology, female sex, and East Asian ethnicity, often receive TKI therapy. To address this issue, prospective clinical trials designed to incorporate the

TABLE 2. Clinicopathological and Biological Factors Reported to be Predictive for Response to Tyrosine Kinase Inhibitors (TKIs)

Factor	Association with TKI response	Reference
Clinicopathological factor		
Nonsmoking	p (+)	Shepherd et al.,[37] Tsao et al.[38]
Female sex	p (+)	Fukuoka et al.,[35] Kris et al.,[36] Shepherd et al.,[37] Tsao et al.[38]
Asian ethnicity	p (+)	Shepherd et al.,[37] Tsao et al.[38]
Adenocarcinoma	p (+)	Shepherd et al.,[37] Tsao et al.[38]
Biological factor		
EGFR mutation	p (+)	Lynch et al.,[10] Paez et al.,[11] Pao et al.,[39] Cappuzzo et al.[45]
EGFR amplification	p (+)	Tsao et al.,[38] Cappuzzo et al.[45]
EGFR protein	p (+)	Tsao et al.,[38] Cappuzzo et al.[45]
EGFR T790M	N (+)	Kobayashi et al.,[40] Pao et al.[41]
HER2 amplification	P (+)	Cappuzzo et al.[48]
KRAS mutation	N (+)	Pao et al.[49]
p-AKT	P (+)	Cappuzzo et al.[46]

EGFR, epidermal growth factor receptor. P (+), positively associated with response to TKIs; N (+), negatively associated with response to TKIs.

patient's clinicopathological data and the molecular biological features of the tumors, such as *EGFR* mutation and amplification, are currently underway.

TKIs have been tested extensively, both alone and in combination with cytotoxic chemotherapy. To evaluate the efficacy of erlotinib and gefitinib as monotherapy, two well-controlled phase III studies were conducted for these drugs. The results of these studies show that erlotinib prolonged survival of previously treated NSCLC patients by 2 months (BR.21 trial), whereas gefitinib failed to show survival benefit (Iressa Survival Evaluation in Lung Cancer trial).[37,54] Because docetaxel is the only cytotoxic drug that prolonged survival for previously treated NSCLC patients, the result of BR.21 is encouraging.[55] Despite positive preclinical studies of the combination of TKI and chemotherapy, several phase III studies have failed to show a survival benefit of adding erlotinib or gefitinib to conventional chemotherapy.[56,57] Clearly, we need to improve strategies that integrate TKIs with chemotherapy and to explore combinations with other molecularly targeted drugs.

The most common adverse effect of both TKIs (gefitinib and erlotinib) and cetuximab is cutaneous rash, which generally occurs in a dose-dependent manner and is usually grade 1/2 rash, with no report of grade 4 (life threatening) rash.[58] Studies have suggested a correlation between the development of rash and response/survival, suggesting the potential use of rash as a surrogate marker for response and a prognosis marker.[58] Diarrhea is the most common nondermatologic adverse event associated with TKIs, but it rarely occurs in patients treated with cetuximab. TKI-induced diarrhea can be controlled by loperamid treatment in most cases, but it is occasionally (1%) life threatening. Interstitial lung disease (ILD) is a rare but serious adverse effect of gefitinib. Gefitinib-induced ILD occurs more frequently in East Asian countries (3.5%–6%) than the United States and Europe (1.0%–1.1%), suggesting the existence of ethnicity-related susceptibility to the development of gefitinib-induced ILD.[59] Studies have reported that approximately one third of patients who developed gefitinib-induced ILD died.[60] Male sex, a history of smoking, and coincidence of interstitial pneumonia are reported to be predictors for development of gefitinib-induced ILD.[61]

HER2 mutations occur in 2% (16/791, pooled from two studies) of NSCLCs.[62,63] All reported *HER2* mutations have been in-frame insertions in exon 20 and have targeted the corresponding TK domain region, as in *EGFR*-insertion mutations. Interestingly, these mutations have frequently occurred in the same subpopulation as those with *EGFR* mutations (adenocarcinoma, never-smoker, East Asian, and female sex).[34] It should be noted that this *HER2* mutation analysis was performed using an unselected sample set; thus, it represents the penetrance of this mutation in the general population of lung cancer patients and is not a result of selection bias. So far, no small-molecule inhibitors have been reported to show similar potency against *HER2* mutations as seen with EGFR TKIs. Future studies will examine whether mutant *HER2* lung cancers respond to trastuzumab.

HER4 mutations were found in five (two squamous cell carcinomas, two adenocarcinomas, and one large-cell carcinoma) of 217 (2.3%) NSCLC tumor samples from Asian patients.[64] By contrast with *EGFR* and *HER2* lung cancer mutations, four patients with *HER4* mutations were male and smokers.[64]

Using laser-capture microdissection Tang et al.[65] have demonstrated that *EGFR* mutations occur in histologically normal bronchial epithelial cells adjacent to tumors with *EGFR* mutations. This finding suggests that *EGFR* mutational status could be useful as an early detection marker and chemoprevention target. Two independent groups have developed transgenic mice harboring either the point or the deletion mutation of *EGFR*.[66,67] Both groups have demonstrated that the mice developed lung adenocarcinomas with very similar histology to those seen in patients with *EGFR* mutations. Moreover, the persistence of the adenocarcinoma

absolutely requires continued mutant EGFR function. These results suggest that mutant *EGFR* is required for both initiation and maintenance of the tumors. Finally, lung cancers with *EGFR* mutations are more sensitive to ionizing radiation than those without *EGFR* mutations; this might provide a molecular basis for combined-modality treatment involving TKIs and radiotherapy.[68]

c-Kit

SCLC, but not NSCLC, frequently (40%–70%) express both the receptor c-KIT and its ligand, stem cell factor.[69] High-level coexpression of this receptor and its ligand suggests that an autocrine or a paracrine loop may promote the growth of SCLC cells. Nevertheless, unlike gastrointestinal stromal tumors, activating *c-KIT* mutations in lung cancer are very rare.[70,71] Imatinib (Gleevec), an inhibitor of c-KIT kinase, inhibited cell growth in some c-KIT–expressing SCLC cell lines by inducing cell cycle arrest and/or apoptosis.[72] Nevertheless, two phase II clinical studies and a mouse xenograft study failed to show tumor regression in SCLC by monotherapy with imatinib.[73–75] Thus, if imatinib is used in lung cancer, it would need to be combined with other agents.

RAS/RAF/MEK/ERK Pathway

The *RAS* family of protooncogenes (*HRAS*, *KRAS*, and *NRAS*) encode 21-kDa plasma membrane–associated G-proteins that regulate key signal-transduction pathways involved in normal cellular differentiation, proliferation, and survival.[76] Activating oncogenic mutations in the *RAS* genes are common in several human cancers, including lung cancer.[76] *RAS* mutations are found in 10% to 15% of NSCLCs, especially in adenocarcinoma (20%–30%), but almost never in SCLCs.[7] The mutations occur at several hot spots in the genes affecting codons 12, 13, and 61, all of which influence intrinsic GTPase activity.[76] Although it has yet to be established what the distinctive functions of HRAS, KRAS, and NRAS are, approximately 90% of *RAS* mutations in lung cancer are *KRAS* mutations. Multiple studies have shown that oncogenic *KRAS* (e.g., $KRAS^{V12}$ mutant) activates cell-signaling pathways important to cellular transformation.[77] As a result, KRAS abnormalities represent an important therapeutic target. A number of drugs that target different aspects of RAS function and metabolism have been developed and are currently under investigation in clinical trials.[76] Farnesyl transferase inhibitors are the best-studied drugs, and two orally bioavailable farnesyl transferase inhibitors (tipifarnib and lonafarnib) are being tested in the combination with cytotoxic drugs in phase III clinical trials in lung cancer.[78]

BRAF protein serine/threonine kinase is a downstream effecter of the RAS pathway. Mutations of *BRAF* occur frequently in melanoma (70%) but only rarely in lung cancers (3% of NSCLCs, 12/437; pooled from three studies).[79–81] Nevertheless, for those lung cancers, mutated BRAF protein is an important and specific therapeutic target. It should be noted that the types of *BRAF* mutations found in melanoma and lung cancers are different. Thus, there may be differences in the responses to BRAF targeted drugs between these two types of cancers. An orally administered Raf kinase inhibitor, sorafenib, is currently being tested in phase I and phase II trials in a variety of cancers, including melanoma and lung cancer.[82,83]

Activated BRAF phosphorylates and activates MEK1 and MEK2, which, in turn, phosphorylate and activate ERK1 and ERK2. Substrates of ERK1/2 include several proteins involved in mitogenic signal transduction, including ELK1 and c-JUN.[76] ERK1/ERK2 are constitutively activated in a subset of lung cancer cell lines, and thus MEK and ERK are therapeutic targets for lung cancer treatment.[84] An oral MEK inhibitor, CI-1040, and its derivative, PD03255901, are being tested in clinical trials for lung cancer.

Phosphatidylinositol 3-Kinase/AKT/PTEN Pathway

Phosphatidylinositol 3-kinases (PI3Ks) are lipid kinases that regulate several cellular processes such as proliferation, growth, apoptosis, and cytoskeletal rearrangement.[85] These proteins are constitutively activated at a high frequency in human cancers.[85] *PIK3CA*, which encodes the 110-kDa alpha catalytic subunit of PI3K, is mutated in several human cancers, including 3% of NSCLCs (8/259, pooled from two studies).[86,87] Mutation in *PIK3CA* results in elevated lipid kinase levels and is a therapeutic target in tumor cells with such mutations.[87] AKT is a downstream effecter of PI3Ks, and its activation has oncogenic effects.[85] Constitutive activation of AKT was reported in 16 of 17 NSCLC cell lines.[88] A PI3K inhibitor, LY294002, which reduces AKT phosphorylation, enhances the sensitivity of NSCLCs to chemotherapeutic agents and radiation therapy. Thus, PI3K inhibitors may be useful as cytotoxic and/or chemosensitizing agents for NSCLCs.[88] By contrast, the TSG phosphatase and tensin homolog deleted on chromosome 10 (*PTEN*) is a negative regulator of AKT. Whereas *PTEN* is infrequently mutated in SCLC and NSCLC,[89] reduced or lost PTEN protein expression is common in lung cancers.[90] Loss of PTEN activity provides another way of activating the AKT pathway in lung cancer, and lung tumors with loss of PTEN activity are candidates for therapy targeting this pathway.

