BASIC SCIENCE REVIEW

MOLECULAR PATHOGENESIS OF LUNG CANCER

Kwun M. Fong, MD, PhD[a]
Yoshitaka Sekido, MD, PhD[b]
John D. Minna, MD[c]

Lung cancer is the largest cancer killer of men and women in the United States. In addition to the progress made from antismoking primary prevention measures, new tools to help treat patients with lung cancer are emerging from the rapid advances in knowledge of the molecular pathogenesis of lung cancer. These tools include molecular and cellular biology and are starting to provide an insight into how the tumor cell, by altering oncogenes and tumor suppressor genes, achieves growth advantage, uncontrolled proliferation and metastatic behavior via disruption of key cell-cycle regulators and signal transduction cascades. Moreover, new knowledge is being developed in terms of the molecular definition of individual susceptibility to tobacco smoke carcinogens. These tools are being translated into clinical strategies to complement surgery, radiotherapy, and chemotherapy and also to assist in primary and secondary prevention efforts. This review summarizes current knowledge of the molecular pathogenesis of lung cancer. From this we know that respiratory epithelial cells require many genetic alterations to become invasive and metastatic cancer. We can detect cells with a few such changes in current and former smokers, offering the opportunity to intercede with a biomarker-monitored prevention and early detection effort. This will be coupled with new advances in computed tomography–based screening. Finally, because the molecular alterations are known, new mechanism-based therapies are being developed and brought to the clinic, including new drugs, vaccines, and gene therapy, which also must be integrated with standard therapies. (J Thorac Cardiovasc Surg 1999;118:1136-52)

Although much is known about the causes and clinical features of lung cancer, including outcome according to TNM stages and histologic features, only recently have physicians been able to delve into the molecular basis of lung cancer pathogenesis and relate these to clinical factors. Here we summarize the major findings resulting from the explosion of knowledge from molecular studies of lung cancer, and we attempt to provide some insight as to how these findings may affect clinical practice in the future. All of these efforts are predicated on continued efforts in preventing smoking initiation and assisting in smoking cessation. They also need to be coupled to recent advances in computed tomography–guided screening for early diagnosis.

From the Hamon Center for Therapeutic Oncology Research,[c] University of Texas Southwestern Medical Center, Dallas, Tex, The Prince Charles Hospital,[a] Brisbane, Australia, and the Department of Clinical Preventive Service,[b] Nagoya University Hospital, Nagoya, Japan.

Supported by Lung Cancer SPORE P50 CA70907.

Received for publication Sept 9, 1999; accepted for publication Sept 21, 1999.

Address for reprints: John D. Minna, MD, Hamon Center for Therapeutic Oncology Research, University of Texas Southwestern Medical Center, Dallas, TX 75235-8593 (E-mail: minna@simmons.swmed.edu).

0022-5223/99 $8.00 + 0 **12/1/103150**

Molecular alterations in lung cancer cells (Table I)

Markers of genetic instability. Lung cancer is the end stage of multiple-step carcinogenesis, in most cases driven by genetic and epigenetic damage caused by chronic exposure to tobacco smoke carcinogens. The genetic instability in human cancers appears to exist at two levels: at the chromosomal level, including large-scale losses and gains, and at the nucleotide level,

Table I. *Major molecular alterations in lung cancer*

	SCLC	*NSCLC*
Putative autocrine loops	GRP/GRP receptor	HGF/MET
	SCF/KIT	NDF/ERBB
RAS mutation	<1%	15%-20%
MYC amplification*	15%-30%	5%-10%
p53 mutation (with 17p13 LOH)	75%-100%	~50%
Abnormal p53 expression (IHC)	40%-70%	40%-60%
Absent RB expression (with 13q14 LOH)	~90%	15%-30%
p16 mutation (with 9p21 LOH)	<1%	10%-40%
Absent p16 expression (IHC)	0%-10%	30%-70%
3p LOH various markers	100%	90%
4p LOH	50%	~20%
4q LOH	80%	30%
8p21-23	80%-90%	80%-100%
BCL-2 expression	75%-95%	10%-35%
Telomerase activity	~100%	80%-85%
Microsatellite instability	~35%	~22%
Promoter hypermethylation	Not studied	10%-40% various genes†

GRP, Gastrin-releasing peptide; *HGF,* hepatocyte growth factor; *MET,* MET proto-oncogene; *SCF,* stem cell factor; *KIT,* KIT proto-oncogene; *NDF,* neu differentiation factor; *ERBB,* neuregulin receptor; *LOH,* loss of heterozygosity; *IHC,* immunohistochemistry; *BCL-2,* BCL-2 anti-apoptotic proto-oncogene.
*Overexpression without amplification is observed in other cases. SCLC amplifications include *MYC, MYCN,* and *MYCL*.
†p16, death-associated protein (DAP) kinase, glutathione S transferase P1 (GSTP1), and O6-methylguanine-DNA methyltransferase (MGMT).

including single or several base changes.[1] Lung cancers harbor many numeric chromosome abnormalities (aneuploidy) and structural cytogenetic abnormalities including deletions and nonreciprocal translocations. Chromosomal instability leading to aneuploidy is associated with the loss of function of a mitotic checkpoint. Exactly how this loss comes about in lung cancer is not known. Tumor DNA copy number aberrations can be more finely mapped by means of newer molecular cytogenetic techniques such as comparative genomic hybridization, which also demonstrated multiple abnormalities in lung cancer.[2,3]

At least three classes of cellular genes are involved: proto-oncogenes, tumor suppressor genes (TSGs), and DNA repair genes. Oncogenic activation often occurs via point mutation, gene amplification, or rearrangement, whereas TSGs are classically inactivated by loss of one parental allele combined with a point or small mutation, or methylation inactivation of a target TSG in the remaining allele. Additionally, dysregulated gene expression (either increased or decreased expression) can occur by other, as yet unknown, mechanisms. Current studies have not yet confirmed a prominent role for abnormalities of DNA repair genes in lung cancer including DNA mismatch repair genes; however, alterations in simple repeat sequences are seen. The phenotype seen in lung cancers appears different from the typical replication error repair (RER+) phenotype seen in tumors with mutations in DNA mismatch repair genes. In lung cancer the instability affects a relatively smaller proportion of markers and causes a single "shift" of individual allelic bands in contrast to "RER+ laddering." We thus refer to the phenotype seen in lung cancer as "microsatellite alteration." Microsatellite alteration frequencies have been reported in approximately 35% of small cell lung cancers (SCLCs) and 22% of non–small cell lung cancers (NSCLCs)[4] and have been reported to be associated with younger age,[5] reduced survival,[6] and advanced tumor stage.[6,7] There are reports implicating other DNA repair pathways in lung cancer. These include occasional mutations in a gene involved in the repair of oxidative DNA damage *(OGG1)*[8] and inactivation of the DNA repair gene O6-methylguanine-DNA methyltransferase (MGMT) by the epigenetic mechanism of promoter hypermethylation.[9]

Specific molecular alterations in lung cancer cells

Growth stimulation and oncogenes. Autocrine and paracrine growth stimulatory loops exist in lung cancers as a consequence of the expression of growth factors, regulatory peptides, and their receptors by either the cancerous or adjacent normal cells. Several but not all components of these stimulatory pathways are proto-oncogene products.

Gastrin-releasing peptide (GRP)/bombesin (BN) autocrine loop. GRP is the 27 amino acid mammalian homologue of the amphibian peptide BN. GRP/BN functions include a role in lung development and

repair.[10] Immunohistochemical studies show that approximately 20% to 60% of SCLC cancers express GRP/BN, whereas non-SCLC (NSCLC) express GRP/BN less frequently.[11] The human GRP/BN receptor subtypes belong to the G-protein–coupled receptor superfamily and include GRP-, neuromedin B–, and BN subtype–3 receptors; all of these can be expressed in SCLC, NSCLC, and in some biopsy specimens of bronchial epithelium of smokers.[12,13] However, lung cancers so far have not been found to have mutations of either GRP/BN or the GRP/BN receptor; thus the mechanisms for "reactivation" of these embryonic regulatory loops are yet unidentified. The GRP/BN autocrine loop is an important growth stimulatory loop in lung cancer, particularly SCLC. The in vitro formation of soft agar clones and the in vivo growth of nude mouse xenografts of SCLC cell lines are inhibited by a neutralizing monoclonal antibody directed against GRP/BN, as well as by antagonists of BN.[14,15] A clinical trial of the anti-BN monoclonal antibody has shown some antitumor activity in previously treated patients with SCLC.[16] This system appears to play an early role in pathogenesis, since there is an increased likelihood of expression of the GRP receptor messenger RNA in the respiratory epithelium of some individuals with a history of prolonged tobacco exposure, and expression of the GRP receptor mRNA is accompanied by responsiveness of these respiratory epithelial cells in vitro to the mitogenic effects of BN-like peptides.[13] These effects also appear to persist after smoking cessation.

ERBB family. NSCLCs but not SCLCs often demonstrate abnormalities of the neuregulin receptors ERBB2 and ERBB1, a family of transmembrane receptor tyrosine kinases. On ligand binding, ERBB receptors homodimerize or heterodimerize, thereby inducing intrinsic kinase activities that initiate intracellular signal transduction cascades including the MAP kinase pathway. *ERBB2 (HER2/neu)* is highly expressed in approximately 30% of NSCLCs, especially adenocarcinomas.[17,18] Transfection experiments suggest that *ERBB2* overexpression contributes to tumorigenicity in immortalized human bronchial epithelial cells.[19] An anti-ERBB2 monoclonal antibody inhibited the in vitro growth of ERBB2 expressing NSCLC cell lines.[20] High ERBB2 levels are associated with the multiple drug resistance phenotype[21] and increased metastatic potential in NSCLC,[22] which may help explain the poor clinical outcome linked to *ERBB2* overexpression reported by some investigators.[23,24] Monoclonal antibodies against the ERBB2 receptor (Herceptin) have entered clinical trials combined with chemotherapy in both breast cancer and lung cancer.

ERBB1 (epidermal growth factor receptor) regulates epithelial proliferation and differentiation and is usually activated in lung cancer cells by overexpression by an unknown mechanism. The production of ERBB1 ligands such as epidermal growth factor and transforming growth factor β by lung cancer cells expressing cognate receptors indicates an autocrine loop.[18,25] More common in NSCLC, ERBB1 activation may be related to tumor stage and differentiation.[26,27] Monoclonal antibodies against the ERBB1 receptor (C225, ImClone Systems Incorporated, New York, NY) are entering clinical trials combined with chemotherapy. In addition, several tyrosine kinase inhibitors that have some selectivity as epidermal growth factor receptor (ERBB1) blockers (CP358774, ZD1839) are also entering clinical trials.