Mammalian target of rapamycin (mTOR) is a key downstream target of AKT kinase activity and is a central regulator of cell growth.[85] mTOR is another potential therapeutic target in the PI3K pathway. mTOR inhibitors, such as the macrolide antibiotic rapamycin (sirolimus) and its derivatives (CCI-779, RAD001, and AP23576), have antitumor activity in lung cancer, and these drugs are now being evaluated in clinical trials for lung cancer.[91]

Signal Transducers and Activators of Transcription Family

The signal transducers and activators of transcription (*STAT*) family consists of seven different members: *STAT1, 2, 3, 4, 5A, 5B*, and *6*.[92] The STATs are cytoplasmic proteins that are activated by tyrosine phosphorylation, resulting in dimer formation and translocation to the nucleus to regulate the expression of target genes. Constitutive activation of STAT3 and STAT5 contributes to oncogenesis in a wide range of malignancies, including lymphoma and breast and lung cancers, by stimulating cell proliferation and inhibiting apoptosis through up-regulation of genes involved in these

pathways, such as *BCL-X_L* , *Cyclin D1*, and *MYC*.[92–94] Constitutive DNA-binding activity of STAT3 were shown in six of seven NSCLC cell lines, and introduction of antisense oligonucleotides against *STAT3* or an adenoviral vector expressing a dominant-negative *STAT3* resulted in apoptosis in NSCLC cell lines with constitutively activated STAT3.[94] In addition, it has been shown that STAT3 activation is required for cell survival and growth of NSCLC cell lines with mutant *EGFRs*.[93] Thus, activated STAT3 is another therapeutic target, perhaps in combination with EGFR targeted therapy.

MYC Family

The *MYC* gene family encodes three nuclear phosphoproteins (MYC, MYCN, and MYCL), which heterodimerize with MAX proteins and function as transcription factors for genes in a variety of cellular processes, including cell growth, cell proliferation, and apoptosis.[95] Amplification of one member of the *MYC* family occurs in 18% to 31% of SCLCs and in 8% to 20% of NSCLCs.[96] *MYC* amplification occurs in both SCLC and NSCLC, whereas *MYCN* and *MYCL* amplifications nearly always occur in SCLC.

ABNORMALITIES IN GROWTH-INHIBITORY TUMOR-SUPPRESSOR PATHWAYS: TSGS

The p53 Pathway

The TSG *p53*, located on chromosome 17p13.1, encodes a protein that functions as a transcription factor. p53 protein is stabilized in response to multiple stimuli including oncogenes, hypoxia, and DNA damage. p53 activity leads to the expression of downstream genes involved in a cell cycle arrest to permit repair or initiate apoptosis.[97] *p53* is the most frequently mutated gene in human cancers; *p53* is inactivated by mutation in approximately 90% of SCLCs and in approximately 50% of NSCLCs, respectively.[98,99] Most inactivating mutations are point mutations in the DNA binding domain (missense mutation, 70%–80%), but homozygous deletions also occur. Some point mutations (missense) in *p53* confer a gain-of-function phenotype that contributes to increased aggressiveness in several types of cancer, including lung cancer.[100,101] *p53* mutations in lung cancer correlate with cigarette smoking and, more specifically, G–T transversions, which are the classic type of mutation caused by tobacco smoke carcinogens.[98]

When wild-type p53 is reexpressed in lung cancer cells with mutant or deleted *p53*, the tumor cells undergo apoptosis.[102] These findings have led to clinical trials investigating *p53* gene–replacement therapy. In fact, clinical trials of *p53* gene replacement using a retrovirus *p53*-expression vector in patients with NSCLCs have shown evidence of antitumor activity and the feasibility and safety of gene therapy.[103] INGN 201 (Ad5CMV-*p53*, Advexin), a replication-impaired *p53* adenoviral vector, has been evaluated in clinical trials; it is both safe and effective for the treatment of several different types of cancer, including lung cancer.[104] Nevertheless, a clear clinical benefit of these *p53* gene therapies in randomized controlled studies has yet to be demonstrated. As with any type of gene therapy (virus based), one needs to consider the potential risk of treating patients with live virus, because many aspects of biology of the wild-type adenovirus remain to be explored.[105]

There are two important upstream regulators in the p53 pathway: MDM2 and $p14^{ARF}$. MDM2 is an E3 ubiquitin ligase that functions as an oncogene by reducing p53 levels through enhancing proteasome-dependent degradation. Amplifications of *MDM2* have been reported in approximately 6% of NSCLCs, resulting in loss of p53 function.[106] The TSG *$p14^{ARF}$* is an alternative reading frame variant of the *$p16^{INK4a}$* locus (9p21) and encodes a protein that enhances p53 activity by binding to MDM2, leading to the stabilization of p53. Immunohistochemical analyses of $p14^{ARF}$ on lung cancers have shown that $p14^{ARF}$ protein expression is lost in approximately 65% of SCLCs and approximately 40% of NSCLCs.[107,108] Thus, loss of $p14^{ARF}$ may contribute to loss of p53 expression and function.

The $p16^{INK4a}$–Cyclind1-CDK4-RB Pathway

The $p16^{INK4a}$–CyclinD1-CDK4-RB pathway regulates the cell cycle at the G1/S transition. The *RB* gene was initially identified as a TSG in retinoblastoma and was the first TSG to be cloned.[109] Subsequently, it was found that alteration of one of the four components in this pathway occurs in nearly all human cancers.[110] Hypophosphorylated RB exerts its tumor-suppressor activity by binding to E2F transcription factor, which is essential for G1/S transition. Once RB is hyperphosphorylated by the CyclinD1/CDK4 complex, it releases E2F, resulting in transition from G1 to S. Thus, absent or mutated RB leads to loss of the G1/S checkpoint. Absent or mutant RB protein is found in approximately 90% of SCLCs and in approximately 15% to 30% of NSCLCs.[111,112]

Another regulator of RB function, $p16^{INK4a}$, keeps RB in the unphosphorylated state (and growth-suppressing mode) by preventing CDK4 from phosphorylating RB. Thus, loss of $p16^{INK4a}$ function results in loss of function of the RB pathway. In contrast to RB, *$p16^{INK4a}$* is very frequently inactivated in NSCLCs (70%) but is rarely altered in SCLCs. Inactivation of *$p16^{INK4a}$* is caused by homozygous deletion, coding region mutations, and promoter hypermethylation. Finally, overexpression of either CDK4 or Cyclin D1 inhibits RB pathway function by blocking the growth-suppressing activity of $p16^{INK4a}$.[110] *CDK4* is amplified in a subset of NSCLCs.[113,114] Cyclin D1 is overexpressed in more than 40% of NSCLCs, as assessed by immunohistochemistry.[115] Overexpression of Cyclin D1 in normal-appearing bronchial epithelium of patients with NSCLCs has been found to be associated with smoking and correlates with shorter survival, suggesting the possible utility of Cyclin D1 as a molecular marker to identify high-risk individuals.[116]

Transforming Growth Factor Beta Signaling

Transforming growth factor beta (TGF-β) is a multifunctional cytokine that regulates several cellular process, including proliferation, cell survival, and immunosurveillance.[117] The mechanism for TGF-β signaling has been elucidated. TGF-β ligands bind to type II TGF-β receptor (TβRII) directly or through type III TGF-β receptor (TβRIII), leading TβRII to form heterodimers with type I

TGF-β receptors (TβRI). Then, TβRII activates TβRI by phosphorylation. Activated TβRI phosphorylates two downstream cytoplasmic transducers, SMAD2 and SMAD3. Phosphorylated SMAD2 or SMAD3 associates with SMAD4, and they translocate into the nucleus, leading to the activation of target genes.

TGF-β plays a paradoxical role in lung cancer. TGF-β inhibits cell proliferation in both SCLC and NSCLC cells and induces apoptosis. Nevertheless, at the later stage of lung cancer tumorigenesis, TGF-β induces angiogenesis, and at this stage, the growth-inhibitory effect of TGF-β is lost by several different mechanisms. In SCLCs, this can be accounted for by the frequent decrease of TβRII in SCLC cells.[118] In NSCLCs, decreased expression of TβRII is not frequent, but mutations in SMAD2 and SMAD4 occur, leading to inhibition of TGF-β signaling.[119,120]

3p TSGs

3p Loss as an Early Event in Lung Cancer Pathogenesis

Allele loss involving chromosome arm 3p is one of the most frequent (100% in SCLC and >90% in NSCLC) and earliest genetic alterations found in lung cancer and may affect more than one TSG. Three discrete regions of 3p loss have been identified by allelotyping in lung cancers, including a 600-kb segment at the 3p21.3, 3p14.2 (*FHIT/FRAB3*), and 3p12 (*ROBO1/DUTT1*) regions. Wistuba et al.[8] performed high-resolution chromosome allelotyping using a panel of 28 3p markers and showed that 3p losses were found in 96% of lung cancers and 78% of preneoplastic/preinvasive lesions, with the size and the frequency of 3p allele loss progressively increasing as severity of histopathological preneoplastic/preinvasive changes increased. Thus, these 3p genetic changes occur early in lung cancer pathogenesis. There are several genes in the 3p21.3 region closely associated with one another that have tumor-suppressor activity.[121] These rarely mutate, but they often lose their expression by epigenetic mechanisms. The four that have been best studied are *RASSF1A, FUS1, SEMA3B*, and *SEMA3F* while two others are *NPRL2* and *101F6.*

RASSF1A

RASSF1A rarely mutates in lung cancer, but its expression is lost by tumor-acquired promoter methylation in approximately 90% of SCLCs and approximately 50% of NSCLCs.[122,123] RASSF1A has the ability to suppress the growth of lung cancer cell lines in tissue culture and in immunodeprived mice.[122,123] Functional analysis of RASSF1A has shown that it is involved in multiple pathways critical to cancer pathogenesis, including cell cycle, apoptosis, and microtubule stability.[124]

FUS1

FUS1 is located directly adjacent to RASSF1A [125] *FUS1* also rarely mutates in lung cancers, and its mRNA is usually expressed.[126] Nevertheless, FUS1 protein expression is frequently lost in lung cancer for unknown mechanisms but detected in normal lung tissues. Wild-type FUS1, but not tumor-acquired mutant FUS1, induces G1 growth arrest and apoptosis.[126,127] Administration of the *FUS1* gene with a nonviral vector, DOTAP: cholesterol, inhibits cancer cell growth in vitro and in vivo, providing the rationale for using *FUS1* gene therapy for local control and for systemic treatment of lung tumors in clinical trials now underway.[128]

SEMA3B and SEMA3F

SEMA3B and *SEMA3F* are also located at 3p21.3 (very near *RASSF1A* and *FUS1*). They are secreted, soluble members of the semaphorin family, important in axonal guidance.[129,130] Wild-type SEMA3B, but not tumor-acquired single–amino acid missense mutants of SEMA3B, induces apoptosis when reexpressed in lung cancers or added as soluble molecules.[131,132] One mechanism of such tumor inhibition is through its ability to block VEGF autocrine activity.[131] The growth-inhibitory effects of SEMA3F are also observed in rat xenografts of NSCLC.[133] Because both SEMA3B and SEMA3F are soluble, secreted proteins, they are promising candidates as drugs for systemic treatment.

Other 3p genes that are located at regions other than 3p21.3 and that have good evidence for tumor-suppressor activity include *FHIT* and *RARβ.*

FHIT

FHIT is located in 3p14.2, one of the most common fragile sites of the human genome. *FHIT* is homozygously deleted in lung cancer, and its aberrant transcripts are frequently found in lung cancers.[134] Also, expression of FHIT protein is lost in approximately 50% of lung cancers.[135] Several studies have shown the ability of reexpressed FHIT to induce apoptosis in lung cancer.[136]

RARβ

The *RARβ* gene is located in the 3p24 region and functions as a receptor for retinoic acid. Although *RARβ* is not mutated in lung cancer, it is methylated in 72% of SCLCs and in 41% of NSCLCs, leading to loss of its expression.[137] Reintroduction of *RARβ*2 into epidermoid lung cancer cell lines suppresses their growth in the culture and in nude mice.[138] For RARβ-expressing lung cancers, retinoic acid ligands have long been considered for therapy, but no results of consistent therapeutic efficacy have been reported for overt lung cancers.

In summary, 3p allele losses occur almost universally in lung cancer and also in preneoplastic/preinvasive lesions involving at least three very small, defined regions (3p21, 3p12, and 3p14.2), and some of the genes located in these regions have tumor-suppressor activity. These observations have led to the conclusion that 3p allele loss contributes to lung cancer initiation and development through inactivating multiple TSGs. Because of the early changes in the 3p chromosome regions (occurring in histologically normal lung epithelium), the presence of 3p allele loss and inactivation of expression of these 3p TSGs may be of use in determining smoking-related field effects. The successful results of systemic administration of *FUS1* in immunocompromised mice have led to the development of a clinical trial and treatment of patients with systemically administered (via lipid-based nanoparticles) *FUS1* gene therapy.

Epigenetic Changes in Lung Cancer: DNA Promoter Methylation Changes as a Mechanism for Inactivating TSGs

CpG dinucleotides are clustered in the promoter regions of approximately 50% of all protein coding genes in the DNA elements that are called CpG islands. Hypermethylation of cytosine in CpG islands in the promoter regions of TSGs leads to loss of expression of the associated genes, contributing to the initiation and progression of human cancer.[139] Promoter hypermethylation has been observed in nearly all human cancers.[139] More than 80 genes have been reported to be hypermethylated in lung cancer, including *RARB, TIMP3, $p16^{INK4a}$, RASSF1A, MGMT, FHIT, DAPK, ECAD*, and *GSTP1*.[140] Several studies have analyzed the methylation status of multiple genes in lung cancer and have shown that most lung cancers have multiple aberrantly methylated genes.[141]

Detecting methylated DNA sequences in biological fluids (sputum, blood) is potentially a powerful tool for early detection of lung cancer. Some recent studies have shown that hypermathylation of $p16^{INK4a}$ is detectable in sputum or exfoliated lung cells before lung cancer diagnosis.[142] Belinsky et al.[5] conducted a seminested case-controlled study to evaluate the ability of examining a panel of genes in sputum to identify people at high risk of lung cancer. They have demonstrated that detection of methylation of three or more genes out of the six selected genes correlated with a 6.5-fold increased risk of developing lung cancer, with a sensitivity and specificity of 64%. This result clearly warrants further prospective studies, perhaps including a larger set of genes. Recently, by using a genome-wide approach, we have identified 132 genes, many of which were shown to be methylated in lung cancers compared with normal lungs with high specificity, thus providing a large new panel of genes to use for early detection.[143] Interestingly, most of these genes have not been previously studied in lung cancer.

In contrast to gene mutation, promoter hypermethylation is a reversible process, making it a very attractive target for cancer therapy. In fact, an inhibitor of DNA methylation, azacitidine (Vidaza), prolongs survival in patients with myelodysplastic syndrome[144], but its efficacy for lung cancer treatment is unknown. Histone deacetylation is another epigenetic change that inhibits gene expression. Drugs that reverse gene silencing by inhibiting histone deacetylation, such as suberoylanilide hydroxamic acid and depsipeptide, are either in or are being considered for lung cancer clinical trials.

ABNORMALITIES LEADING TO EVASION OF APOPTOSIS

In addition to uncontrolled growth, evading apoptosis is another important property of cancer cells.[152] The BCL2 protein inhibits apoptosis and is overexpressed in both SCLC (75%–95%) and NSCLC (10%–35%).[7] Antisense oligonucleotides targeted to *BCL2*, oblimersen sodium (Genasense), enhance the efficacy of standard chemotherapy in several animal models of cancer.[145] Randomized Phase II trials of oblimersen in combination with cytotoxic chemotherapy for SCLC and NSCLC are currently being conducted.[146] Recently, ABT-737, a potent inhibitor of BCL-2, BCL-X_L, and BCL-w, has shown efficacy in xenograft models of SCLC and enhances the activity of paclitaxel against A549 NSCLC cells, providing a rationale for its use in clinical trials for lung cancer as monotherapy or in combination with cytotoxic drugs.[147] BAX is a BCL-2–related protein that promotes apoptosis and is a downstream transcription target of p53.[148] An inverse relationship between BAX and BCL-2 expression is seen in SCLC but not in carcinoids, suggesting that the aggressiveness of neuroendocrine lung tumors may be correlated with apoptosis-related factors.[149]

CELL IMMORTALIZATION AND THE ACTIVATION OF TELOMERASE

Telomeres are repetitive sequences, composed of TTAGGG and localized at the end of mammalian chromosome, that protect chromosomes from degradation and loss of essential genes.[150] Telomeres shorten after each round of cell division, limiting the life span of the cells.[151] The enzyme telomerase maintains telomeric repeats by elongating telomeric DNA by reverse transcription, and its activity is largely determined by the expression levels of hTERT, which is the protein catalytic subunit of telomerase.[150] Up-regulation of telomerase is almost universal in human cancers and is thought to contribute to the early immortalization steps of tumorigenesis, which is one of the hallmarks of human malignancies.[152] Approximately 80% of NSCLCs and nearly 100% of SCLCs have detectable levels of telomerase.[153] High telomerase activity in primary NSCLCs is detected frequently in tumors with high tumor cell–proliferation rates and advanced pathological stage, implying that expression of telomerase may also contribute to the later stages of lung cancer progression.[153] The novel telomerase template antagonist GRN163L, which targets RNA template region of hTR, inhibits anchorage-independent growth and in vivo xenograft tumor growth of lung cancer cells,[154] providing the rationale for its use in clinical trials of lung cancer treatment. This drug may be effective against lung cancer stem cells (discussed later).

SUSTAINED ANGIOGENESIS

One of the hallmarks of cancer is the ability to stimulate angiogenesis; tumor growth beyond the size of 2 mm^3 requires this activity.[152,155] Several studies have demonstrated that elevated angiogenesis in lung cancer measured by microvessel density significantly correlates with the incidence of metastasis and poor survival.[156]

VEGF

VEGF plays the most critical role in angiogenesis, and lung cancers frequently produce high levels of VEGF.[157] Bevacizumab, a monoclonal antibody that neutralizes all VEGF isoforms, has been tested clinically. Recently, the addition of bevacizumab to chemotherapy regimens with paclitaxel and carboplatin in patients with advanced nonsquamous NSCLC provided a significant survival advantage.[9] The results of this study were dramatic and provided a major

impetus to continue work on VEGF antagonists in lung cancer. An occasional but serious life-threatening side effect (pulmonary hemorrhage) of bevacizumab treatment has been observed, showing the importance of patient selection and monitoring for this therapy.[9] ZD6474 is a dual kinase inhibitor that targets both VEGF receptor and EGFR. The combination of ZD6474 and docetaxel as a second-line therapy in a phase II clinical trial improved progression-free survival in patients with advanced disease.[158] The results from this trial provide further confirmation of the importance of this target for lung cancer.

ABNORMALITIES IN IMMUNE RESPONSE IN LUNG CANCER AND IMMUNOTHERAPY FOR LUNG CANCER

Molecular Abnormalities in Immune Response Found in Lung Cancer

Evidence suggests that immune responses against solid tumors exist. For example, spontaneous tumor regression suggests that tumors were rejected by immunologic host response. Nevertheless, cancers usually escape host immune responses, using several different mechanisms. For example, the major histocompatibility complex class I molecule, which mediates presentation of endogenous antigen peptides to cytotoxic T-lymphocytes, can be down-regulated in lung cancer.[159] Another mechanism used by lung tumors to escape immune surveillance is the expression of Fas ligand.[160] Soluble Fas ligand causes activated T cells, but not lung cancer cells, to undergo apoptosis.

Immunotherapy for Lung Cancer

Therapeutic interventions that kill cancer cells by inducing immune responses against cancer cells are attractive because of the potential of the immune response to be both tumor specific and tumor systemic in nature. Two active areas of immunotherapy include active vaccination and adoptive T-cell transfer (ACT) therapies.

Active vaccination therapy for lung cancer has been a challenge because of the poor antigenic characterization of lung tumors and their ability to escape the immune response. Recently, Nemunaitis et al.[161] have shown encouraging clinical results in patients with NSCLC immunized with autologous tumor cell vaccines expressing granulocyte macrophage colony–stimulating factor. Granulocyte macrophage colony–stimulating factor was introduced to enhance tumor antigen recognition. Nemunaitis et al.[161] conducted a phase I/II multicenter trial in patients with NSCLC and demonstrated proof of principle for this treatment modality, with three durable complete response cases out of 33 advanced stage patients. These results warrant further investigation.