Other membrane tyrosine kinases. Hepatocyte growth factor stimulates epithelial cells to proliferate, move, and undergo complex differentiation programs. During fetal lung development, hepatocyte growth factor acts as a mesenchyme-derived morphogenic factor.[28] The constitutively low levels of hepatocyte growth factor increase in response to lung or distant injury.[29] Hepatocyte growth factor stimulates mitogenesis and/or motogenesis of human bronchial epithelial, alveolar type II, and SCLC cells in vitro. The receptor for hepatocyte growth factor is the MET proto-oncogene; it is generally expressed in normal lung and both SCLC and NSCLC; hepatocyte growth factor, however, is expressed mostly in NSCLCs, suggesting an autocrine loop specific for NSCLC.[30,31] Clinically, high hepatocyte growth factor levels were associated with a poor outcome in patients with resectable NSCLC.[32] The *KIT* proto-oncogene encodes the tyrosine kinase receptor, CD117. It is co-expressed together with its ligand, stem cell factor, in many SCLCs,[33,34] representing another autocrine loop that may provide a growth advantage or mediate chemo-attraction. Other putative loops involve insulin-like growth factor 1, insulin-like growth factor 2, and the type I insulin-like growth factor receptor, which are frequently co-expressed in both SCLC and NSCLC,[35] as well as platelet-derived growth factor and its receptor.[36]

Nicotine and opioid receptors. Lung cancer cells express nicotine receptors and a variety of opioid receptors, as well as being able to produce several opioid peptides.[37] Intriguingly, opioids can inhibit lung cancer cell growth and cause apoptosis.[38] This leads to the apparent paradox whereby lung cancer cells express a negative opioid autocrine regulatory loop. Regardless, nicotine acting through specific, high-affinity, nicotinic acetylcholine receptors on lung cancer cells can antag-

onize the apoptotic effect of opioids. Thus it is possible that inhaled nicotine from smoking may play a role in lung cancer pathogenesis by inhibiting apoptosis, for example, in precursor lesions.[38]

RAS. The *RAS* proto-oncogene family *(KRAS, HRAS,* and *NRAS)* encodes plasma membrane proteins and is activated in some lung cancers by point mutations, and *RAS* mutations result in inappropriate prolonged signaling for continued cell division. *KRAS* is the most frequently activated *RAS* gene in lung cancer, usually by mutations at codon 12 but occasionally codons 13 and 61. *KRAS* mutations affect approximately 20% to 30% of lung adenocarcinomas and 15% to 20% of all NSCLC but rarely SCLCs.[11] There is subtype heterogeneity; for example, *KRAS* mutations are present in parenchymal but not bronchial adenocarcinomas,[39] and goblet-cell subtypes of adenocarcinoma have the highest frequency of *KRAS* mutations.[40] *KRAS* mutations correlate with smoking[41] and most mutations comprise the G-T transversions expected from bulky DNA adducts caused by the polycyclic hydrocarbons and nitrosamines in tobacco smoke.[42] The presence of *KRAS* mutations in a patient's tumor appears to portend a poor prognosis in NSCLC, although this is debated.[13,43-46] However, *KRAS* mutations were not associated with in vitro resistance against a range of chemotherapeutic agents in NSCLC cell lines.[47] Moreover, neither chemotherapy sensitivity nor survival correlated with *KRAS* mutations in a prospective study of advanced lung adenocarcinoma.[48] To be active in the cell, RAS has to have a lipid modification (farnesylation) regulated by an enzyme (farnesyltransferase). Several farnesyltransferase inhibitors (from Bristol Myers Squibb, Janssen, and Merck) are currently in clinical trials against lung cancer.

MYC. RAS signaling ultimately activates nuclear proto-oncogene products like MYC, which on heterodimerization transcriptionally activates downstream genes, which drives cells to grow. The *myc* family comprises *MYC, MYCN,* and *MYCL* (originally isolated from a lung cancer[49]). *MYC* is the most frequently activated and affects SCLC and NSCLC, whereas the others usually only affect SCLC. Activation occurs by gene amplification (~20-115 copies per cell) or by transcriptional dysregulation, both of which lead to protein overexpression. Richardson and Johnson[11] concluded from 17 studies that 18% to 31% of SCLCs had amplification of one *MYC* family member. Conversely, only 8% to 20% of NSCLCs were affected. *MYC* amplification appears to occur more frequently in chemotherapy-treated patients and the "variant" SCLC subtype[50] and may correlate with adverse survival. Last, in vitro growth inhibition of an SCLC cell line by all-*trans*-retinoic acid was associated with increased neuroendocrine differentiation, increased *MYCL,* and decreased *MYC* expression.[51]

Tumor suppressor genes (TSGs)

p53. p53 maintains genomic integrity in the face of DNA damage from γ or ultraviolet irradiation and carcinogens. DNA damage or hypoxia results in a rapid increase of p53, which acts as a sequence-specific transcription factor regulating downstream genes including *p21, MDM2, GADD45,* and *BAX,* thereby helping to regulate the G1/S cell cycle transition, G2/M DNA damage checkpoint, and apoptosis. Dysfunction of p53 allows the inappropriate survival of genetically damaged cells, setting the stage for the accumulation of multiple mutations and the subsequent evolution of a cancer cell. p53 plays a critical role in lung cancer; its chromosome 17p13 locus is frequently hemizygously deleted, and mutational inactivation of the remaining allele occurs in 75% or more of SCLCs and about 50% of NSCLCs.[42,52,53] *p53* mutations in lung tumors correlate with cigarette smoking and are mostly the G-T transversions expected of tobacco smoke carcinogens.[42] Furthermore, benzo(*a*)pyrene, a major tobacco smoke carcinogen, selectively forms adducts at the major *p53* mutational hot spots in bronchial epithelial cells.[54] Missense *p53* mutations can prolong the protein half-life, leading to easily detected mutant p53 protein by immunohistochemical studies.[55] However, other types of *p53* mutations do not correlate with immunohistochemical staining. Studies have shown abnormal p53 expression by immunohistochemistry in 40% to 70% of SCLCs and 40% to 60% of NSCLCs (squamous cell carcinomas higher than adenocarcinomas).[56-58] Although the prognostic value of p53 immunohistochemistry or mutational abnormalities is controversial,[59] mutations have been linked to response to cisplatin-based chemotherapy in NSCLC[60] and response to radiotherapy.[61]

Can a mutant *p53* be replaced with a normal wild-type copy with beneficial effect? The in vitro reintroduction of wild-type *p53* into lung cancer cells that also have various other genetic abnormalities besides *p53* blocks tumor cell growth by inducing apoptosis.[62] Direct injection, using a retroviral vector containing wild-type *p53,* into tumors (either by bronchoscopy or by a computed tomography–guided needle) in 9 patients with NSCLC in whom conventional treatment had failed led to tumor regression in a third of patients.[63] Other promising alternative strategies for delivering *p53* as gene replacement therapy include liposomal-*p53* complexes delivered endobronchially

and adenovirus-mediated *p53* transfer given locally, endobronchially, or even systemically.[64]

Antibodies against the p53 protein develop in some 15% to 25% of patients with lung cancer, indicating that mutant p53 protein overexpression can lead to a humoral immune response. Whereas the development of p53 antibodies appears associated with squamous histology, the value of the antibodies as diagnostic or prognostic markers is controversial.[65-68] Nonetheless, p53 antibodies appear to be reduced during chemotherapy.[69] DNA from tumor cells can be released into patients' plasma. Thus additional future early detection strategies may involve screening by means of polymerase chain reaction technology for the presence of DNA in patient plasma that contains mutant p53 sequences.[70]

There are other proteins homologous to p53, including p51 at chromosome loci 3q28 and p73 at lp36, both of which can induce growth suppression and apoptosis. Mutations of p51 appear to be infrequent in lung cancer.[71] Similarly, p73 mutations are absent or infrequent in lung cancers despite frequent allele loss of the 1p36 region (42%).[72,73] In fact, it may be that p73 "activation" is important since wild-type p73 is highly expressed in lung tumors.[74]

p21 is a p53-responsive gene that inhibits cyclin/cyclin-dependent kinase (CDK) complexes at the G1 phase. Although not somatically mutated in lung cancer, p21 was overexpressed in 65% to 75% of NSCLCs, especially in well-differentiated tumors.[75] One NSCLC study reported that p21 expression was linked to a favorable outcome,[76] whereas another suggested that concordant expression of p21 and transforming growth factor β1 predicts better survival than discordant expression.[77]

The MDM2 oncogene product inhibits p53 function by blocking its regulation of target genes and also enhances proteasome-dependent degradation of p53. Conversely, p53 regulates (increases) the expression of *MDM2* by directly binding and activating the *MDM2* promoter. The MDM2 protein is overexpressed in 25% of NSCLCs,[78] and *MDM2* expression without abnormal p53 expression (mutation) has been reported to be a favorable prognostic factor. Thus different ways of inactivating the p53 pathway may have different clinical outcomes.

p16-cyclin D1-CDK4-RB pathway

The retinoblastoma gene, RB. The pl6-cyclin D1-CDK4-RB pathway is central to controlling the G1-S transition of the cell cycle, and its components are functionally altered or mutated in many cancers. The major growth suppressing function of RB is by blocking G1-S progression. Inactivation of both *RB* alleles at chromosome region l3ql4 is common in lung cancers,[79] with protein abnormalities detected in about 90% of SCLCs and 15% to 30% of NSCLCs.[58,80-82] Functional RB loss can include deletion, nonsense mutations, or splicing abnormalities, frequently leading to a truncated RB protein. Whether absent RB expression is associated with poor prognosis in NSCLCs is controversial.[58,82-84] Functionally, in vitro reintroduction into tumor cells of a wild-type *RB* suppresses SCLC growth.[85] The inherited germline form of mutant *RB* is associated with the development of the childhood disease retinoblastoma, and relatives of retinoblastoma patients carrying germline *RB* mutation are about 15 times more likely to die of lung cancer than the general population.[86] Thus in this rare inherited disorder there appears to be an example of genetic predisposition to lung cancer. Two *RB*-related genes also have been implicated in lung cancer including, *p107* and *pRB2/p130*, where decreased expression of these proteins is associated with more aggressive histologic behavior.[87]

Cyclin D1 and CDK4. Cyclin D1 inhibits the activity of RB by stimulating its phosphorylation by CDK4. Thus cyclin D1 overexpression is an alternative mechanism to RB mutation for disrupting the pl6-cyclin D1-CDK4-RB pathway. Cyclin D1 was overexpressed in 25% to 47% of primary NSCLCs and in some cases has been associated with poor prognosis.[88-90] Transfection of a cyclin D1 antisense construct into lung cancer cell lines (and thus inhibiting any tumor-related cyclin D1 production) induces destabilization of RB and retards growth.[91] Although amplification of *CDK4* has been reported in other human malignancies, its role in lung cancer has not yet been studied. However, a CDK inhibitor, flavopiridol, is in clinical trials against lung and other cancers.

p16. Chromosome 9p allele loss is observed in many lung cancers.[92] *p16 (p16^{INK4} CDKN2)* is situated at 9p21 and frequently undergoes allele loss and mutation in lung cancer.[4] As p16 regulates RB function by inhibiting CDK4 and CDK6 kinase activity, p16 inactivation is thus another tumor strategy for disrupting the pl6-cyclin D1-CDK4-RB cell cycle control pathway. *p16* abnormalities are frequent in NSCLC but rare in SCLC (where the pathway is usually disrupted by an *RB* mutation). Homozygous deletion or point mutations occur in 10% to 40% of NSCLCs.[93,94] Hypermethylation in the 5′ CpG island of the retained allele may also cause loss of expression of *pl6* from the remaining allele in lung cancer.[95,96] These mechanisms contribute to the loss of *p16* expression in NSCLC,

functionally analogous to the preferential RB inactivation in SCLC, with the common end result of pl6-cyclin D1-CDK4-RB pathway disruption.[84,97-101] Co-inactivation of RB and p16 in any one tumor is rare, but cyclin D1 overexpression can coexist with these abnormalities in the same tumor.[102] Notably, 10% to 30% of NSCLCs appear normal for RB and p16, indicating involvement of cyclin D1, CDK4, or other pathway members in these cases. Perhaps 30% to 50% of early-stage primary NSCLCs do not express p16. *p16* alteration and loss of function in lung cancer may be associated with tumor progression, clinical stage, and survival, although not all studies concur.[58,84,101,103,104]

p19^{ARF}. The *p16* locus also encodes a second alternative reading frame protein, p19^{ARF}, that overlaps with p16. The amino acid sequence of these two alternatively spliced messages are different. However, they both appear to be important in growth regulation. p19^{ARF} binds to the MDM2-p53 complex and prevents p53 degradation, resulting in p53 activation. Immunohistochemical analysis suggests that the p19^{ARF} protein expression was more frequently lost in tumors with neuroendocrine features.[105] Thus one genetic locus at 9p21 produces two products, p16 and p19^{ARF}, both of which play a critical role in growth regulation: p16 with the RB pathway and p19^{ARF} with the p53 pathway.