ACT involves obtaining tumor-reactive T cells (tumor-infiltrating cells or circulating blood lymphocytes), expanding the cells in vitro, and then reinfusing them into the patient.[162] ACT has been shown to have clinical benefits in patients with malignant melanoma.[163] Nevertheless, because of several difficulties in obtaining enough tumor-reactive T cells from patients with lung cancer, the effectiveness of ACT for lung cancer has yet to be demonstrated.

NEW TECHNOLOGIES AND NOVEL CONCEPTS IN LUNG CANCER RESEARCH

Genome-wide Approaches for Identifying Regions of Genetic Changes

A variety of genome-wide approaches with increasingly higher resolution have been used to identify genomic areas of amplification, deletion, and loss of heterozygosity in human lung cancer. These include karyotypic studies, chromosome-based comparative genomic hybridization (CGH), array CGH (array-based version of CGH), genome-wide microsatellite analysis, and single-nucleotide polymorphism array analysis.[114,164–168] The goal of these studies is to systemically identify genes that have been genetically altered during lung cancer pathogenesis, including classic oncogenes and TSGs, and those involved in other oncogenic capabilities of lung cancer described above.

Karyotypic analysis provided the first information on genetic changes in lung cancer (Table 3).[167] Subsequent studies using chromosome-based CGH have better defined chromosomal gains and losses and have revealed nonrandom gains and losses of particular portions of chromosomes that were not identified by conventional karyotypic analysis (Table 3).[164] Modern genetic analysis techniques such as array CGH and single-nucleotide polymorphism array have helped identify even smaller regions of copy number alterations.[114,165,168] Besides the well-known regions, such as amplifications of *MYC* family genes, several novel recurrently altered loci, including homozygous deletions of 9p23 and 3q25 in SCLC and NSCLC, and amplifications of 8q12-13 in SCLC, 8p12, 12p11, 20q11, and 22q11in NSCLC, have been found.[114,168] Many groups have focused on identifying specific genes with abnormalities in these regions (Table 3). Yet, despite these efforts, a number of well-defined loci still require the genes involved in malignancy to be identified. These are summarized in Table 3.

Gene Expression Profiling by Microarray Technology

Oligonucleotide and cDNA microarrays are now widely available and are powerful tools for analyzing global gene expression changes in lung cancers compared with normal tissues. Possible applications of this technology in cancer research include (1) to classify subtypes of cancer, (2) to predict prognosis, (3) to predict the response of cancers to therapeutic interventions, (4) to find markers for early detection of cancer, and (5) to find new TSGs or oncogenes involved in cancer pathogenesis.

As reviewed by Meyerson et al.,[169] three studies have demonstrated that gene expression patterns using microarray technology are able to recapitulate the conventional morphologic classification of lung tumors into squamous, large cell, small cell, and adenocarcinoma. Many of the differentially regulated genes in different lung cancer histologic types overlapped between the studies. In addition, two of these studies found that adenocarcinomas could be placed in sub-

TABLE 3. Recurrently Altered Genetic Regions Found in Lung Cancer

Analysis	SCLC		NSCLC	
	Gain	Loss	Gain	Loss
Karyotypic analysis[167]		3p, 5q, 13q, 17p		3p, 9p, 17p
Chromosome-based CGH[164]	3q26-29, 5p12-13, 8q23-24	3p13-14, 4q32-35, 5q32-35, 8p21-22, 10q25, 13q13-14, 17p12-13	1q31, 3q25-27, 5p13-14, 8q23-24	3p21, 8p22, 9p21-22, 13q22, 17p12-13
Array-CGH[168]	ND	ND	1p36.32, 1p34.3, 1q32.2, 2q11.2, 2q31.1, 5p15.33, 5q31.3, 8p12–8p11.22, 10q24.1, 10q26.3, 12q13.2, 14q32.13, 16q22.2, 18q12.1, 19q13.33, 20q11.21	7q34, 11q11, 13q12-11, 13q32.2, 21p11.2-21p11.1
SNP array[114]	1p34.2, 2q24.3-p24.2, 8q24.13-q24.21, 19q12	3q25.1, 9p23, 10q23.31	3q26.31-q27.1, 7p12.1-q11.22, 8p12-p11.22, 8q24.13-q24.21, 12p11.21, 12q13.3-q14.1, 19q12, 22q11.21-q11.22	2q22.1, 3p14.2, 3q25.1, 9p23, 9p21.3

SCLC, small cell lung cancer; NSCLC, non-small cell lung cancer; CGH, comparative genomic hybridization; SNP, single nucleotide polymorphism.

groups that correlated with patient survival.[169] Furthermore, using different microarray platforms, three independent cohorts of patients with adenocarcinoma were classified into three subtypes correlated to clinically relevant outcomes, demonstrating the reproducible ability of DNA microarray to identify clinically relevant subtypes of adenocarcinomas.[170]

Adjuvant chemotherapy for NSCLCs is becoming a standard therapy because of its survival benefit. Thus, there is a strong need to identify patients at high risk of recurrence who will benefit from adjuvant chemotherapy. The current clinical stage-based classification method is not precise, and there is need for a method to identify patients in such a subgroup. To predict the prognosis of early-stage NSCLC patients accurately, Potti et al.[171] developed a new model, termed "metagene," which integrates various forms of data, including clinical variables and multiple gene-expression profiles. They have shown that the model predicted recurrence significantly better than a model that included only clinical data. This promising result warrants testing of this model in prospective phase III clinical trials.

Several studies have identified the sets of genes in lung cancer whose expression levels are associated with responses of tumors to antitumor drugs.[172] Furthermore, gene expression signatures involved in oncogenic pathways such as RAS and MYC pathways were described and were found to be significantly correlated with the prognosis of patients and sensitivity to cytotoxic drugs in three types of cancers, including NSCLC.[173] This provides a basis for using gene expression signatures of oncogenic pathways that are deregulated in clinical tumors as a guide to select therapeutics for patients with these tumors.

Spira et al.[174] compared the gene expression profiles of human airway epithelial cells obtained by bronchoscopy from current, former, and never-smokers and identified approximately 100 genes differentially expressed between current smokers and never-smokers. They also found that changes in expression levels of several smoking-induced genes, including potential TSGs and oncogenes, persisted after cessation of smoking, which could be a possible explanation for the fact that approximately 50% of all new lung cancer cases occur in former smokers. The SIEGE (Smoking Induced Epithelial Gene Expression) database, which has Affymetrix array data on bronchial epitheliums from current, former, and never-smokers, in addition to their relevant clinical data, was created to facilitate the identification of an airway gene expression signature to predict the risk of lung cancer development among smokers.[175]

These studies have shown that microarray technology is a robust tool for classifying tumors and may be useful for predicting the prognosis of patients and their sensitivity to therapies.

Transgenic Mouse Models of Lung Cancer

Mouse models that recapitulate the carcinogenic process of human lung cancer have substantial advantages over in vitro tissue culture systems. For example, mouse models have a complete physiological environment and allow analysis of host–tumor interactions and angiogenesis, which cannot be studied in tissue culture. There are also several limi-

tations of mouse models. Most importantly, there are significant differences in the process of tumor development between humans and mice. Whereas introduction of one to three genetic changes is sufficient to transform murine cells from several different types of tissues, additional genetic changes (more than three) are required to transform corresponding human cells; thus, there is a significant difference in the susceptibility to transformation between murine and human cells. We need to understand the advantages and disadvantages of mouse and human models to let them complement each other to facilitate the study of carcinogenic process of lung cancer.

Several different types of transgenic mouse models of lung cancer have been developed with recent innovative strategies. In particular, bitransgenic models using Cre/LoxP recombination or tetracycline-inducible gene expression systems have enabled regulation of gene expression in mice in a timely, spatially controlled manner. Two groups have engineered mouse strains harboring conditional mutant *Kras* alleles that are expressed only after Cre/LoxP-mediated recombination occurs. Both groups have shown that somatic activation of oncogenic *Kras* induces lung adenocarcinoma, demonstrating the contributions of oncogenic *Kras* to lung cancer pathogenesis.[176] Also, using a Cre/LoxP-mediated recombination system, Olive et al.[177] have shown that somatic activation of point mutations of *p53* caused lung adenocarcinoma in mice, whereas loss of *p53* did not cause any cancer, demonstrating the gain-of-function nature of mutant p53 in vivo. Moreover, Meuwissen et al.[178] developed a mouse model of SCLC by inactivating both *Rb* and *p53*, using a Cre/LoxP recombination system.

Immortalized HBEC Models

To systemically test the importance of the multiple different gene alterations (including expression changes) found in lung cancer, we developed a series of HBECs, immortalized with *cdk4* (providing a bypass of $p16^{INK4a}$) and *hTERT* (providing for maintenance of the ends of chromosomes). Both $p16^{INK4a}$ loss of function and telomerase expression are almost universally altered in human lung cancers.[179] These HBECs are immortal, can be cloned, and can be genetically manipulated, but they do not form soft agar colonies or tumors in nude mice, and they are able to differentiate into a pseudostratified epithelium structure, with a histology very similar to that of normal human bronchial epithelium in organotypic three-dimensional culture.[180,181] A great advantage of this system is that the immortalization is performed without the use of viral oncoproteins.

This is an attractive model system for analyzing the multistep pathogenesis of lung cancer. For example, HBECs manipulated to have mutant $KRAS^{V12}$, p53 knockdown, or mutant *EGFR*, alone or in various combinations, acquired the ability to grow in soft agar and invade in three-dimensional organotypic cultures.[180] Nevertheless, the combination of four alterations (*p16* bypass, telomerase, p53 abrogation, and mutant *KRAS* or mutant *EGFR*) was not sufficient to induce tumor formation in nude mice.[180] Thus, more than four such oncogenic changes are needed. These results indicate that the HBEC system is a powerful new approach to assess the contribution of individual and combinatorial genetic alterations to lung cancer pathogenesis. Another possible use of the HBEC system is to evaluate the ability of tobacco smoke and other carcinogens to transform normal epithelial cells to malignant or premalignant cells.

The Concept of Lung Cancer Stem Cells

The cancer stem cell hypothesis posits that a cancer stem cell has the ability of self-renewal: dividing to give rise to another malignant stem cell and a cancer "progenitor" cell that give rise to the phenotypically diverse tumor cell population.[182] In fact, a gene expression study found that lung cancers that expressed a "stem cell gene program" had worse survival than those that did not.[183] The concept of cancer stem cells is very important for cancer treatment. It is hypothesized that cancer stem cells can escape from the cytotoxic effects of chemotherapy and radiotherapy because of their low proliferation rate and potential drug resistance related to drug transporter expression. If this is true, then therapeutic interventions that target cancer stem cells are needed.

Evidence for cancer stem cells was first demonstrated in hematologic malignancies.[184] Subsequently, cancer stem-like tumor-initiating cells have been identified in breast and central nervous system tumors.[185,186] Although direct evidence for the existence of cancer stem cells in lung cancer has yet to be shown, in the *Kras* mouse model of lung cancer, Kim et al.[187] isolated a stem cell population at the region of the bronchioalveolar duct junction that had the ability to undergo self-renewal and differentiation; these are referred as to bronchioalveolar stem cells. Furthermore, Kim et al.[187] have shown that introduction of oncogenic *Kras* caused these putative stem cells to expand, suggesting that these cells are the precursors of adenocarcinoma of the lung. Clearly, more work is needed to prove the existence of lung cancer stem cells, and methodologies for isolating and characterizing lung cancer stem cells are under development.

Conclusions and Future Perspectives

Molecular analysis of lung cancer has provided a great deal of information on the molecular abnormalities in lung cancer. On the basis of this information, new methods of early detection, prevention, and therapeutic design for lung cancer have been developed, and some of these methods have shown promising results. Nevertheless, future effort needs to be focused on important points. First, it is an important priority to develop methods of detecting lung cancer at early stage. Combinations of spiral computed tomography screening, genetic epidemiology, serum proteomics, and biomarkers for lung cancer, such as methylated DNA in sputum, could serve such methods. Second, to find better molecular targets for therapeutic design, global searches for targets that have the most impact on malignant behaviors of lung cancer are necessary. The HBEC system will serve as a powerful tool for such studies. Third, because lung cancers are heterogeneous diseases, both at the molecular level and in terms of clinical behavior, the development of more individualized treatment is necessary. The recent discovery of the correlation between *EGFR* mutations and responses to TKI therapy

shows the possibility of molecular typing of tumors to aid in therapy selection for individual lung cancer patients. Nevertheless, it remains unclear whether *EGFR* mutation and other biological features can predict survival of patients treated with TKIs; prospective clinical trials are being conducted to validate these biological predictors. A long-term goal is that treatments for individual patients could be decided on the basis of molecular profiling of the tumors, using global strategies such as microarray and proteomics analyses on tumor species or in blood from patients. Fourth, it is necessary to develop methods for the efficient exploration of better combinations of targeted drugs. Because lung cancers have multiple changes, it would be reasonable to combine several targeted drugs. Finally, identification of the molecular targets that are important for a cancer stem cell population will be crucial for developing curative systemic therapy.

ACKNOWLEDGMENTS

This work was supported by Lung Cancer SPORE P50CA75907, NO1-CN-43301, DOD VITAL and PROSPECT grants, NASA/NSCOR (NNJ05HD36G), and the Gillson Longenbaugh Foundation.

REFERENCES

1. Jemal A, Siegel R, Ward E, et al. Cancer statistics, 2006. *CA Cancer J Clin* 2006;56:106–130.
2. Shopland DR. Tobacco use and its contribution to early cancer mortality with a special emphasis on cigarette smoking. *Environ Health Perspect* 1995;103(Suppl 8):131–142.
3. Bach PB, Kelley MJ, Tate RC, McCrory DC. Screening for lung cancer: a review of the current literature. *Chest* 2003;123:72S–82S.
4. Henschke CI, Yankelevitz DF, Libby DM, Pasmantier MW, Smith JP, Miettinen OS. Survival of patients with stage I lung cancer detected on CT screening. *N Engl J Med* 2006;355:1763–1771.
5. Belinsky SA, Liechty KC, Gentry FD, et al. Promoter hypermethylation of multiple genes in sputum precedes lung cancer incidence in a high-risk cohort. *Cancer Res* 2006;66:3338–3344.
6. Chemotherapy in non-small cell lung cancer: a meta-analysis using updated data on individual patients from 52 randomised clinical trials. Non-small Cell Lung Cancer Collaborative Group. *BMJ* 1995;311: 899–909.
7. Sekido Y, Fong KM, Minna JD. Molecular genetics of lung cancer. *Annu Rev Med* 2003;54:73–87.
8. Wistuba, II, Behrens C, Virmani AK, et al. High resolution chromosome 3p allelotyping of human lung cancer and preneoplastic/preinvasive bronchial epithelium reveals multiple, discontinuous sites of 3p allele loss and three regions of frequent breakpoints. *Cancer Res* 2000;60:1949–1960.
9. Sandler AB, Gray R, Brahmer J,et al. Randomized phase II/III trial of paclitaxel (P) plus carboplatin (C) with or without bevacizumab (NSC # 704865) in patients with advanced non-squamous non-small cell lung cancer (NSCLC): an Eastern Cooperative Oncology Group (ECOG) trial - E4599. *Pro Am Soc Clin Onc* 2005;23:16S (abstract 14).
10. 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;350:2129–2139.
11. Paez JG, Janne PA, Lee JC, et al. EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. *Science* 2004; 304:1497–1500.
12. Hecht SS. Tobacco carcinogens, their biomarkers and tobacco-induced cancer. *Nat Rev Cancer* 2003;3:733–744.
13. Hecht SS. Tobacco smoke carcinogens and lung cancer. *J Natl Cancer Inst* 1999;91:1194–1210.
14. Amos CI, Xu W, Spitz MR. Is there a genetic basis for lung cancer susceptibility? *Recent Results Cancer Res* 1999;151:3–12.
15. Caporaso NE. Why have we failed to find the low penetrance genetic constituents of common cancers? *Cancer Epidemiol Biomarkers Prev* 2002;11:1544–1549.
16. Paz-Elizur T, Krupsky M, Blumenstein S, Elinger D, Schechtman E, Livneh Z. DNA repair activity for oxidative damage and risk of lung cancer. *J Natl Cancer Inst* 2003;95:1312–1319.
17. Bailey-Wilson JE, Amos CI, Pinney SM, et al. A major lung cancer susceptibility locus maps to chromosome 6q23-25. *Am J Hum Genet* 2004;75:460–474.
18. Smith LT, Lin M, Brena RM, et al. Epigenetic regulation of the tumor suppressor gene TCF21 on 6q23-q24 in lung and head and neck cancer. *Proc Natl Acad Sci U S A* 2006;103:982–987.
19. Lengauer C Kinzler KW, Vogelstein B. Genetic instabilities in human cancers. *Nature* 1998;396:643–649.
20. Cahill DP, Lengauer C, Yu J, et al. Mutations of mitotic checkpoint genes in human cancers. *Nature* 1998;392:300–303.
21. Sato M, Sekido Y, Horio Y, et al. Infrequent mutation of the hBUB1 and hBUBR1 genes in human lung cancer. *Jpn J Cancer Res* 2000;91: 504–509.
22. Michel LS Liberal V, Chatterjee A, et al. MAD2 haplo-insufficiency causes premature anaphase and chromosome instability in mammalian cells. *Nature* 2001;409:355–359.
23. Boland CR, Thibodeau SN, Hamilton SR, et al. A National Cancer Institute Workshop on Microsatellite Instability for cancer detection and familial predisposition: development of international criteria for the determination of microsatellite instability in colorectal cancer. *Cancer Res* 1998;58:5248–5257.
24. Adachi J, Shiseki M, Okazaki T, et al. Microsatellite instability in primary and metastatic lung carcinomas. *Genes Chromosomes Cancer* 1995;14:301–306.
25. Chen XQ, Stroun M, Magnenat JL, et al. Microsatellite alterations in plasma DNA of small cell lung cancer patients. *Nat Med* 1996;2:1033–1035.
26. Peltomaki P, Lothe RA, Aaltonen LA, et al. Microsatellite instability is associated with tumors that characterize the hereditary non-polyposis colorectal carcinoma syndrome. *Cancer Res* 1993;53:5853–5855.
27. Rosell R, Pifarre A, Monzo M, et al. Reduced survival in patients with stage-I non-small-cell lung cancer associated with DNA-replication errors. *Int J Cancer* 1997;74:330–334.
28. Sozzi G, Musso K, Ratcliffe C, Goldstraw P, Pierotti MA, Pastorino U. Detection of microsatellite alterations in plasma DNA of non-small cell lung cancer patients: a prospect for early diagnosis. *Clin Cancer Res* 1999;5:2689–2692.
29. Weinstein IB. Cancer. Addiction to oncogenes—the Achilles heal of cancer. *Science* 2002;297:63–64.
30. Sordella R, Bell DW, Haber DA, Settleman J. Gefitinib-sensitizing EGFR mutations in lung cancer activate anti-apoptotic pathways. *Science* 2004;305:1163–1167.
31. Rowinsky EK. The erbB family: targets for therapeutic development against cancer and therapeutic strategies using monoclonal antibodies and tyrosine kinase inhibitors. *Annu Rev Med* 2004;55:433–457.
32. Franklin WA, Veve R, Hirsch FR, Helfrich BA, Bunn JrPA Epidermal growth factor receptor family in lung cancer and premalignancy. *Semin Oncol* 2002;29:3–14.
33. Shigematsu H, Gazdar AF. Somatic mutations of epidermal growth factor receptor signaling pathway in lung cancers. *Int J Cancer* 2006; 118:257–262.
34. Shigematsu H, Lin L, Takahashi T, et al. Clinical and biological features associated with epidermal growth factor receptor gene mutations in lung cancers. *J Natl Cancer Inst* 2005;97:339–346.
35. Fukuoka M, Yano S, Giaccone G, et al. Multi-institutional randomized phase II trial of gefitinib for previously treated patients with advanced non-small-cell lung cancer (the IDEAL 1 trial) [corrected]. *J Clin Oncol* 2003;21:2237–2246.
36. Kris MG, Natale RB, Herbst RS, et al. Efficacy of gefitinib, an inhibitor of the epidermal growth factor receptor tyrosine kinase, in symptomatic patients with non-small cell lung cancer: a randomized trial. *JAMA* 2003;290:2149–2158.
37. Shepherd FA, Rodrigues Pereira J, Ciuleanu T, et al. Erlotinib in previously treated non-small-cell lung cancer. *N Engl J Med* 2005;353: 123–132.
38. Tsao MS, Sakurada A, Cutz JC, et al. Erlotinib in lung cancer -