Reduced expression of another CDK inhibitor gene, p27^{KIP1}, was correlated with poor prognosis in NSCLC.[106,107] In contrast, most SCLCs exhibit increased p27^{KIP1} staining, suggesting a possible link with neuronal differentiation.[107] *p57^{KIP2}* at chromosome region 11p15 is imprinted with maternal expression and *p57^{KIP2}* expression is down-regulated by selective loss of the maternal alleles in some lung cancers.[108]

Other candidate TSGs. Cytogenetics and allelotyping have revealed many hemizygous and some homozygous deletions at multiple chromosomal regions in lung cancers. In the classic TSG paradigm, the presence of underlying TSGs is suggested as the target of these genetic losses. The chromosomal regions showing hemizygous deletions include 1p, 1q, 2q, 3p, 4p, 4q, 5q, 6p, 6q, 8p, 8q, 11p, 11q, 14q, 17q, 18q, and 22q.[109-118] Although several of these chromosomal arms contain known or candidate TSGs (such as *APC* at 5q21, *WT1* at 11p13, and *NF2* at 22q12), these genes are not known to be mutated in lung cancer.

Very frequent hemizygous loss of one chromosome 3p allele occurs in lung cancer,[119] implicating multiple TSGs in at least three regions including 3p25-26, 3p21.3-22, and 3p14-cen.[120] Moreover, five separate homozygously deleted regions at 3p12-13, 3p14.2, and 3p21 occur in several lung cancer cell lines.[4] One candidate is *FHIT* at 3p14.2, which undergoes frequent hemizygous and occasional homozygous deletion in lung cancer cells and encodes a dinucleoside hydrolase. Lung cancer cells frequently (40%-80%) express abnormal mRNA transcripts of *FHIT* but nearly always also express wild-type *FHIT* transcripts.[121,122] However, unlike classic TSG inactivation, *FHIT* point mutations are rare,[121,122] and abnormal transcripts can be found in normal lung tissue.[123] Notwithstanding, Fhit is expressed in normal lung but absent Fhit protein occurs in primary lung tumors.[124] Moreover, *FHIT* allele loss is more common in smokers than nonsmokers[125] and may be associated with a poorer survival in NSCLC.[126] Furthermore, reintroduction of exogenous wild-type *FHIT* suppressed tumorigenicity of lung cancer cell lines in nude mice,[127,128] although others have reported that *FHIT* transfection does not suppress tumor growth of human cancer cell lines.[129] The 3p21.3 region has been extensively examined for putative TSGs,[119] particularly at a 600-kb region homozygously deleted in three SCLC cell lines,[130,131] while another 800-kb deletion region exists at 3p21.[132]

Protein tyrosine phosphatases may play a role in tumor suppression because of their ability to antagonize the growth-promoting protein kinases. A candidate is the phosphatase encoded by the *PTEN* gene at chromosome 10q23.[133] *PTEN* is mutated in a few primary lung cancers, and several lung cancer cell lines show homozygous deletions of this gene.[134] Another candidate at 10q, region 25.3-26.1, is *DBMT1*, which is frequently down-regulated and occasionally homozygously deleted in lung cancer.[135]

11q23-24 chromosome is a region containing frequent allelic loss including 71.4% of lung cancer cell lines, with two distinct minimal regions of loss.[136] One region contains the gene encoding the beta isoform of the A subunit of the human protein phosphatase 2A *(PPP2R1B).* Recently, mutations were described in lung and colon cancers, suggesting that *PPP2R1B* functions as a TSG.[137]

Apoptosis. Tumor cells often escape the physiologic response (programmed cell death or apoptosis) to cellular and DNA damage. Expression of the *BCL-2* anti-apoptotic proto-oncogene is relatively higher in squamous cell carcinoma (25%-35%) than in adenocarcinoma (~10%).[138-141] It also correlates with neuroendocrine differentiation and is higher in SCLCs (75%-95%) than in NSCLCs.[138,142] The latter is interesting inasmuch as SCLCs are more responsive to chemotherapy, which usually results in tumor apoptosis. Clinically, there is a trend toward longer survival in SCLCs that express BCL-2,[142] but this relationship is

controversial in NSCLC.[139-141,143,144] BAX, which counteracts BCL-2 and promotes apoptosis, is a downstream transcription target of p53. Expression of BAX and BCL-2 are inversely related in neuroendocrine cancers; most typical and atypical carcinoids have low BCL-2 and high BAX expression, with the reverse in most SCLCs and large cell neuroendocrine cancers.[56] There are multiple anti-apoptosis approaches that are in preclinical development or in early clinical trials. These include antisense BCL2 in SCLC (to inhibit the translation of BCL2 mRNA and down-regulate BCL2 protein expression), BCL-xL antisense in NSCLC, and a bispecific BCL2-BCLxL antisense to target both SCLC and NSCLC.

The receptor Fas (CD95) and the Fas ligand (FasL) also play key roles in the initiation of apoptotic pathways. FasL can induce apoptosis in activated T cells, a mechanism thought to contribute to immune privilege sites. Thus it is notable that lung cancer cell lines and tumors express FasL, and co-culture of lung cancer lines with a Fas-sensitive human T-cell line induces T-cell apoptosis.[145] Meanwhile, cell surface Fas expression has been shown to be reduced in adenocarcinomas, which may also account for resistance to Fas-mediated apoptosis.[146] Thus it appears that lung cancers can make FasL but cannot respond to it because they lack the receptor, Fas. However, the FasL they produce can function to inactivate T cells, providing for a mechanism of escape from a patient's immune response.

Telomerase activity. Human telomeres are specialized structures located at the ends of chromosomes comprising TTAGGG tandem repeats bound to specific proteins. During normal cell division, the absence of telomerase activity is associated with progressive telomere shortening, perhaps representing an intrinsic "cellular clock," leading to cell senescence and normal cell "mortality." In contrast, germ cells, some stem cells, and most cancer cells have telomerase activity, which is able to compensate for telomere shortening by replacing the hexameric repeats, leading to potential cellular "immortality." The telomerase holoenzyme is composed of both RNA and protein (enzymatic) subunits. Both of these components are required for activity and can be overexpressed in cancer, with the final measure being telomerase enzyme activity. Nearly all SCLCs and 80% to 85% of NSCLCs have high levels of telomerase activity.[147,148] High telomerase activity was associated with increased cell proliferation rates and advanced stage in NSCLCs.[148] Additionally, telomerase activity and/or dysregulated RNA expression are frequent in carcinoma in situ lesions, implicating involvement early in lung cancer development.[149]

Aberrant methylation. Methylation of the promoter regions of genes is frequently used to regulate gene expression with hypermethylation of the promoter region, resulting in loss of expression. Abnormalities of DNA methylation occur consistently in human neoplasia, and promoter region hypermethylation in 5′ CpG islands may transcriptionally silence and thus inactivate TSGs such as the *RB, VHL,* and *p16* genes. In NSCLCs, p16 hypermethylation contributes to down-regulation of p16 expression and occurs at an early stage in lung cancer development.[95,96,150] A series of genes have been found to undergo promoter methylation in lung cancer but not in the associated normal lung. In a small series of 22 patients, the following methylation frequencies were observed in NSCLC but not in the normal lung tissue: p16 (41%), death-associated protein (DAP) kinase (23%), glutathione S transferase (GSTP1) (9%), MGMT (27%), or any of the markers (68%).[151] In addition, DNA containing these methylated sequences could be detected in patients' sera at the following frequencies when the tumors were positive for the methylated marker: p16 (33%), DAP kinase (80%), GSTP1 (50%), MGMT (66%), or any marker (73%).[151] Because the methylated DNA sequences can be found even when they represent a small fraction within total normal DNA, they are very attractive candidates for early molecular detection tools and for following chemoprevention studies. Other regional sites of hypermethylation have been found in lung cancer, including sites at 3p, 4q34, 10q26, and 17p13, although the precise gene targets at these sites are uncertain and the significance is not yet apparent.[152,153] Methylation also plays a role in mediating genomic imprinting, which is a gamete-specific modification causing differential expression of the two alleles of a gene. Loss of genomic imprinting of the *IGF2* and *H19* genes at 11p15 occurs in some lung cancers, and in the case of *IGF2* this could lead to IGF2 expression and lung cancer growth.[154,155]

Angiogenesis and metastases. Tumors cannot exceed l- to 2-mm^3 volume without new blood vessels developing; thus they require angiogenic factors early in their pathogenesis. Microvessel density may be regarded as a morphologic measure of tumor angiogenesis and has been variably linked to metastases and impaired survival in lung cancer.[156-159] Tumor angiogenesis is complex and controlled by a diverse family of inducers and inhibitors governing angiogenesis, regulating endothelial cell proliferation and migration.[160] Vascular endothelial growth factor (VEGF) and basic fibroblast growth factor are among the more important angiogenesis inducers. Expression of VEGF was asso-

ciated with intratumoral microvessel density and tumor cell proliferation in squamous lung cancer.[161-163] Expression was higher in lung cancers with nodal metastasis than in those without[164] and was associated with poor prognosis in NSCLC.[162,165] In squamous cell carcinomas, the VEGF receptor, Flt-1, is frequently expressed, suggesting a possible autocrine role of VEGF.[165] Mutant p53 acts synergistically with hypoxia to induce VEGF expression[166] whereas wild-type p53 up-regulates the expression of the anti-angiogenic protein TSP-1.[167] Basic fibroblast growth factor is expressed in about 70% of NSCLCs, but its prognostic significance is controversial.[168,169] In addition, expression of the angiogenic platelet-derived endothelial cell growth factor is correlated with tumor angiogenesis and a worse prognosis in N0-stage NSCLCs.[170] Angiogenic CXC chemokines such as interleukin 8 (IL-8) and epithelial neutrophil attractant 78 (ENA-78), and angiostatic chemokines such as gamma interferon–inducible protein 10 (IP-10), may also be relevant in lung cancer.[171] IL-8 may be an angiogenic factor for SCLC, and IL-8 inhibition by passive immunization reduces tumorigenesis of NSCLC xenografts in SCID mice.[172] In contrast, IP-10 was shown to inhibit NSCLC tumorigenesis and spontaneous metastases.[173]

There are multiple new therapeutic strategies in lung cancer directed against angiogenesis in general and the VEGF system in particular. Currently, ongoing clinical trials are testing a recombinant, "humanized," monoclonal antibody against VEGF (rhuMVEGF, Genentech, Inc, South San Francisco, Calif) that blocks the binding of VEGF to its receptor. The "humanization" process takes a mouse monoclonal antibody and replaces human sequences within it so that no immune response is elicited when the antibody is given to patients. Other preclinical studies or clinical trials involve a monoclonal antibody directed against VEGF receptor 2 (KDR/FLK1, ImClone Systems Incorporated, New York, NY), a tyrosine kinase inhibitor (SU5416, SUGEN, Inc, South San Francisco, Calif), and a ribozyme (Angiozyme) directed against the VEGF receptor 1 (Flt-l) mRNA and thus inactivating the receptor by inactivating the mRNA.

Tumor metastasis is a complex multistep host-dependent process. Cell adhesion molecules have been implicated in epithelial differentiation, carcinogenesis, and metastasis. E-cadherin is responsible for the organization, maintenance, and morphogenesis of epithelial tissues, and reduced expression has been associated with tumor dedifferentiation, increased lymphogenous metastasis, and poor survival in NSCLC.[174] The integrins that bind to the major basement membrane components (collagen and laminin) are down-regulated in NSCLC.[175] Reduced integrin α3 expression is linked with poor prognosis in adenocarcinomas.[176] Others have reported that the v6 isoform of the CD44 lymphocyte homing receptor correlates with adverse prognosis in NSCLC.[177] The degradation of the extracellular matrix and basement membrane, particularly type IV collagen, by tumor cells is essential for invasion and metastasis. Type IV collagen expression of adenocarcinomas is progressively lost with increasing tumor dedifferentiation.[178] The matrix metalloproteinase enzymes that degrade stroma and thus could lead to tumor invasion and metastasis include collagenases, gelatinases, stromelysins, membrane-type matrix metalloproteinases, matrilysin, and metalloelastase. Gelatinase A expression has been shown in both SCLCs (~50%) and NSCLCs (~65%).[179] The activation of gelatinase A in lung cancer was correlated with membrane-type matrix metalloproteinase mRNA expression, whose product is one of the pro-gelatinase activators.[180] Stromelysin-3 has been shown to be more strongly expressed by stromal elements in primary NSCLCs than in adjacent normal lung specimens.[181] Several drug companies have matrix metalloproteinase inhibitors (Prinomastat from Agouron Pharmaceuticals, Inc, [La Jolla, Calif] and BAY129566 from Bayer AG [Leverkusen, Germany]) currently in clinical trials.