molecular and clinical predictors of outcome. *N Engl J Med* 2005;353: 133–144.
39. Pao W, Miller V, Zakowski M, et al. EGF receptor gene mutations are common in lung cancers from "never smokers" and are associated with sensitivity of tumors to gefitinib and erlotinib. *Proc Natl Acad Sci U S A* 2004;101:13306–13311.
40. Kobayashi S, Boggon TJ, Dayaram T, et al. EGFR mutation and resistance of non-small-cell lung cancer to gefitinib. *N Engl J Med* 2005;352:786–792.
41. Pao W, Miller VA, Politi KA, et al. Acquired resistance of lung adenocarcinomas to gefitinib or erlotinib is associated with a second mutation in the EGFR kinase domain. *PLoS Med* 2005;2:e73.
42. Inukai M, Toyooka S, Ito S, et al. Presence of epidermal growth factor receptor gene T790M mutation as a minor clone in non-small cell lung cancer. *Cancer Res* 2006;66:7854–7858.
43. Bell DW, Gore I, Okimoto RA, et al. Inherited susceptibility to lung cancer may be associated with the T790M drug resistance mutation in EGFR. *Nat Genet* 2005;37:1315–1316.
44. Kobayashi S, Ji H, Yuza Y, et al. An alternative inhibitor overcomes resistance caused by a mutation of the epidermal growth factor receptor. *Cancer Res* 2005;65:7096–7101.
45. Cappuzzo F, Hirsch FR, Rossi E, et al. Epidermal growth factor receptor gene and protein and gefitinib sensitivity in non-small-cell lung cancer. *J Natl Cancer Inst* 2005;97:643–655.
46. Cappuzzo F, Magrini E, Ceresoli GL, et al. Akt phosphorylation and gefitinib efficacy in patients with advanced non-small-cell lung cancer. *J Natl Cancer Inst* 2004;96:1133–1141.
47. Cappuzzo F, Toschi L, Domenichini I, et al. HER3 genomic gain and sensitivity to gefitinib in advanced non-small-cell lung cancer patients. *Br J Cancer* 2005;93:1334–1340.
48. Cappuzzo F, Varella-Garcia M, Shigematsu H, et al. Increased HER2 gene copy number is associated with response to gefitinib therapy in epidermal growth factor receptor-positive non-small-cell lung cancer patients. *J Clin Oncol* 2005;23:5007–5018.
49. Pao W, Wang TY, Riely GJ, et al. KRAS mutations and primary resistance of lung adenocarcinomas to gefitinib or erlotinib. *PLoS Med* 2005;2:e17.
50. Cappuzzo F, Gregorc V, Rossi E, et al. Gefitinib in pretreated non-small-cell lung cancer (NSCLC): analysis of efficacy and correlation with HER2 and epidermal growth factor receptor expression in locally advanced or metastatic NSCLC. *J Clin Oncol* 2003;21:2658–2663.
51. Bell DW, Lynch TJ, Haserlat SM, et al. Epidermal growth factor receptor mutations and gene amplification in non-small-cell lung cancer: molecular analysis of the IDEAL/INTACT gefitinib trials. *J Clin Oncol* 2005;23:8081–8092.
52. Eberhard DA, Johnson BE, Amler LC, et al. Mutations in the epidermal growth factor receptor and in KRAS are predictive and prognostic indicators in patients with non-small-cell lung cancer treated with chemotherapy alone and in combination with erlotinib. *J Clin Oncol* 2005;23:5900–5909.
53. Mitsudomi T, Kosaka T, Endoh H, et al. Mutations of the epidermal growth factor receptor gene predict prolonged survival after gefitinib treatment in patients with non-small-cell lung cancer with postoperative recurrence. *J Clin Oncol* 2005;23:2513–2520.
54. Thatcher N, Chang A, Parikh P, et al. Gefitinib plus best supportive care in previously treated patients with refractory advanced non-small-cell lung cancer: results from a randomised, placebo-controlled, multicentre study (Iressa Survival Evaluation in Lung Cancer). *Lancet* 2005;366:1527–1537.
55. Barlesi F, Jacot W, Astoul P, Pujol JL. Second-line treatment for advanced non-small cell lung cancer: a systematic review. *Lung Cancer* 2006;51:159–172.
56. Giaccone G, Herbst RS, Manegold C, et al. Gefitinib in combination with gemcitabine and cisplatin in advanced non-small-cell lung cancer: a phase III trial—INTACT 1. *J Clin Oncol* 2004;22:777–784.
57. Herbst RS, Giaccone G, Schiller JH, et al. Gefitinib in combination with paclitaxel and carboplatin in advanced non-small-cell lung cancer: a phase III trial—INTACT 2. *J Clin Oncol* 2004;22:785–794.
58. Perez-Soler R, Saltz L. Cutaneous adverse effects with HER1/EGFR-targeted agents: is there a silver lining? *J Clin Oncol* 2005;23:5235–5246.
59. Kato T, Nishio K. Clinical aspects of epidermal growth factor receptor inhibitors: benefit and risk. *Respirology* 2006;11:693–698.
60. Sandler AB. Nondermatologic adverse events associated with anti-EGFR therapy. *Oncology (Williston Park)* 2006;20:35–40.
61. Ando M, Okamoto I, Yamamoto N, et al. Predictive factors for interstitial lung disease, antitumor response, and survival in non-small-cell lung cancer patients treated with gefitinib. *J Clin Oncol* 2006;24: 2549–2556.
62. Shigematsu H, Takahashi T, Nomura M, et al. Somatic mutations of the HER2 kinase domain in lung adenocarcinomas. *Cancer Res* 2005;65: 1642–1646.
63. Stephens P, Hunter C, Bignell G, et al. Lung cancer: intragenic ERBB2 kinase mutations in tumours. *Nature* 2004;431:525–526.
64. Soung YH, Lee JW, Kim SY, et al. Somatic mutations of the ERBB4 kinase domain in human cancers. *Int J Cancer* 2006;118:1426–1429.
65. Tang X, Shigematsu H, Bekele BN, et al. EGFR tyrosine kinase domain mutations are detected in histologically normal respiratory epithelium in lung cancer patients. *Cancer Res* 2005;65:7568–7572.
66. Ji H, Li D, Chen L, et al. The impact of human EGFR kinase domain mutations on lung tumorigenesis and in vivo sensitivity to EGFR-targeted therapies. *Cancer Cell* 2006;9:485–495.
67. Politi K, Zakowski MF, Fan PD, Schonfeld EA, Pao W, Varmus HE. Lung adenocarcinomas induced in mice by mutant EGF receptors found in human lung cancers respond to a tyrosine kinase inhibitor or to down-regulation of the receptors. *Genes Dev* 2006;20:1496–1510.
68. Das AK, Sato M, Story MD, et al. Non-small cell lung cancers with kinase domain mutations in the epidermal growth factor receptor are sensitive to ionizing radiation. *Cancer Res* 2006;66:9601–9608.
69. Sekido Y, Obata Y, Ueda R, et al. Preferential expression of c-kit protooncogene transcripts in small cell lung cancer. *Cancer Res* 1991; 51:2416–2419.
70. Boldrini L, Ursino S, Gisfredi S, et al. Expression and mutational status of c-kit in small-cell lung cancer: prognostic relevance. *Clin Cancer Res* 2004;10:4101–4108.
71. Hirota S, Isozaki K, Moriyama Y, et al. Gain-of-function mutations of c-kit in human gastrointestinal stromal tumors. *Science* 1998;279:577–580.
72. Wang WL, Healy ME, Sattler M, et al. Growth inhibition and modulation of kinase pathways of small cell lung cancer cell lines by the novel tyrosine kinase inhibitor STI 571. *Oncogene* 2000;19:3521–3528.
73. Johnson BE, Fischer T, Fischer B, et al. Phase II study of imatinib in patients with small cell lung cancer. *Clin Cancer Res* 2003;9:5880–5887.
74. Krug LM, Crapanzano JP, Azzoli CG, et al. Imatinib mesylate lacks activity in small cell lung carcinoma expressing c-kit protein: a phase II clinical trial. *Cancer* 2005;103:2128–2131.
75. Wolff NC, Randle DE, Egorin MJ, Minna JD, Ilaria RL Imatinib mesylate efficiently achieves therapeutic intratumor concentrations in vivo but has limited activity in a xenograft model of small cell lung cancer. *Clin Cancer Res* 2004;10:3528–3534.
76. Downward J. Targeting RAS signalling pathways in cancer therapy. *Nat Rev Cancer* 2003;3:11–22.
77. Stacey DW, Kung HF. Transformation of NIH 3T3 cells by microinjection of Ha-ras p21 protein. *Nature* 1984;310:508–511.
78. Isobe T, Herbst RS, Onn A. Current management of advanced non-small cell lung cancer: targeted therapy. *Semin Oncol* 2005;32:315–328.
79. Brose MS, Volpe P, Feldman M, et al. BRAF and RAS mutations in human lung cancer and melanoma. *Cancer Res* 2002;62:6997–7000.
80. Davies H, Bignell GR, Cox C, et al. Mutations of the BRAF gene in human cancer. *Nature* 2002;417:949–954.
81. Naoki K, Chen TH, Richards WG, Sugarbaker DJ, Meyerson M. Missense mutations of the BRAF gene in human lung adenocarcinoma. *Cancer Res* 2002;62:7001–7003.
82. Liu B, Barrett T, Choyke P, et al. A phase II study of BAY 43-9006 (Sorafenib) in patients with relapsed non-small cell lung cancer (NSCLC). *Pro Am Soc Clin Onc* 2005;24:18S (abstract 17119).
83. Tuveson DA, Weber BL, Herlyn M. BRAF as a potential therapeutic target in melanoma and other malignancies. *Cancer Cell* 2003;4:95–98.
84. Hoshino R, Chatani Y, Yamori T, et al. Constitutive activation of the