Molecular alterations in normal and preneoplastic at-risk respiratory cells

The concept of cancers arising from precursor lesions occurring in a multistep fashion has long been suggested and underpinned by the notion of somatically acquired genetic alterations in preneoplastic cells, which subsequently evolve into invasive cancer by clonal expansion. Morphologically distinct preneoplastic changes (hyperplasia, metaplasia, dysplasia, and carcinoma in situ) can be observed in bronchial epithelium before overt, invasive cancer develops. The sequential morphologic changes that occur in preneoplastic lesions are better described for central squamous cell carcinomas than for adenocarcinomas or SCLCs. Some reversal of morphology can occur after smoking cessation even though the elevated risk of lung cancer does not completely return to baseline, raising the possibility that there may be persistent areas of at-risk bronchial epithelium, perhaps from being irreversibly genetically altered. These preneoplastic cells and even macroscopically normal bronchial epithelium adjacent to cancers can contain genetic abnormalities identical to those in invasive cancer cells. These include allele loss at several loci (3p, 9p, 8p,

17p), *MYC* and *RAS* up-regulation, cyclin D1 expression, p53 immunoreactivity, BCL-2 overexpression, and DNA aneuploidy.[182-186] Examination of microdissected, preneoplastic lesions suggests that the earliest change is allele loss at chromosome regions 3p, then 9p, 8p, 17p (with *p53* mutation), 5q, and then *RAS* mutations.[187-190] However, topographic analyses by others have indicated that *KRAS* activation also can occur at early stages.[185] Moreover, a potential precursor lesion of adenocarcinomas, atypical alveolar hyperplasia, can also have *KRAS* mutations.[39,191]

Recent studies have suggested that the genetic changes found in invasive cancers and preneoplasia can also be identified in morphologically normal appearing bronchial epithelium from current or former smokers.[190,192,193] In general, such genetic changes are not found in the bronchial epithelium from true, lifetime never smokers. The earliest change appearing in histologically normal epithelium appears to be allele loss at one of several different 3p sites. Approximately 50% of histologically normal specimens from current or former smokers show 3p allele loss. There is also a progressive increase in the frequency and size of the areas undergoing allele loss as the specimens progress from normal, to hyperplasia or metaplasia (mildly abnormal), to dysplasia, to carcinoma in situ.[190,193] Moreover, some smokers exhibited allele loss at multiple sites, a molecular picture frequently observed in carcinoma in situ and invasive cancers.[193]

These observations are consistent with the multistep model of carcinogenesis and a "field cancerization" process, whereby the whole tissue region is repeatedly exposed to carcinogenic damage (tobacco smoke) and is at risk for the development of multiple, separate, clonally unrelated foci of neoplasia. The widespread aneuploidy that occurs throughout the respiratory tree of smokers supports this notion.[194] Interestingly, in patients with lung cancer, geographically separate regions of bronchial mucosa may demonstrate loss of the same allele of a polymorphic marker ("allele-specific loss"). Although this could be consistent with a clone populating a large part of the respiratory tree, other mechanisms are not excluded.[187,188] However, the presence of the same somatic *p53* gene point mutation at widely dispersed preneoplastic lesions in a smoker without invasive lung cancer is consistent with the clonal expansion of a single progenitor clone throughout the respiratory tree.[195]

Inherited lung cancer susceptibility

The major classes of carcinogens in tobacco smoke are the polycyclic hydrocarbons (such as benzo[a]pyrene), the nitrosamines, and the aromatic amines. Tobacco smoke carcinogens may be activated enzymatically to chemically reactive electrophiles that form carcinogen DNA adducts. It has been hypothesized that an individual's susceptibility to cancer may partially be affected by the balance between the capacity to activate inhaled procarcinogens (phase I enzymes) and the capacity to detoxify carcinogens (phase II enzymes).[196] It is increasingly recognized that genetic polymorphisms common in the population can affect each of these processes. Thus an individual's susceptibility to lung cancer could be determined by these inherited factors coupled with exposure through cigarette smoking. Familial factors predisposing to lung cancer have long been suggested.[197,198] The phenotypic variations that are thought to be important in lung cancer include polymorphisms at the P450 gene loci, such as *CYP1A1*, *CYP2D6*, and the *GST* gene cluster.[199,200] Early evidence suggests that different metabolic enzymes may be associated with tumor subtype susceptibility to various tobacco smoke carcinogens.[201] Another indication of individual variation was the difference in the number of chromosome breaks in peripheral blood lymphocytes after they were exposed in vitro to benzo(a)pyrene diol-epoxide, a very carcinogenic derivative of benzopyrene. Lung cancer cases and controls had lymphocytes exposed to benzo(a)pyrene diol-epoxide in vitro and then had the chromosomes assayed by fluorescent in situ hybridization for deletions in chromosome region 3p21.3. More deletions were found in the lung cancer cases than in the controls such that the phenotype of high numbers of 3p21.3 deletions was associated with almost a 14-fold increase in risk of having lung cancer.[202] This could prove to be a very useful marker for risk assessment of which individuals are likely to have lung cancer develop. It also provides a correlation between 3p deletions in the target tissue (lung) compared with a surrogate tissue (lymphocytes) after exposure to a respiratory carcinogen.

Clinical implications

We have given examples of how the new molecular tools may change clinical practice in the future and these are summarized in Table II. For instance, the potential of molecular epidemiology is increasingly recognized, with the aim of identifying individual genetic susceptibility factors to lung cancer and individuals at the highest risk for the development of lung cancer. This will be particularly important as we begin large-scale spiral computed tomographic lung cancer screening trials.[203,204] Next, somatic mutational events occur in airways exposed to tobacco smoke carcinogens and may lead to further refinement of risk predic-

Table II. *Translational application of molecular alterations in lung cancer*

Issue	*Possible approaches*
Molecular epidemiology	Identify individual susceptibility
	Target high-risk groups for prevention and screening
Early detection	Molecular screening approach
	Detection of mutations, methylation, and other genetic alterations in sputum, blood, urine, bronchoscopy specimens
Chemoprevention	Identify valid intermediate molecular markers that can be monitored as an indication of response to chemoprevention treatment
Diagnosis	Refine diagnosis
	Confirm malignancy
	Subtype differentiation
	Confirm genetic changes in CT-detected abnormalities
	Generate new imaging (eg, PET scan) probes
Prognosis	Refine outcome prediction and prediction of metastatic disease
	Predict individual response to conventional chemotherapy/radiotherapy
	Predict individual risk of complications from therapy
Therapy (alone or, more likely, in combination with or adjuvant to conventional therapy)	Matrix metalloprotease inhibitors
	Monoclonal antibodies against receptors and growth factors
	Growth factor or receptor antagonists (eg, *ERBB2 [HER2/neu])*
	EGF receptor blockers
	CDK inhibitors
	Cyclo-oxgenase inhibitors
	Farnesyltransferase inhibitors
	Replacement gene therapy (eg, p53)
	Protein phosphatase modulators
	Dominant negative constructs
	Protein kinase inhibitors/modulators
	Antisense molecules
	Apoptosis modulators
	Angiogenesis inhibitors
	VEGF and VEGF receptor inhibitors
	Vaccines
	Antibody- or ligand-toxin conjugates

CT, Computed tomography; *PET,* positron emission tomography; *EGF,* epidermal growth factor; *CDK,* cyclin-dependent kinase; *VEGF,* vascular endothelial growth factor.

tion in genetically susceptible individuals. However, any such molecular markers used for this and other purposes such as intermediate markers for chemoprevention have to be tested and validated in prospective clinical trials. Apart from this, early detection of lesions remains a high priority for lung cancer workers,[205] and highly sensitive molecular methods are now able to detect changes in one cancer cell among thousands. Bodily fluids are suitable for molecular analysis, but the challenge will be to identify a panel of markers that are highly sensitive and specific for lung cancer. Refinement of histologic diagnosis and categorization of lung cancer subtypes may also be improved with increasing knowledge of subtype-specific molecular changes. Moreover, the molecular basis for tests such as F-18 fluorodeoxyglucose positron emission tomography (FDG-PET) scan may lead to more advanced refinements in their use.[206,207] We have also given examples of some links to prognosis, but in general most studies have until now been relatively small, retrospective, and quite variable in methodology. Currently, there is no molecular marker that has major clinical prognostic predictive value. Clinically valid conclusions will need to come from large, adequately powered prospective studies. Finally, there is much therapeutic optimism for a variety of rationally based new therapies. Thus, in the next millennium, the molecular basis of lung cancer should provide us with the new tools to counter the clinical nihilism that affects lung cancer treatment. It is likely that these tools will complement existing strategies for lung cancer control.

REFERENCES

1. Lengauer C, Kinzler KW, Vogelstein B. Genetic instabilities in human cancers. Nature 1998;396:643-9.
2. Levin NA, Brzoska PM, Warnock ML, Gray JW, Christman MF. Identification of novel regions of altered DNA copy number in small cell lung tumors. Genes Chromosomes Cancer 1995;13: 175-8.