41-/43-kDa mitogen-activated protein kinase signaling pathway in human tumors. *Oncogene* 1999;18:813–822.
85. Vivanco I, Sawyers CL. The phosphatidylinositol 3-kinase AKT pathway in human cancer. *Nat Rev Cancer* 2002;2:489–501.
86. Kawano O, Sasaki H, Endo K, et al. PIK3CA mutation status in Japanese lung cancer patients. *Lung Cancer* 2006;54:209–215.
87. Samuels Y, Wang Z, Bardelli A, et al. High frequency of mutations of the PIK3CA gene in human cancers. *Science* 2004;304:554.
88. Brognard J, Clark AS, Ni Y, Dennis PA. Akt/protein kinase B is constitutively active in non-small cell lung cancer cells and promotes cellular survival and resistance to chemotherapy and radiation. *Cancer Res* 2001;61:3986–3997.
89. Forgacs E, Biesterveld EJ, Sekido Y, et al. Mutation analysis of the PTEN/MMAC1 gene in lung cancer. *Oncogene* 1998;17:1557–1565.
90. Marsit CJ, Zheng S, Aldape K, et al. PTEN expression in non-small-cell lung cancer: evaluating its relation to tumor characteristics, allelic loss, and epigenetic alteration. *Hum Pathol* 2005;36:768–776.
91. Gomez-Martin C, Rubio-Viqueira B, Hidalgo M. Current status of mammalian target of rapamycin inhibitors in lung cancer. *Clin Lung Cancer* 2005;7(Suppl 1):S13–S18.
92. Darnell JrJE STATs and gene regulation. *Science* 1997;277:1630–1635.
93. Alvarez JV, Greulich H, Sellers WR, Meyerson M, Frank DA. Signal transducer and activator of transcription 3 is required for the oncogenic effects of non-small-cell lung cancer-associated mutations of the epidermal growth factor receptor. *Cancer Res* 2006;66:3162–3168.
94. Song L, Turkson J, Karras JG, Jove R, Haura EB. Activation of Stat3 by receptor tyrosine kinases and cytokines regulates survival in human non-small cell carcinoma cells. *Oncogene* 2003;22:4150–4165.
95. Adhikary S, Eilers M. Transcriptional regulation and transformation by Myc proteins. *Nat Rev Mol Cell Biol* 2005;6:635–645.
96. Richardson GE, Johnson BE. The biology of lung cancer. *Semin Oncol* 1993;20:105–127.
97. Vousden KH, Lu X. Live or let die: the cell's response to p53. *Nat Rev Cancer* 2002;2:594–604.
98. Hainaut P, Hernandez T, Robinson A, et al. IARC database of p53 gene mutations in human tumors and cell lines: updated compilation, revised formats and new visualisation tools. *Nucleic Acids Res* 1998;26:205–213.
99. Takahashi T, Nau MM, Chiba I, et al. p53: a frequent target for genetic abnormalities in lung cancer. *Science* 1989;246:491–494.
100. Jackson EL, Olive KP, Tuveson DA, et al. The differential effects of mutant p53 alleles on advanced murine lung cancer. *Cancer Res* 2005;65:10280–10288.
101. van Oijen MG, Slootweg PJ. Gain-of-function mutations in the tumor suppressor gene p53. *Clin Cancer Res* 2000;6:2138–2145.
102. Takahashi T, Carbone D, Takahashi T, et al. Wild-type but not mutant p53 suppresses the growth of human lung cancer cells bearing multiple genetic lesions. *Cancer Res* 1992;52:2340–2343.
103. Roth JA, Nguyen D, Lawrence DD, et al. Retrovirus-mediated wild-type p53 gene transfer to tumors of patients with lung cancer. *Nat Med* 1996;2:985–991.
104. Gabrilovich DI. INGN 201 (Advexin): adenoviral p53 gene therapy for cancer. *Expert Opin Biol Ther* 2006;6:823–832.
105. Merten OW, Geny-Fiamma C, Douar AM. Current issues in adeno-associated viral vector production. *Gene Ther* 2005;12(Suppl 1):S51–S61.
106. Higashiyama M, Doi O, Kodama K, et al. MDM2 gene amplification and expression in non-small-cell lung cancer: immunohistochemical expression of its protein is a favourable prognostic marker in patients without p53 protein accumulation. *Br J Cancer* 1997;75:1302–1308.
107. Gazzeri S, Della Valle V, Chaussade L, Brambilla C, Larsen CJ, Brambilla E. The human p19ARF protein encoded by the beta transcript of the p16INK4a gene is frequently lost in small cell lung cancer. *Cancer Res* 1998;58:3926–3931.
108. Vonlanthen S, Heighway J, Tschan MP, et al. Expression of p16INK4a/p16alpha and p19ARF/p16beta is frequently altered in non-small cell lung cancer and correlates with p53 overexpression. *Oncogene* 1998;17:2779–2785.
109. Friend SH, Bernards R, Rogelj S, et al. A human DNA segment with properties of the gene that predisposes to retinoblastoma and osteosarcoma. *Nature* 1986;323:643–646.
110. Sherr CJ, McCormick F. The RB and p53 pathways in cancer. *Cancer Cell* 2002;2:103–112.
111. Reissmann PT, Koga H, Takahashi R, et al. Inactivation of the retinoblastoma susceptibility gene in non-small-cell lung cancer. The Lung Cancer Study Group. *Oncogene* 1993;8:1913–1919.
112. Shimizu E, Coxon A, Otterson GA, et al. RB protein status and clinical correlation from 171 cell lines representing lung cancer, extrapulmonary small cell carcinoma, and mesothelioma. *Oncogene* 1994;9:2441–2448.
113. Wikman H, Nymark P, Vayrynen A, et al. CDK4 is a probable target gene in a novel amplicon at 12q13.3-q14.1 in lung cancer. *Genes Chromosomes Cancer* 2005;42:193–199.
114. Zhao X, Weir BA, LaFramboise T, et al. Homozygous deletions and chromosome amplifications in human lung carcinomas revealed by single nucleotide polymorphism array analysis. *Cancer Res* 2005;65:5561–5570.
115. Betticher DC, Heighway J, Hasleton PS, et al. Prognostic significance of CCND1 (cyclin D1) overexpression in primary resected non-small-cell lung cancer. *Br J Cancer* 1996;73:294–300.
116. Ratschiller D, Heighway J, Gugger M, et al. Cyclin D1 overexpression in bronchial epithelia of patients with lung cancer is associated with smoking and predicts survival. *J Clin Oncol* 2003;21:2085–2093.
117. Elliott RL, Blobe GC. Role of transforming growth factor beta in human cancer. *J Clin Oncol* 2005;23:2078–2093.
118. de Jonge RR, Garrigue-Antar L, Vellucci VF, Reiss M. Frequent inactivation of the transforming growth factor beta type II receptor in small-cell lung carcinoma cells. *Oncol Res* 1997;9:89–98.
119. Nagatake M, Takagi Y, Osada H, et al. Somatic in vivo alterations of the DPC4 gene at 18q21 in human lung cancers. *Cancer Res* 1996;56:2718–2720.
120. Uchida K, Nagatake M, Osada H, et al. Somatic in vivo alterations of the JV18-1 gene at 18q21 in human lung cancers. *Cancer Res* 1996;56:5583–5585.
121. Lerman MI, Minna JD. The 630-kb lung cancer homozygous deletion region on human chromosome 3p21.3: identification and evaluation of the resident candidate tumor suppressor genes. The International Lung Cancer Chromosome 3p21.3 Tumor Suppressor Gene Consortium. *Cancer Res* 2000;60:6116–6133.
122. Burbee DG, Forgacs E, Zochbauer-Muller S, et al. Epigenetic inactivation of RASSF1A in lung and breast cancers and malignant phenotype suppression. *J Natl Cancer Inst* 2001;93:691–699.
123. Dammann R, Li C, Yoon JH, Chin PL, Bates S, Pfeifer GP. Epigenetic inactivation of a RAS association domain family protein from the lung tumour suppressor locus 3p21.3. *Nat Genet* 2000;25:315–319.
124. Agathanggelou A, Cooper WN, Latif F. Role of the Ras-association domain family 1 tumor suppressor gene in human cancers. *Cancer Res* 2005;65:3497–3508.
125. Zabarovsky ER, Lerman MI, Minna JD. Chromosome 3 abnormalities in lung cancer. In: Pass HI, Carbone DP, Johnson DH, Minna JD, Turrisi AT, Eds. Lung cancer: principles and practice. Philadelphia, PA: Lippincott Williams & Wilkins, 2005:118–134.
126. Kondo M, Ji L, Kamibayashi C, et al. Overexpression of candidate tumor suppressor gene FUS1 isolated from the 3p21.3 homozygous deletion region leads to G1 arrest and growth inhibition of lung cancer cells. *Oncogene* 2001;20:6258–6262.
127. Ji L, Nishizaki M, Gao B, et al. Expression of several genes in the human chromosome 3p21.3 homozygous deletion region by an adenovirus vector results in tumor suppressor activities in vitro and in vivo. *Cancer Res* 2002;62:2715–2720.
128. Ito I, Ji L, Tanaka F, et al. Liposomal vector mediated delivery of the 3p FUS1 gene demonstrates potent antitumor activity against human lung cancer in vivo. *Cancer Gene Ther* 2004;11:733–739.
129. Nakamura F, Kalb RG, Strittmatter SM. Molecular basis of semaphorin-mediated axon guidance. *J Neurobiol* 2000;44:219–229.
130. Sekido Y, Bader S, Latif F, et al. Human semaphorins A(V) and IV reside in the 3p21.3 small cell lung cancer deletion region and demonstrate distinct expression patterns. *Proc Natl Acad Sci U S A* 1996;93:4120–4125.
131. Castro-Rivera E, Ran S, Thorpe P, Minna JD. Semaphorin 3B (SEMA3B) induces apoptosis in lung and breast cancer, whereas VEGF165 antagonizes this effect. *Proc Natl Acad Sci U S A* 2004;101:11432–11437.