3. Petersen I, Bujard M, Petersen S, Wolf G, Goeze A, Schwendel A, et al. Patterns of chromosomal imbalances in adenocarcinoma and squamous cell carcinoma of the lung. Cancer Res 1997; 57:2331-5.
4. Sekido Y, Fong KM, Minna JD. Progress in understanding the molecular pathogenesis of human lung cancer. Biochem Biophys Acta 1998;1378:F21-59.
5. Ryberg D, Lindstedt BA, Zienolddiny S, Haugen A. A hereditary genetic marker closely associated with microsatellite instability in lung cancer. Cancer Res 1995;55:3996-9.
6. Rosell R, Pifarré A, Monzó M, Astudillo J, López-Cabrerizo MP, Calvo R, et al. Reduced survival in patients with stage-I non-small-cell lung cancer associated with DNA-replication errors. Int J Cancer 1997;74:330-4.
7. Adachi J, Shiseki M, Okazaki T, Ishimaru G, Noguchi M, Hirohashi S, et al. Microsatellite instability in primary and metastatic lung carcinomas. Genes Chromosomes Cancer 1995; 14:301-6.
8. Chevillard S, Radicella JP, Levalois C, Lebeau J, Poupon M-F, Oudard S, et al. Mutations in OGG1, a gene involved in the repair of oxidative DNA damage, are found in human and kidney tumors. Oncogene 1998;16:3083-6.
9. Esteller M, Hamilton SR, Burger PC, Baylin SB, Herman JG. Inactivation of the DNA repair gene O6-methylguanine-DNA methyltransferase by promoter hypermethylation is a common event in primary human neoplasia. Cancer Res 1999;59:793-7.
10. Spurzem JR, Rennard SI, Romberger DJ. Bombesin-like peptides and airway repair: a recapitulation of lung development. Am J Respir Cell Mol Biol 1997;16:209-11.
11. Richardson GE, Johnson BE. The biology of lung cancer. Semin Oncol 1993;20:105-27.
12. Fathi Z, Way JW, Corjay MH, Viallet J, Sausville EA, Battey JF. Bombesin receptor structure and expression in human lung carcinoma cell lines. J Cell Biochem Suppl 1996;24:237-46.
13. Siegfried JM, Gillespie AT, Mera R, Casey TJ, Keohavong P, Testa JR, et al. Prognostic value of specific KRAS mutations in lung adenocarcinomas. Cancer Epidemiol Biomarkers Prev 1997;6:841-7.
14. Cuttitta F, Carney DN, Mulshine J, Moody TW, Fedorko J, Fischler A, et al. Bombesin-like peptides can function as autocrine growth factors in human small-cell lung cancer. Nature 1985;316:823-6.
15. Halmos G, Schally AV. Reduction in receptors for bombesin and epidermal growth factor in xenografts of human small-cell lung cancer after treatment with bombesin antagonist RC-3095. Proc Natl Acad Sci U S A 1997;94:956-60.
16. Kelley MJ, Linnoila RI, Avis IL, Georgiadis MS, Cuttitta F, Mulshine JL, et al. Antitumor activity of a monoclonal antibody directed against gastrin-releasing peptide in patients with small cell lung cancer. Chest 1997;112:256-61.
17. Weiner DB, Nordberg J, Robinson R, Nowell PC, Gazdar A, Greene MI, et al. Expression of the *neu* gene-encoded protein ($P185^{neu}$) in human non-small cell carcinomas of the lung. Cancer Res 1990;50:421-5.
18. Rachwal WJ, Bongiorno PF, Orringer MB, Whyte RI, Ethier SP, Beer DG. Expression and activation of erbB-2 and epidermal growth factor receptor in lung adenocarcinomas. Br J Cancer 1995;72:56-64.
19. Noguchi M, Murakami M, Bennett W, Lupu R, Hui F Jr, Harris CC, et al. Biological consequences of overexpression of a transfected c-*erb*B-2 gene in immortalized human bronchial epithelial cells. Cancer Res 1993;53:2035-43.
20. Kern JA, Torney L, Weiner D, Gazdar A, Shepard HM, Fendly B. Inhibition of human lung cancer cell line growth by an anti-p185HER2 antibody. Am J Respir Cell Mol Biol 1993;9:448-54.
21. Tsai C-M, Chang K-T, Wu L-H, Chen J-Y, Gazdar AF, Mitsudomi T, et al. Correlations between intrinsic chemoresistance and *HER-2/neu* gene expression, *p53* gene mutations, and cell proliferation characteristics in non-small cell lung cancer cell lines. Cancer Res 1996;56:206-9.
22. Yu D, Wang S-S, Dulski KM, Tsai C-M, Nicolson GL, Hung M-C. c-*erb*B-2/*neu* overexpression enhances metastatic potential of human lung cancer cells by induction of metastasis-associated properties. Cancer Res 1994;54:3260-6.
23. Kern JA, Slebos RJC, Top B, Rodenhuis S, Lager D, Robinson RA, et al. C-*erb*B-2 expression and codon 12 K-*ras* mutations both predict shortened survival for patients with pulmonary adenocarcinomas. J Clin Invest 1994;93:516-20.
24. Pfeiffer P, Clausen PP, Andersen K, Rose C. Lack of prognostic significance of epidermal growth factor receptor and the oncoprotein p 185HER-2 in patients with systemically untreated non-small-cell lung cancer: an immunohistochemical study on cryosections. Br J Cancer 1996;74:86-91.
25. Rusch V, Baselga J, Cordon-Cardo C, Orazem J, Zaman M, Hoda S, et al. Differential expression of the epidermal growth factor receptor and its ligands in primary non-small cell lung cancers and adjacent benign lung. Cancer Res 1993;53:2379-85.
26. Tateishi M, Ishida T, Mitsudomi T, Kaneko S, Sugimachi K. Immunohistochemical evidence of autocrine growth factors in adenocarcinoma of the human lung. Cancer Res 1990;50:7077-80.
27. Damstrup L, Rygaard K, Spang-Thomsen M, Poulsen HS. Expression of the epidermal growth factor receptor in human small cell lung cancer cell lines. Cancer Res 1992;52:3089-93.
28. Ohmichi H, Koshimizu U, Matsumoto K, Nakamura T. Hepatocyte growth factor (HGF) acts as a mesenchyme-derived morphogenic factor during fetal lung development. Development 1998;125:1315-24.
29. Yanagita K, Matsumoto K, Sekiguchi K, Ishibashi H, Niho Y, Nakamura T. Hepatocyte growth factor may act as a pulmotrophic factor on lung regeneration after acute lung injury. J Biol Chem 1993;268:21212-7.
30. Harvey P, Warn A, Newman P, Perry LJ, Ball RY, Warn RM. Immunoreactivity for hepatocyte growth factor/scatter factor and its receptor, met, in human lung carcinomas and malignant mesotheliomas. J Pathol 1996;180:389-94.
31. Olivero M, Rizzo M, Madeddu R, Casadio C, Pennacchietti S, Nicotra MR, et al. Overexpression and activation of hepatocyte growth factor/scatter factor in human non-small-cell lung carcinomas. Br J Cancer 1996;74:1862-8.
32. Siegfried JM, Weissfeld LA, Singh-Kaw P, Weyant RJ, Testa JR, Landreneau RJ. Association of immunoreactive hepatocyte growth factor with poor survival in resectable non-small cell lung cancer. Cancer Res 1997;57:433-9.
33. Sekido Y, Takahashi T, Ueda R, Takahashi M, Suzuki H, Nishida K, et al. Recombinant human stem cell factor mediates chemotaxis of small-cell lung cancer cell lines aberrantly expressing the c-*kit* protooncogene. Cancer Res 1993;53:1709-14.
34. Krystal GW, Hines SJ, Organ CP. Autocrine growth of small cell lung cancer mediated by coexpression of c-*kit* and stem cell factor. Cancer Res 1996;56:370-6.
35. Quinn KA, Treston AM, Unsworth EJ, Miller MJ, Vos M,

Grimley C, et al. Insulin-like growth factor expression in human cancer cell lines. J Biol Chem 1996;271:11477-83.

36. Antoniades HN, Galanopoulos T, Neville-Golden J, O'Hara CJ. Malignant epithelial cells in primary human lung carcinomas coexpress in vivo platelet-derived growth factor (PDGF) and PDGF receptor mRNAs and their protein products. Proc Natl Acad Sci U S A 1992;89:3942-6.
37. Maneckjee R, Minna JD. Opioid and nicotine receptors affect growth regulation of human lung cancer cell lines. Proc Natl Acad Sci U S A 1990;87:3294-8.
38. Maneckjee R, Minna JD. Opioids induce while nicotine suppresses apoptosis in human lung cancer cells. Cell Growth Differ 1994;5:1033-40.
39. Cooper CA, Carey FA, Bubb VJ, Lamb D, Kerr KM, Wyllie AH. The pattern of K-*ras* mutation in pulmonary adenocarcinoma defines a new pathway of tumour development in the human lung. J Pathol 1997;181:401-4.
40. Tsuchiya E, Furuta R, Wada N, Nakagawa K, Ishikawa Y, Kawabuchi B, et al. High K-*ras* mutation rates in goblet-cell-type adenocarcinomas of the lungs. J Cancer Res Clin Oncol 1995;121:577-81.
41. Slebos RJ, Hruban RH, Dalesio O, Mooi WJ, Offerhaus GJ, Rodenhuis S. Relationship between K-ras oncogene activation and smoking in adenocarcinoma of the human lung. J Natl Cancer Inst 1991;83:1024-7.
42. Greenblatt MS, Bennett WP, Hollstein M, Harris CC. Mutations in the *p53* tumor suppressor gene: clues to cancer etiology and molecular pathogenesis. Cancer Res 1994;54:4855-78.
43. Slebos RJ, Kibbelaar RE, Dalesio O, Kooistra A, Stam J, Meijer CJLM, et al. K-*ras* oncogene activation as a prognostic marker in adenocarcinoma of the lung. N Engl J Med 1990;323:561-5.
44. Mitsudomi T, Steinberg SM, Oie HK, Mulshine JL, Phelps R, Viallet J, et al. *ras* gene mutations in non-small cell lung cancers are associated with shortened survival irrespective of treatment intent. Cancer Res 1991;51:4999-5002.
45. Rosell R, Li S, Skacel Z, Mate JL, Maestre J, Canela M, et al. Prognostic impact of mutated K-ras gene in surgically resected non-small cell lung cancer patients. Oncogene 1993;8:2407-12.
46. Graziano SL, Gamble GP, Newman NB, Abbott LZ, Rooney M, Mookherjee S, et al. Prognostic significance of K-ras codon 12 mutations in patients with resected stage I and II non-small-cell lung cancer. J Clin Oncol 1999;17:668-75.
47. Tsai C-M, Chang K-T, Perng R-P, Mitsudomi T, Chen M-H, Kadoyama C, et al. Correlation of intrinsic chemoresistance of non-small-cell lung cancer cell lines with HER-2/neu gene expression but not with ras gene mutations. J Natl Cancer Inst 1993;85:897-901.
48. Rodenhuis S, Boerrigter L, Top B, Slebos RJ, Mooi WJ, van't Veer L, et al. Mutational activation of the K-*ras* oncogene and the effect of chemotherapy in advanced adenocarcinoma of the lung: a prospective study. J Clin Oncol 1997;15:285-91.
49. Nau MM, Brooks BJ, Battey J, Sausville E, Gazdar AF, Kirsch IR, et al. L-myc, a new myc-related gene amplified and expressed in human small cell lung cancer. Nature 1985;318:69-73.
50. Johnson BE, Russell E, Simmons AM, Phelps R, Steinberg SM, Ihde DC, et al. *MYC* family DNA amplification in 126 tumor cell lines from patients with small cell lung cancer. J Cell Biochem Suppl 1996;24:210-7.
51. Ou X, Campau S, Slusher R, Jasti RK, Mabry M, Kalemkerian GP. Mechanism of all-*trans*-retinoic acid-mediated L-*myc* gene regulation in small cell lung cancer. Oncogene 1996;13:1893-9.
52. Takahashi T, Nau MM, Chiba I, Birrer MJ, Rosenberg RK, Vinocour M, et al. p53: a frequent target for genetic abnormalities in lung cancer. Science 1989;246:491-4.
53. Bennett WP, Hussain SP, Vahakangas KH, Khan MA, Shields PG, Harris CC. Molecular epidemiology of human cancer risk: gene-environment interactions and p53 mutation spectrum in human lung cancer. J Pathol 1999;187:8-18.
54. Denissenko MF, Pao A, Tang M, Pfeifer GP. Preferential formation of benzo[*a*]pyrene adducts at lung cancer mutational hotspots in *P53.* Science 1996;274:430-2.
55. Casey G, Lopez ME, Ramos JC, Plummer SJ, Arboleda MJ, Shaughnessy M, et al. DNA sequence analysis of exons 2 through 11 and immunohistochemical staining are required to detect all known p53 alterations in human malignancies. Oncogene 1996;13:1971-81.
56. Brambilla E, Negoescu A, Gazzeri S, Lantuejoul S, Moro D, Brambilla C, et al. Apoptosis-related factors p53, Bcl2, and Bax in neuroendocrine lung tumors. Am J Pathol 1996;149:1941-52.
57. Nishio M, Koshikawa T, Kuroishi T, Suyama M, Uchida K, Takagi Y, et al. Prognostic significance of abnormal p53 accumulation in primary, resected non-small-cell lung cancers. J Clin Oncol 1996;14:497-502.
58. Geradts J, Fong KM, Zimmerman PV, Maynard R, Minna JD. Correlation of abnormal RB, p16ink4a, and p53 expression with 3p loss of heterozygosity, other genetic abnormalities, and clinical features in 103 primary non-small cell lung cancers. Clin Cancer Res 1999;5:791-800.
59. Graziano SL. Non-small cell lung cancer: clinical value of new biological predictors. Lung Cancer 1997;17:S37-58.
60. Kandioler-Eckersberger D, Kappel S, Mittlböck M, Dekan G, Ludwig C, Janschek E, et al. The TP53 genotype but not immunohistochemical result is predictive of response to cisplatin-based neoadjuvant therapy in stage III non–small cell lung cancer. J Thorac Cardiovasc Surg 1999;117:744-50.
61. Matsuzoe D, Hideshima T, Kimura A, Inada K, Watanabe K, Akita Y, et al. p53 mutations predict non-small cell lung carcinoma response to radiotherapy. Cancer Lett 1999;135:189-94.
62. Adachi J, Ookawa K, Shiseki M, Okazaki T, Tsuchida S, Morishita K, et al. Induction of apoptosis but not G1 arrest by expression of the wild-type p53 gene in small cell lung carcinoma. Cell Growth Differ 1996;7:879-86.
63. Roth JA, Nguyen D, Lawrence DD, Kemp BL, Carrasco CH, Ferson DZ, et al. Retrovirus-mediated wild-type *p53* gene transfer to tumors of patients with lung cancer. Nature Med 1996; 2:985-91.
64. Zou Y, Zong G, Ling YH, Hao MM, Lozano G, Hong WK, et al. Effective treatment of early endobronchial cancer with regional administration of liposome-p53 complexes [see comments]. J Natl Cancer Inst 1998;90:1130-7.
65. Lubin R, Zalcman G, Bouchet L, Tredanel J, Legros Y, Cazals D, et al. Serum p53 antibodies as early markers of lung cancer. Nature Med 1995;1:701-2.
66. Rosenfeld MR, Malats N, Schramm L, Graus F, Cardenal F, Vinolas N, et al. Serum anti-p53 antibodies and prognosis of patients with small-cell lung cancer. J Natl Cancer Inst 1997;89: 381-5.
67. Mitsudomi T, Suzuki S, Yatabe Y, Nishio M, Kuwabara M, Gotoh K, et al. Clinical implications of p53 autoantibodies in the sera of patients with non-small-cell lung cancer. J Natl Cancer Inst 1998;90:1563-8.
68. Lai CL, Tsai CM, Tsai TT, Kuo BI, Chang KT, Fu HT, et al.

Presence of serum antip53 antibodies is associated with pleural effusion and poor prognosis in lung cancer patients. Clin Cancer Res 1998;4:3025-30.

69. Zalcman G, Schlichtholz B, Tredaniel J, Utban T, et al. Monitoring of p53 autoantibodies in lung cancer during therapy: relationship to treatment. Clin Cancer Res 1998;4:1359-66.
70. Silva JM, Gonzalez R, Dominguez G, Garcia JM, España P, Bonilla F. TP53 gene mutations in plasma DNA of cancer patients. Genes Chromosomes Cancer 1999;24:160-1.
71. Osada M, Ohba M, Kawahara C, Ishioka C, Kanamaru R, Katoh T, et al. Cloning and functional analysis of human *p51*, which structurally and functionally resembles *p53.* Nature Med 1998; 4:839-43.
72. Nomoto S, Haruki N, Kondo M, Konishi H, Takahashi T, Takahashi T, et al. Search for mutations and examination of allelic expression imbalance of the p73 gene at lp36.33 in human lung cancers. Cancer Res 1998;58:1380-3.
73. Yoshikawa H, Nagashima M, Khan MA, McMenamin MG, Hagiwara K, Harris CC. Mutational analysis of p73 and p53 in human cancer cell lines. Oncogene 1999;18:3415-21.
74. Mai M, Yokomizo A, Qian C, Yang P, Tindall DJ, Smith DI, et al. Activation *of p73* silent allele in lung cancer. Cancer Res 1998;58:2347-9.
75. Marchetti A, Doglioni C, Barbareschi M, Buttitta F, Pellegrini S, Bertacca G, et al. p21 RNA and protein expression in non-small cell lung carcinomas: evidence of p53-independent expression and association with tumoral differentiation. Oncogene 1996; 12:1319-24.
76. Caputi M, Esposito V, Baldi A, De LA, Dean C, Signoriello G, et al. p21wafl/cip1mda-6 expression in non-small-cell lung cancer: relationship to survival. Am J Respir Cell Mol Biol 1998; 18:213-7.
77. Bennett WP, El-Deiry WS, Rush WL, Guinee DGJ, et al. p21 waf1/cip1 and transforming growth factor β1 protein expression correlate with survival in non-small cell lung cancer. Clin Cancer Res 1998;4:1499-506.
78. Higashiyama M, Doi O, Kodama K, Yokouchi H, Kasugai T, Ishiguro S, 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;75:1302-8.
79. Harbour JW, Lai SL, Whang-Peng J, Gazdar AF, Minna ID, Kaye FJ. Abnormalities in structure and expression of the human retinoblastoma gene in SCLC. Science 1988;241:353-7.
80. Reissmann PT, Koga H, Takahashi R, Figlin RA, Holmes EC, Piantadosi S, et al. Inactivation of the retinoblastoma susceptibility gene in non-small-cell lung cancer. Oncogene 1993;8: 1913-9.
81. Cagle PT, El-Naggar AK, Xu H-J, Hu S-X, Benedict WF. Differential retinoblastoma protein expression in neuroendocrine tumors of the lung: potential diagnostic implications. Am J Pathol 1997;150:393-400.
82. Dosaka-Akita H, Hu S-X, Fujino M, Harada M, Kinoshita I, Xu H-J, et al. Altered retinoblastoma protein expression in nonsmall cell lung cancer: its synergistic effects with altered ras and p53 protein status on prognosis. Cancer 1997;79:1329-37.
83. Xu H-J, Quinlan DC, Davidson AG, Hu S-X, Summers CL, Li J, et al. Altered retinoblastoma protein expression and prognosis in early-stage non-small-cell lung carcinoma. J Natl Cancer Inst 1994;86:695-9.
84. Kratzke RA, Greatens TM, Rubins JB, Maddaus MA, Niewoehner DE, Niehans GA, et al. *Rb* and p 16^{INK4a} expression in resected non-small cell lung tumors. Cancer Res 1996;56: 3415-20.
85. Ookawa K, Shiseki M, Takahashi R, Yoshida Y, Terada M, Yokota J. Reconstitution of the *RB* gene suppresses the growth of small-cell lung carcinoma cells carrying multiple genetic alterations. Oncogene 1993;8:2175-81.
86. Sanders BM, Jay M, Draper GJ, Roberts EM. Non-ocular cancer in relatives of retinoblastoma patients. Br J Cancer 1989; 60:358-65.
87. Minimo C, Bibbo M, Claudio PP, De Luca A, Giordano A. The role of pRb2/pl30 protein in diagnosing lung carcinoma on fine needle aspiration biopsies. Pathol Res Pract 1999;195:67-70.
88. Betticher DC, Heighway J, Hasleton PS, Altermatt HJ, Ryder WD, Cerny T, et al. Prognostic significance of *CCND1* (cyclin D1) overexpression in primary resected non-small-cell lung cancer. Br J Cancer 1996;73:294-300.
89. Mishina T, Dosaka-Akita H, Kinoshita I, Hommura F, Morikawa T, Katoh H, et al. Cyclin D1 expression in non-small-cell lung cancers: its association with altered p53 expression, cell proliferation and clinical outcome. Br J Cancer 1999;80: 1289-95.
90. Caputi M, Groeger AM, Esposito V, Dean C, De Luca A, Pacilio C, et al. Prognostic role of cyclin D1 in lung cancer: relationship to proliferating cell nuclear antigen. Am J Respir Cell Mol Biol 1999;20:746-50.
91. Driscoll B, Wu L, Buckley S, Hall FL, Anderson KD, Warburton D. Cyclin D1 antisense RNA destabilizes pRb and retards lung cancer cell growth. Am J Physiol 1997;273:L941-9.
92. Merlo A, Gabrielson E, Askin F, Sidransky D. Frequent loss of chromosome 9 in human primary non-small cell lung cancer. Cancer Res 1994;54:640-2.
93. Rusin MR, Okamoto A, Chorazy M, Czyzewski K, Harasim J, Spillare EA, et al. Intragenic mutations of the *$p16^{INK4}$, $p15^{INK4B}$ and p18* genes in primary non-small-cell lung cancers. Int J Cancer 1996;65:734-9.
94. Marchetti A, Buttitta F, Pellegrini S, Bertacca G, Chella A, Carnicelli V, et al. Alterations *of P16 (MTS1)* in node-positive non-small cell lung carcinomas. J Pathol 1997;181:178-82.
95. Merlo A, Herman JG, Mao L, Lee DJ, Gabrielson E, Burger PC, et al. 5´CpG island methylation is associated with transcriptional silencing of the tumour suppressor *pl6/CDKN2/MTS1* in human cancers. Nature Med 1995;1:686-92.
96. Otterson GA, Khleif SN, Chen W, Coxon AB, Kaye FJ. *CDKN2* gene silencing in lung cancer by DNA hypermethylation and kinetics of $p16^{INK4}$ protein induction by 5-aza 2´deoxycytidine. Oncogene 1995;11:1211-6.
97. Shapiro GI, Park JE, Edwards CD, Mao L, Merlo A, Sidransky D, et al. Multiple mechanisms of $p16^{INK4A}$ inactivation in non-small cell lung cancer cell lines. Cancer Res 1995;55:6200-9.
98. Okamoto A, Demetrick DJ, Spillare EA, Hagiwara K, Hussain SP, Bennett WP, et al. Mutations and altered expression of $p16^{INK4}$ in human cancer. Proc Natl Acad Sci U S A 1994;91: 11045-9.
99. Nakagawa K, Conrad NK, Williams JP, Johnson BE, Kelley MJ. Mechanism of inactivation of *CDKN2* and *MTS2* in non-small cell lung cancer and association with advanced stage. Oncogene 1995;11:1843-51.
100. Kelley MJ, Nakagawa K, Steinberg SM, Mulshine JL, Kamb A, Johnson BE. Differential inactivation of CDKN2 and Rb protein

in non-small-cell and small-cell lung cancer cell lines. J Natl Cancer Inst 1995;87:756-61.