132. Tomizawa Y, Sekido Y, Kondo M, et al. Inhibition of lung cancer cell growth and induction of apoptosis after reexpression of 3p21.3 candidate tumor suppressor gene SEMA3B. *Proc Natl Acad Sci U S A* 2001;98:13954–13959.
133. Kusy S, Nasarre P, Chan D, et al. Selective suppression of in vivo tumorigenicity by semaphorin SEMA3F in lung cancer cells. *Neoplasia* 2005;7:457–465.
134. Sozzi G, Veronese ML, Negrini M, et al. The FHIT gene 3p14.2 is abnormal in lung cancer. *Cell* 1996;85:17–26.
135. Sozzi G, Tornielli S, Tagliabue E, et al. Absence of Fhit protein in primary lung tumors and cell lines with FHIT gene abnormalities. *Cancer Res* 1997;57:5207–5212.
136. Sard L, Accornero P, Tornielli S, et al. The tumor-suppressor gene FHIT is involved in the regulation of apoptosis and in cell cycle control. *Proc Natl Acad Sci U S A* 1999;96:8489–8492.
137. Virmani AK, Rathi A, Zochbauer-Muller S, et al. Promoter methylation and silencing of the retinoic acid receptor-beta gene in lung carcinomas. *J Natl Cancer Inst* 2000;92:1303–1307.
138. Houle B, Rochette-Egly C, Bradley WE. Tumor-suppressive effect of the retinoic acid receptor beta in human epidermoid lung cancer cells. *Proc Natl Acad Sci U S A* 1993;90:985–989.
139. Jones PA, Baylin SB. The fundamental role of epigenetic events in cancer. *Nat Rev Genet* 2002;3:415–428.
140. Tsou JA, Hagen JA, Carpenter CL, Laird-Offringa IA. DNA methylation analysis: a powerful new tool for lung cancer diagnosis. *Oncogene* 2002;21:5450–5461.
141. Zochbauer-Muller S, Fong KM, Virmani AK, Geradts J, Gazdar AF, Minna JD. Aberrant promoter methylation of multiple genes in non-small cell lung cancers. *Cancer Res* 2001;61:249–255.
142. Palmisano WA, Divine KK, Saccomanno G, et al. Predicting lung cancer by detecting aberrant promoter methylation in sputum. *Cancer Res* 2000;60:5954–5958.
143. Shames DS, Girard L, Gao B, et al. A genome-wide screen for promoter methylation in lung cancer identifies novel methylation markers for multiple malignancies. *PLoS Med* 2006;3:e486.
144. Silverman LR, Demakos EP, Peterson BL, et al. Randomized controlled trial of azacitidine in patients with the myelodysplastic syndrome: a study of the cancer and leukemia group B. *J Clin Oncol* 2002;20:2429–2440.
145. Hu Y, Bebb G, Tan S, et al. Antitumor efficacy of oblimersen Bcl-2 antisense oligonucleotide alone and in combination with vinorelbine in xenograft models of human non-small cell lung cancer. *Clin Cancer Res* 2004;10:7662–7670.
146. Herbst RS, Frankel SR. Oblimersen sodium (Genasense bcl-2 antisense oligonucleotide): a rational therapeutic to enhance apoptosis in therapy of lung cancer. *Clin Cancer Res* 2004;10:4245s–4248s.
147. Oltersdorf T, Elmore SW, Shoemaker AR, et al. An inhibitor of Bcl-2 family proteins induces regression of solid tumours. *Nature* 2005;435:677–681.
148. Yin C, Knudson CM, Korsmeyer SJ, Van Dyke T. Bax suppresses tumorigenesis and stimulates apoptosis in vivo. *Nature* 1997;385:637–640.
149. Brambilla E, Negoescu A, Gazzeri S, et al. Apoptosis-related factors p53, Bcl2, and Bax in neuroendocrine lung tumors. *Am J Pathol* 1996;149:1941–1952.
150. Shay JW, Wright WE. Telomerase therapeutics for cancer: challenges and new directions. *Nat Rev Drug Discov* 2006;5:577–584.
151. Meyerson M. Role of telomerase in normal and cancer cells. *J Clin Oncol* 2000;18:2626–2634.
152. Hanahan D, Weinberg RA. The hallmarks of cancer. *Cell* 2000;100:57–70.
153. Hiyama K, Hiyama E, Ishioka S, et al. Telomerase activity in small-cell and non-small-cell lung cancers. *J Natl Cancer Inst* 1995;87:895–902.
154. Dikmen ZG, Gellert GC, Jackson S, et al. In vivo inhibition of lung cancer by GRN163L: a novel human telomerase inhibitor. *Cancer Res* 2005;65:7866–7873.
155. Folkman J. What is the evidence that tumors are angiogenesis dependent? *J Natl Cancer Inst* 1990;82:4–6.
156. Macchiarini P, Fontanini G, Hardin MJ, Squartini F, Angeletti CA. Relation of neovascularisation to metastasis of non-small-cell lung cancer. *Lancet* 1992;340:145–146.
157. O'Byrne KJ, Koukourakis MI, Giatromanolaki A, et al. Vascular endothelial growth factor, platelet-derived endothelial cell growth factor and angiogenesis in non-small-cell lung cancer. *Br J Cancer* 2000;82:1427–1432.
158. Heymach JV, Johnson BE, Rowbottom JA, et al. A randomized, placebo-controlled phase II trial of ZD6474 plus docetaxel, in patients with NSCLC. *Pro Am Soc Clin Oncol* 2005;23:16S (abstract 3023).
159. Korkolopoulou P, Kaklamanis L, Pezzella F, Harris AL, Gatter KC. Loss of antigen-presenting molecules (MHC class I and TAP-1) in lung cancer. *Br J Cancer* 1996;73:148–153.
160. Niehans GA, Brunner T, Frizelle SP, et al. Human lung carcinomas express Fas ligand. *Cancer Res* 1997;57:1007–1012.
161. Nemunaitis J, Sterman D, Jablons D, et al. Granulocyte-macrophage colony-stimulating factor gene-modified autologous tumor vaccines in non-small-cell lung cancer. *J Natl Cancer Inst* 2004;96:326–331.
162. Blattman JN, Greenberg PD. Cancer immunotherapy: a treatment for the masses. *Science* 2004;305:200–205.
163. Morgan RA, Dudley ME, Wunderlich JR, et al. Cancer regression in patients after transfer of genetically engineered lymphocytes. *Science* 2006;314:126–129.
164. Balsara BR, Testa JR. Chromosomal imbalances in human lung cancer. *Oncogene* 2002;21:6877–6883.
165. Coe BP, Lockwood WW, Girard L, et al. Differential disruption of cell cycle pathways in small cell and non-small cell lung cancer. *Br J Cancer* 2006;94:1927–1935.
166. Girard L, Zochbauer-Muller S, Virmani AK, Gazdar AF, Minna JD. Genome-wide allelotyping of lung cancer identifies new regions of allelic loss, differences between small cell lung cancer and non-small cell lung cancer, and loci clustering. *Cancer Res* 2000;60:4894–4906.
167. Testa JR, Liu Z, Feder M, et al. Advances in the analysis of chromosome alterations in human lung carcinomas. *Cancer Genet Cytogenet* 1997;95:20–32.
168. Tonon G, Wong KK, Maulik G, et al. High-resolution genomic profiles of human lung cancer. *Proc Natl Acad Sci U S A* 2005;102:9625–9630.
169. Meyerson M, Franklin WA, Kelley MJ. Molecular classification and molecular genetics of human lung cancers. *Semin Oncol* 2004;31:4–19.
170. Hayes DN, Monti S, Parmigiani G, et al. Gene expression profiling reveals reproducible human lung adenocarcinoma subtypes in multiple independent patient cohorts. *J Clin Oncol* 2006;24:5079–5090.
171. Potti A, Mukherjee S, Petersen R, et al. A genomic strategy to refine prognosis in early-stage non-small-cell lung cancer. *N Engl J Med* 2006;355:570–580.
172. Kikuchi T, Daigo Y, Katagiri T, et al. Expression profiles of non-small cell lung cancers on cDNA microarrays: identification of genes for prediction of lymph-node metastasis and sensitivity to anti-cancer drugs. *Oncogene* 2003;22:2192–2205.
173. Bild AH, Yao G, Chang JT, et al. Oncogenic pathway signatures in human cancers as a guide to targeted therapies. *Nature* 2006;439:353–357.
174. Spira A, Beane J, Shah V, et al. Effects of cigarette smoke on the human airway epithelial cell transcriptome. *Proc Natl Acad Sci U S A* 2004;101:10143–10148.
175. Shah V, Sridhar S, Beane J, Brody JS, Spira A. SIEGE: Smoking Induced Epithelial Gene Expression Database. *Nucleic Acids Res* 2005;33:D573–D579.
176. Meuwissen R, Berns A. Mouse models for human lung cancer. *Genes Dev* 2005;19:643–664.
177. Olive KP, Tuveson DA, Ruhe ZC, et al. Mutant p53 gain of function in two mouse models of Li-Fraumeni syndrome. *Cell* 2004;119:847–860.
178. Meuwissen R, Linn SC, Linnoila RI, Zevenhoven J, Mooi WJ, Berns A. Induction of small cell lung cancer by somatic inactivation of both Trp53 and Rb1 in a conditional mouse model. *Cancer Cell* 2003;4:181–189.
179. Ramirez RD, Sheridan S, Girard L, et al. Immortalization of human bronchial epithelial cells in the absence of viral oncoproteins. *Cancer Res* 2004;64:9027–9034.
180. Sato M, Vaughan MB, Girard L, et al. Multiple oncogenic changes (K-RAS(V12), p53 knockdown, mutant EGFRs, p16 bypass, telomerase) are not sufficient to confer a full malignant phenotype on human bronchial epithelial cells. *Cancer Res* 2006;66:2116–2128.

181. Vaughan MB, Ramirez RD, Wright WE, Minna JD, Shay JW. A three-dimensional model of differentiation of immortalized human bronchial epithelial cells. *Differentiation* 2006;74:141–148.
182. Bjerkvig R, Tysnes BB, Aboody KS, Najbauer J, Terzis AJ. Opinion: the origin of the cancer stem cell: current controversies and new insights. *Nat Rev Cancer* 2005;5:899–904.
183. Glinsky GV, Berezovska O, Glinskii AB. Microarray analysis identifies a death-from-cancer signature predicting therapy failure in patients with multiple types of cancer. *J Clin Invest* 2005;115:1503–1521.
184. Lapidot T, Sirard C, Vormoor J, et al. A cell initiating human acute myeloid leukaemia after transplantation into SCID mice. *Nature* 1994; 367:645–648.
185. Al-Hajj M, Wicha MS, Benito-Hernandez Morrison SJ, Clarke MF. Prospective identification of tumorigenic breast cancer cells. *Proc Natl Acad Sci U S A* 2003;100:3983–3988.
186. Singh SK, Hawkins C, Clarke ID, et al. Identification of human brain tumour initiating cells. *Nature* 2004;432:396–401.
187. Kim CF, Jackson EL, Woolfenden AE, et al. Identification of bronchioalveolar stem cells in normal lung and lung cancer. *Cell* 2005;121: 823–835.