101. Kinoshita I, Dosaka-Akita H, Mishina T, Akie K, Nishi M, Hiroumi H, et al. Altered $p16^{INK4}$ and retinoblastoma protein status in non-small cell lung cancer: potential synergistic effect with altered p53 protein on proliferative activity. Cancer Res 1996;56:5557-62.
102. Shapiro Gl, Edwards CD, Kobzik L, Godleski J, Richards W, Sugarbaker DJ, et al. Reciprocal Rb inactivation and $p16^{INK4}$ expression in primary lung cancers and cell lines. Cancer Res 1995;55:503-9.
103. Okamoto A, Hussain SP, Hagiwara K, Spillare EA, Rusin MR, Demetrick DJ, et al. Mutations in the *pl6^{INK4}/MTS1/CDKN2*, *pl5^{INK4B}/MTS2*, and *p18* genes in primary and metastatic lung cancer. Cancer Res 1995;55:1448-51.
104. Taga S, Osaki T, Ohgami A, Imoto H, Yoshimatsu T, Yoshino I, et al. Prognostic value of the immunohistochemical detection of $p16^{INK4}$ expression in nonsmall cell lung carcinoma. Cancer 1997;80:389-95.
105. Gazzeri S, Della Valle V, Chaussade L, Brambilla C, Larsen CJ, Brambilla E. The human p19ARF protein encoded by the beta transcript of the pl6INK4a gene is frequently lost in small cell lung cancer. Cancer Res 1998;58:3926-31.
106. Esposito V, Baldi A, De Luca A, Groger AM, Loda M, Giordano GG, et al. Prognostic role of the cyclin-dependent kinase inhibitor p27 in non-small cell lung cancer. Cancer Res 1997;57:3381-5.
107. Yatabe Y, Masuda A, Koshikawa T, Nakamura S, Kuroishi T, Osada H, et al. $p27^{KIP1}$ in human lung cancers: differential changes in small cell and non-small cell carcinomas. Cancer Res 1998;58:1042-7.
108. Kondo M, Matsuoka S, Uchida K, Osada H, Nagatake M, Takagi K, et al. Selective maternal-allele loss in human lung cancers of the maternally expressed *p57^{KIP2}* gene at 11p15.5. Oncogene 1996;12:1365-8.
109. Ohata H, Emi M, Fujiwara Y, Higashino K, Nakagawa K, Futagami R, et al. Deletion mapping of the short arm of chromosome 8 in non-small cell lung carcinoma. Genes Chromosomes Cancer 1993;7:85-8.
110. Sato S, Nakamura Y, Tsuchiya E. Difference of allelotype between squamous cell carcinoma and adenocarcinoma of the lung. Cancer Res 1994;54:5652-5.
111. Shiseki M, Kohno T, Nishikawa R, Sameshima Y, Mizoguchi H, Yokota J. Frequent allelic losses on chromosomes 2q, 18q, and 22q in advanced non-small cell lung carcinoma. Cancer Res 1994;54: 5643-8.
112. Otsuka T, Kohno T, Mori M, Noguchi M, Hirohashi S, Yokota J. Deletion mapping of chromosome 2 in human lung carcinoma. Genes Chromosomes Cancer 1996;16:113-9.
113. O'Briant KC, Bepler G. Delineation of the centromeric and telomeric chromosome segment 11p15.5 lung cancer suppressor regions LOH11A and LOH11B. Genes Chromosomes Cancer 1997;18:111-4.
114. Ueno K, Kumagai T, Kijima T, Kishimoto T, Hosoe S. Cloning and tissue expression of cDNAs from chromosome 5q21-22 which is frequently deleted in advanced lung cancer. Hum Genet 1998;102:63-8.
115. Virmani AK, Fong KM, Kodagoda D, McIntire D, Hung J, Tonk V, et al. Allelotyping demonstrates common and distinct patterns of chromosomal loss in human lung cancer types. Genes Chromosomes Cancer 1998;21:308-19.
116. Wistuba II, Behrens C, Virmani AK, Milchgrub S, Syed S, Lam S, et al. Allelic losses at chromosome 8p21-23 are early and frequent events in the pathogenesis of lung cancer. Cancer Res 1999;59:1973-9.
117. Lerebours F, Olschwang S, Thuille B, Schmitz A, Fouchet P, Buecher B, et al. Fine deletion mapping of chromosome 8p in non-small-cell lung carcinoma. Int J Cancer 1999;81:854-8.
118. Shivapurkar N, Virmani AK, Wistuba II, Milchgrub S, Mackay B, Minna JD, et al. Deletions of chromosome 4 at multiple sites are frequent in malignant mesothelioma and small cell lung carcinoma. Clin Cancer Res 1999;5:17-23.
119. Kok K, Naylor SL, Buys CH. Deletions of the short arm of chromosome 3 in solid tumors and the search for suppressor genes. Adv Cancer Res 1997;71:27-92.
120. Hibi K, Takahashi T, Yamakawa K, Ueda R, Sekido Y, Ariyoshi Y, et al. Three distinct regions involved in 3p deletion in human lung cancer. Oncogene 1992;7:445-9.
121. Sozzi G, Veronese ML, Negrini M, Baffa R, Cotticelli MG, Inoue H, et al. The *FHIT* gene 3p14.2 is abnormal in lung cancer. Cell 1996;85:17-26.
122. Fong KM, Biesterveld EJ, Virmani A, Wistuba I, Sekido Y, Bader SA, et al. *FHIT* and FRA3B 3p14.2 allele loss are common in lung cancer and preneoplastic bronchial lesions and are associated with cancer-related *FHIT* cDNA splicing aberrations. Cancer Res 1997;57:2256-67.
123. Tokuchi Y, Kobayashi Y, Hayashi S, Hayashi M, Tanimoto K, Hashimoto T, et al. Abnormal FHIT transcripts found in both lung cancer and normal lung tissue. Genes Chromosomes Cancer 1999;24:105-11.
124. Sozzi G, Tornielli S, Tagliabue E, Sard L, Pezzella F, Pastorino U, et al. Absence of Fhit protein in primary lung tumors and cell lines with *FHIT* gene abnormalities. Cancer Res 1997;57:5207-12.
125. Sozzi G, Sard L, De Gregorio L, Marchetti A, Musso K, Buttitta F, et al. Association between cigarette smoking and FHIT gene alterations in lung cancer. Cancer Res 1997;57:2121-3.
126. Burke L, Khan MA, Freedma AN, Gemma A, Rusin M, et al. Allelic deletion analysis of the *FHIT* gene predicts poor survival in non-small cell lung cancer. Cancer Res 1998;58:2533-6.
127. Siprashvili Z, Sozzi G, Barnes LD, McCune P, Robinson AK, Eryomin V, et al. Replacement of Fhit in cancer cells suppresses tumorigenicity. Proc Natl Acad Sci U S A 1997;94:13771-6.
128. Ji L, Fang B, Yen N, Fong K, Minna JD, Roth JA. Induction of apoptosis and inhibition of tumorigenicity and tumor growth by adenovirus vector-mediated fragile histidine triad (FHIT) gene overexpression [In Process Citation]. Cancer Res 1999;59: 3333-9.
129. Otterson GA, Xiao GH, Geradts J, Jin F, Chen WD, Niklinska W, et al. Protein expression and functional analysis of the FHIT gene in human tumor cells. J Natl Cancer Inst 1998;90:426-32.
130. Kok K, van den Berg A, Veldhuis PMJF, van der Veen AY, Franke M, Schoenmakers EFPM, et al. A homozygous deletion in a small cell lung cancer cell line involving a 3p21 region with a marked instability in yeast artificial chromosomes. Cancer Res 1994;54:4183-7.
131. Wei M-H, Latif F, Bader S, Kashuba V, Chen J-Y, Duh F-M, et al. Construction of a 600-kilobase cosmid clone contig and generation of a transcriptional map surrounding the lung cancer tumor suppressor gene *(TSG)* locus on human chromosome 3p21.3: progress toward the isolation of a lung cancer TSG. Cancer Res 1996;56:1487-92.

132. Yamakawa K, Takahashi T, Horio Y, Murata Y, Takahashi E, Hibi K, et al. Frequent homozygous deletions in lung cancer cell lines detected by a DNA marker located at 3p21.3-p22. Oncogene 1993;8:327-30.
133. Li J, Yen C, Liaw D, Podsypanina K, Bose S, Wang SI, et al. *PTEN*, a putative protein tyrosine phosphatase gene mutated in human brain, breast, and prostate cancer. Science 1997;275: 1943-7.
134. Forgacs E, Biesterveld EJ, Sekido Y, Fong K, Muneer S, Wistuba II, et al. Mutation analysis of the PTEN/MMAC1 gene in lung cancer. Oncogene 1998;17:1557-65.
135. Wu W, Kemp BL, Proctor ML, Gazdar AF, Minna JD, Hong WK, et al. Expression of DMBT1, a candidate tumor suppressor gene, is frequently lost in lung cancer. Cancer Res 1999;59: 1846-51.
136. Wang SS, Virmani A, Gazdar AF, Minna JD, Evans GA. Refined mapping of two regions of loss of heterozygosity on chromosome band 11q23 in lung cancer. Genes Chromosomes Cancer 1999;25:154-9.
137. Wang SS, Esplin ED, Li JL, Huang L, Gazdar A, Minna J, et al. Alterations of the PPP2R1B gene in human lung and colon cancer. Science 1998;282:284-7.
138. Jiang SX, Kameya T, Sato Y, Yanase N, Yoshimura H, Kodama T. Bcl-2 protein expression in lung cancer and close correlation with neuroendocrine differentiation. Am J Pathol 1996;148:837-46.
139. Pezzella F, Turley H, Kuzu I, Tungekar MF, Dunnill MS, Pierce CB, et al. *bcl*-2 protein in non-small-cell lung carcinoma. N Engl J Med 1993;329:690-4.
140. Higashiyama M, Doi O, Kodama K, Yokouchi H, Nakamori S, Tateishi R. *bcl-2* oncoprotein in surgically resected nonsmall cell lung cancer: possibly favorable prognostic factor in association with low incidence of distant metastasis. J Surg Oncol 1997;64:48-54.
141. Apolinario RM, van der Valk P, de Jong JS, Deville W, van Ark-Otte J, Dingemans AM, et al. Prognostic value of the expression of p53, *bcl*-2, and *bax* oncoproteins, and neovascularization in patients with radically resected non-small-cell lung cancer. J Clin Oncol 1997;15:2456-66.
142. Kaiser U, Schilli M, Haag U, Neumann K, Kreipe H, Kogan E, et al. Expression of bcl-2–protein in small cell lung cancer. Lung Cancer 1996;15:31-40.
143. Fontanini G, Vignati S, Bigini D, Mussi A, Lucchi M, Angeletti CA, et al. Bcl-2 protein: a prognostic factor inversely correlated to p53 in non-small-cell lung cancer. Br J Cancer 1995;71: 1003-7.
144. Anton RC, Brown RW, Younes M, Gondo MM, Stephenson MA, Cagle PT. Absence of prognostic significance of bcl-2 immunopositivity in non-small cell lung cancer: analysis of 427 cases. Hum Pathol 1997;28:1079-82.
145. Niehans GA, Brunner T, Frizelle SP, Liston JC, Salerno CT, Knapp DJ, et al. Human lung carcinomas express Fas ligand. Cancer Res 1997;57:1007-12.
146. Nambu Y, Hughes SJ, Rehemtulla A, Hamstra D, Orringer MB, Beer DG. Lack of cell surface Fas/APO-1 expression in pulmonary adenocarcinomas. J Clin Invest 1998;101:1102-10.
147. Hiyama K, Hiyama E, Ishioka S, Yamakido M, Inai K, Gazdar AF, et al. Telomerase activity in small-cell and non-small-cell lung cancers. J Natl Cancer Inst 1995;87:895-902.
148. Albanell J, Lonardo F, Rusch V, Engelhardt M, Langenfeld J, Han W, et al. High telomerase activity in primary lung cancers: association with increased cell proliferation rates and advanced pathologic stage. J Natl Cancer Inst 1997;89:1609-15.
149. Yashima K, Litzky LA, Kaiser L, Rogers T, Lam S, Wistuba II, et al. Telomerase expression in respiratory epithelium during the multistage pathogenesis of lung carcinomas. Cancer Res 1997; 57:2373-7.
150. Belinsky SA, Nikula KJ, Palmisano WA, Michels R, Saccomanno G, Gabrielson E, et al. Aberrant methylation of p16(INK4a) is an early event in lung cancer and a potential biomarker for early diagnosis. Proc Natl Acad Sci 1998;95: 11891-6.
151. Esteller M, Sanchez-Cespedes M, Rosell R, Sidransky D, Baylin SB, Herman JG. Detection of aberrant promoter hypermethylation of tumor suppressor genes in serum DNA from non-small cell lung cancer patients. Cancer Res 1999;59:67-70.
152. Makos M, Nelkin BD, Lerman MI, Latif F, Zbar B, Baylin SB. Distinct hypermethylation patterns occur at altered chromosome loci in human lung and colon cancer. Proc Natl Acad Sci 1992; 89:1929-33.
153. Kohno T, Kawanishi M, Inazawa J, Yokota J. Identification of CpG islands hypermethylated in human lung cancer by the arbitrarily primed-PCR method. Hum Genet 1998;102:258-64.
154. Suzuki H, Ueda R, Takahashi T, Takahashi T. Altered imprinting in lung cancer. Nature Genet 1994;6:332-3.
155. Kondo M, Suzuki H, Ueda R, Osada H, Takagi K, Takahashi T, et al. Frequent loss of imprinting of the *H19* gene is often associated with its overexpression in human lung cancers. Oncogene 1995;10:1193-8.
156. Angeletti CA, Lucchi M, Fontanini G, Mussi A, Chella A, Ribechini A, et al. Prognostic significance of tumoral angiogenesis in completely resected late stage lung carcinoma (stage IIIA-N2): impact of adjuvant therapies in a subset of patients at high risk of recurrence. Cancer 1996;78:409-15.
157. Fontanini G, Lucchi M, Vignati S, Mussi A, Ciardiello F, De Laurentiis M, et al. Angiogenesis as a prognostic indicator of survival in non-small-cell lung carcinoma: a prospective study. J Natl Cancer Inst 1997;89:881-6.
158. Pastorino U, Andreola S, Tagliabue E, Pezzella F, Incarbone M, Sozzi G, et al. Immunocytochemical markers in stage I lung cancer: relevance to prognosis. J Clin Oncol 1997;15:2858-65.
159. Chandrachud LM, Pendleton N, Chisholm DM, Horan MA, Schor AM. Relationship between vascularity, age and survival in non-small-cell lung cancer. Br J Cancer 1997;76:1367-75.
160. Hanahan D, Folkman J. Patterns and emerging mechanisms of the angiogenic switch during tumorigenesis. Cell 1996;86:353-64.
161. Mattern J, Koomägi R, Volm M. Association of vascular endothelial growth factor expression with intratumoral microvessel density and turnout cell proliferation in human epidermoid lung carcinoma. Br J Cancer 1996;73:931-4.
162. Fontanini G, Vignati S, Lucchi M, Mussi A, Calcinai A, Boldrini L, et al. Neoangiogenesis and p53 protein in lung cancer: their prognostic role and their relation with vascular endothelial growth factor (VEGF) expression. Br J Cancer 1997;75: 1295-301.
163. Ambs S, Bennett WP, Merriam WG, Ogunfusika MO, Oser SM, Khan MA, et al. Vascular endothelial growth factor and nitric oxide synthase expression in human lung cancer and the relation to p53. Br J Cancer 1998;78:233-9.
164. Ohta Y, Watanabe Y, Murakami S, Oda M, Hayashi Y, Nonomura A, et al. Vascular endothelial growth factor and lymph node

metastasis in primary lung cancer. Br J Cancer 1997;76: 1041-5.

165. Volm M, Koomägi R, Mattern J. Prognostic value of vascular endothelial growth factor and its receptor Flt-1 in squamous cell lung cancer. Int J Cancer 1997;74:64-8.
166. Kieser A, Weich HA, Brandner G, Marme D, Kolch W. Mutant p53 potentiates protein kinase C induction of vascular endothelial growth factor expression. Oncogene 1994;9:963-9.
167. Mukhopadhyay D, Tsiokas L, Sukhatme VP. Wild-type p53 and v-Src exert opposing influences on human vascular endothelial growth factor gene expression. Cancer Res 1995;55:6161-5.
168. Takanami I, Imamura T, Hashizume T, Kikuchi K, Yamamoto Y, Yamamoto T, et al. Immunohistochemical detection of basic fibroblast growth factor as a prognostic indicator in pulmonary adenocarcinoma. Jpn J Clin Oncol 1996;26:293-7.
169. Volm M, Koomägi R, Mattern J, Stammler G. Prognostic value of basic fibroblast growth factor and its receptor (FGFR-1) in patients with non-small cell lung carcinomas. Eur J Cancer 1997;33:691-3.
170. Koukourakis MI, Giatromanolaki A, O'Byrne KJ, Comley M, Whitehouse RM, Talbot DC, et al. Platelet-derived endothelial cell growth factor expression correlates with tumour angiogenesis and prognosis in non-small-cell lung cancer. Br J Cancer 1997;75:477-81.
171. Arenberg DA, Keane MP, DiGiovine B, Kunkel SL, Morris SB, Xue YY, et al. Epithelial-neutrophil activating peptide (ENA-78) is an important angiogenic factor in non-small cell lung cancer. J Clin Invest 1998;102:465-72.
172. Arenberg DA, Kunkel SL, Polverini PJ, Glass M, Burdick MD, Strieter RM. Inhibition of interleukin-8 reduces tumorigenesis of human non-small cell lung cancer in SCID mice. J Clin Invest 1996;97:2792-802.
173. Arenberg DA, Kunkel SL, Polverini PJ, Morris SB, Burdick MD, Glass MC, et al. Interferon-γ-inducible protein 10 (IP-10) is an angiostatic factor that inhibits human non-small cell lung cancer (NSCLC) tumorigenesis and spontaneous metastases. J Exp Med 1996;184:981-92.
174. Sulzer MA, Leers MP, van Noord JA, Bollen EC, Theunissen PH. Reduced E-cadherin expression is associated with increased lymph node metastasis and unfavorable prognosis in non-small cell lung cancer. Am J Respir Crit Care Med 1998;157:1319-23.
175. Smythe WR, LeBel E, Bavaria JE, Kaiser LR, Albelda SM. Integrin expression in non-small cell carcinoma of the lung. Cancer Metastasis Rev 1995;14:229-39.
176. Adachi M, Taki T, Huang C, Higashiyama M, Doi O, Tsuji T, et al. Reduced integrin alpha3 expression as a factor of poor prognosis of patients with adenocarcinoma of the lung. J Clin Oncol 1998;16:1060-7.
177. Hirata T, Fukuse T, Naiki H, Hitomi S, Wada H. Expression of CD44 variant exon 6 in stage I non-small cell lung carcinoma as a prognostic factor. Cancer Res 1998;58:1108-10.
178. Clarke MR, Landreneau RJ, Finkelstein SD, Wu TT, Ohori P, Yousem SA. Extracellular matrix expression in metastasizing and nonmetastasizing adenocarcinomas of the lung. Hum Pathol 1997;28:54-9.
179. Kawano N, Osawa H, Ito T, Nagashima Y, Hirahara F, Inayama Y, et al. Expression of gelatinase A, tissue inhibitor of metalloproteinases-2, matrilysin, and trypsin(ogen) in lung neoplasms: an immunohistochemical study. Hum Pathol 1997;28:613-22.
180. Tokuraku M, Sato H, Murakami S, Okada Y, Watanabe Y, Seiki M. Activation of the precursor of gelatinase A/72 kDa type IV collagenase/MMP-2 in lung carcinomas correlates with the expression of membrane-type matrix metalloproteinase (MT-MMP) and with lymph node metastasis. Int J Cancer 1995;64: 355-9.
181. Anderson IC, Sugarbaker DJ, Ganju RK, Tsarwhas DG, Richards WG, Sunday M, et al. Stromelysin-3 is overexpressed by stromal elements in primary non-small cell lung cancers and regulated by retinoic acid in pulmonary fibroblasts. Cancer Res 1995;55:4120-6.
182. Hirano T, Franzén B, Kato H, Ebihara Y, Auer G. Genesis of squamous cell lung carcinoma: sequential changes of proliferation, DNA ploidy, and *p53* expression. Am J Pathol 1994;144: 296-302.
183. Betticher DC, Heighway J, Thatcher N, Hasleton PS. Abnormal expression of *CCND1* and *RB1* in resection margin epithelia of lung cancer patients. Br J Cancer 1997;75:1761-8.
184. Satoh Y, Ishikawa Y, Nakagawa K, Hirano T, Tsuchiya E. A follow-up study of progression from dysplasia to squamous cell carcinoma with immunohistochemical examination of p53 protein overexpression in the bronchi of ex-chromate workers. Br J Cancer 1997;75:678-83.
185. Li ZH, Zheng J, Weiss LM, Shibata D. c-k-*ras* and p53 mutations occur very early in adenocarcinoma of the lung. Am J Pathol 1994;144:303-9.
186. Brambilla E, Gazzeri S, Lantuejoul S, Coll JL, Moro D, Negoescu A, et al. p53 mutant immunophenotype and deregulation of p53 transcription pathway (Bcl2, Bax, and Waf1) in precursor bronchial lesions of lung cancer. Clin Cancer Res 1998; 4:1609-18.
187. Hung J, Kishimoto Y, Sugio K, Virmani A, McIntire DD, Minna JD, et al. Allele-specific chromosome 3p deletions occur at an early stage in the pathogenesis of lung carcinoma. JAMA 1995;273:558-63.
188. Kishimoto Y, Sugio K, Hung JY, Virmani AK, McIntire DD, Minna JD, et al. Allele-specific loss in chromosome 9p loci in preneoplastic lesions accompanying non-small-cell lung cancers. J Natl Cancer Inst 1995;87:1224-9.
189. Sugio K, Kishimoto Y, Virmani AK, Hung JY, Gazdar AF. K-*ras* mutations are a relatively late event in the pathogenesis of lung carcinomas. Cancer Res 1994;54:5811-5.
190. Wistuba II, Behrens C, Milchgrub S, Bryant D, Hung J, Minna JD, et al. Sequential molecular abnormalities are involved in the multistage development of squamous cell lung carcinoma. Oncogene 1999;18:643-50.
191. Westra WH, Baas IO, Hruban RH, Askin FB, Wilson K, Offerhaus GJ, et al. K-*ras* oncogene activation in atypical alveolar hyperplasias of the human lung. Cancer Res 1996;56:2224-8.
192. Mao L, Lee JS, Kurie JM, Fan YH, Lippman SM, Lee JJ, et al. Clonal genetic alterations in the lungs of current and former smokers [see comments]. J Natl Cancer Inst 1997;89:857-62.
193. Wistuba II, Lam S, Behrens C, Virmani AK, Fong KM, LeRiche J, et al. Molecular damage in the bronchial epithelium of current and former smokers. J Natl Cancer Inst 1997;89:1366-73.
194. Smith AL, Hung J, Walker L, Rogers TE, Vuitch F, Lee E, et al. Extensive areas of aneuploidy are present in the respiratory epithelium of lung cancer patients. Br J Cancer 1996;73:203-9.
195. Franklin WA, Gazdar AF, Haney J, Wistuba II, La Rosa FG, Kennedy T, et al. Widely dispersed *p53* mutation in respiratory epithelium: a novel mechanism for field carcinogenesis. J Clin Invest 1997;100:2133-7.

196. Spivack SD, Fasco MJ, Walker VE, Kaminsky LS. The molecular epidemiology of lung cancer. Crit Rev Toxicol 1997;27: 319-65.
197. Tokuhata GK, Lilienfeld AM. Familial aggregation of lung cancer among hospital patients. Public Health Rep 1963;78:277-83.
198. Ooi WL, Elston RC, Chen VW, Bailey-Wilson JE, Rothschild H. Increased familial risk for lung cancer. J Natl Cancer Inst 1986;76:217-22.
199. Wolf CR, Smith CA, Forman D. Metabolic polymorphisms in carcinogen metabolising enzymes and cancer susceptibility. Br Med Bull 1994;50:718-31.
200. Jourenkova-Mironova N, Wikman H, Bouchardy C, Voho A, Dayer P, Benhamou S, et al. Role of glutathione S-transferase GSTM1, GSTM3, GSTP1 and GSTT1 genotypes in modulating susceptibility to smoking-related lung cancer. Pharmacogenetics 1998;8:495-502.
201. Le Marchand L, Sivaraman L, Pierce L, Seifried A, Lum A, Wilkens LR, et al. Associations of CYP1A1, GSTM1, and CYP2E1 polymorphisms with lung cancer suggest cell type specificities to tobacco carcinogens. Cancer Res 1998;58:4858-63.
202. Wu X, Zhao Y, Honn SE, Tomlinson GE, Minna JD, Hong WK, et al. Benzo[a]pyrene diol epoxide-induced 3p21.3 aberrations and genetic predisposition to lung cancer. Cancer Res 1998; 58:1605-8.
203. Sone S, Takashima S, Li F, Yang Z, Honda T, Maruyama Y, et al. Mass screening for lung cancer with mobile spiral computed tomography scanner [see comments]. Lancet 1998;351:1242-5.
204. Henschke CI, McCauley DI, Yankelevitz DF, Naidich DP, McGuinness G, Miettinen OS, et al. Early Lung Cancer Action Project: overall design and findings from baseline screening [see comments]. Lancet 1999;354:99-105.
205. Ahrendt SA, Chow JT, Xu LH, Yang SC, Eisenberger CF, Esteller M, et al. Molecular detection of tumor cells in bronchoalveolar lavage fluid from patients with early stage lung cancer [see comments]. J Natl Cancer Inst 1999;91:332-9.
206. Younes M, Brown RW, Stephenson M, Gondo M, Cagle PT. Overexpression of Glut1 and Glut3 in stage I nonsmall cell lung carcinoma is associated with poor survival. Cancer 1997;80: 104651.
207. Ito T, Noguchi Y, Satoh S, Hayashi H, Inayama Y, Kitamura H. Expression of facilitative glucose transporter isoforms in lung carcinomas: its relation to histologic type, differentiation grade, and tumor stage [see comments]. Mod Pathol 1998;11:437-43.