A Subset of Lung Adenocarcinomas and Atypical Adenomatous Hyperplasia–Associated Foci Are Genotypically Related

An *EGFR, HER2*, and K-*ras* Mutational Analysis

Giuliana Sartori, PhD,[1] Alberto Cavazza, MD,[5] Federica Bertolini, MD,[2] Lucia Longo, MD,[3] Alessandro Marchioni, MD,[4] Matteo Costantini, MD,[1] Fausto Barbieri, MD,[2] Mario Migaldi, MD, PhD,[1] and Giulio Rossi, MD[1]

Key Words: Atypical adenomatous hyperplasia; Lung; Adenocarcinoma; Epidermal growth factor receptor; *EGFR*; K-*ras*; *HER2*

DOI: 10.1309/THU13F3JRJVWLM30

Abstract

Atypical adenomatous hyperplasia (AAH) is considered the preinvasive lesion of pulmonary adenocarcinoma, and mutations of EGFR, HER2, *and K-*ras *are involved in the early stage of lung adenocarcinoma carcinogenesis, also predicting clinical response to anti-EGFR small molecule inhibitors. We analyzed 18 cases of primary lung adenocarcinoma with concomitant AAH foci from 13 patients for mutations of* EGFR *(exons 18-21),* HER2 *(exons 19-20), and K-*ras *(exon 2) by direct sequencing polymerase chain reaction. Among mutated cases, concordant mutations of* EGFR *or K-*ras *in adenocarcinoma and related AAH were observed in 5 (63%) of 8 cases. In particular, 3 of 4 adenocarcinomas with* EGFR *mutations (all L858R point mutations in women, never or former smokers) had a concomitant and identical mutation in AAH, and 2 of 4 adenocarcinomas with K-*ras *mutations (both at codon 12 in women, a never and a current smoker) showed the same mutation in concomitant AAH. All cases were wild-type for* HER2. *Mutations of* EGFR *and K-*ras *genes represent an early event in lung adenocarcinomagenesis, and AAH convincingly seems to be a precursor lesion in a subset of cases of adenocarcinoma.*

Atypical adenomatous hyperplasia (AAH) is a recently introduced preinvasive lesion in the 2004 World Health Organization (WHO) lung tumor classification as a possible precursor of adenocarcinoma.[1] AAH is a focal, slightly atypical proliferation of monolayer cuboidal pneumocytes with distinct margins, measuring less than 5 mm in maximum diameter, and appears as a solitary or multifocal small ground-glass nodule in a high-resolution computed tomography scan.[1-6] Some authors subdivide AAH into low and high grade, but this issue remains controversial, and subdivision is not recommended by the 2004 WHO classification. The differential diagnosis between AAH and nonmucinous-type bronchioloalveolar carcinoma (nmBAC) may be difficult, and distinction is basically related to the size of the lesion and the cytologic characteristics of the cells.[1-4] Although AAH foci are mainly found in patients with multiple synchronous lung adenocarcinomas, it is hard to prove that the former is a precursor of the latter. Previous studies have highlighted the presence of several genetic alterations in AAH and then demonstrated the neoplastic nature of this lesion.[1-5,7-23]

Epidermal growth factor receptor (EGFR) is a receptor tyrosine kinase (RTK) deeply involved in the carcinogenesis of non–small cell lung cancer (NSCLC), mainly adenocarcinomas.[24] A subset of patients with NSCLC experienced a significant improvement of symptoms and/or survival using orally dispensed small molecules acting as EGFR selective inhibitors (ie, erlotinib and gefitinib).[25-27] In addition, up to 20% of unselected patients with chemorefractory NSCLC had a partial to complete regression of the mass using EGFR inhibitors.[28] Female sex, a history of never smoking, Asian ethnicity, and a histologic finding of adenocarcinoma with bronchioloalveolar features are the most important

clinicopathologic parameters for predicting tumor response to EGFR RTK inhibitors.[25-27,29-32]

Among the biologic predictive markers studied until now, *EGFR* somatic mutations occurring at exons 19 and 21 (encoding for the EGFR RTK domain) represent the most promising predictive factor for drug efficacy with dramatic clinical responses,[33-45] whereas the finding of a K-*ras* mutation (mainly involving codons 12 and 13 at exon 2) is the single most important parameter for foreseeing primary resistance to tumor drugs.[46-49] In addition, several studies have found *HER2* mutations in a small (about 2%) subset of patients with adenocarcinoma showing the same clinicopathologic characteristics of *EGFR*-mutated tumors, also pointing out that *EGFR*, *HER2*, and K-*ras* mutations are mutually exclusive.[50-52] Mutations involving all of these genes are thought to represent early events in the carcinogenesis of lung adenocarcinoma in never (*EGFR* and *HER2*) and current smokers (K-*ras*).

In this study, we analyzed *EGFR, HER2*, and K-*ras* mutational events in 18 cases of adenocarcinoma and concomitant AAH from 13 patients to compare their genetic alterations and to evaluate their possible role as an early molecular step, which may support the AAH–pulmonary adenocarcinoma sequence.

Materials and Methods

We obtained 18 cases of primary lung adenocarcinoma, associated with foci of AAH obtained from 13 patients, from the files of the Section of Pathologic Anatomy, Hospital Policlinico, Modena, Italy, from January 2003 to March 2007. Case series included 3 patients with multiple tumors (1 patient with 4 distinct tumors in 3 different lobes, 1 with 2 tumors in the same lobe and 1 with 2 tumors in different lobes). All available slides were reviewed at a multiheaded microscope by 3 pathologists (A.C., M.C., and G.R.) according to the criteria set by the WHO lung tumors classification.[1] All cases of AAH consisted of incidental findings observed in pulmonary resections for primary lung cancer.

All cases consisted of a surgical specimen (10 lobectomies, 6 wedge resections, and 1 pneumonectomy) that was routinely fixed in 10% buffered formalin. Only cases in which foci of AAH were present in at least 5 consecutive 5-μm serial sections were included in the study to obtain sufficient cells for DNA isolation. After gross examination, the sampled tissues were embedded in paraffin blocks. A mean of 12 H&E-stained slides (range, 6-22 slides) per case were available.

Patients were subdivided into never smokers, former smokers (stopped smoking at least 3 years before diagnosis), and current smokers. Clinical data were obtained from pathologic reports, clinical charts, referring physicians, or the patients' families. The study did not require approval by the local ethics committee or institutional review board because the samples were coded and the names of patients not revealed.

Mutational Analysis

For the study, 5-μm thick sections obtained from a representative paraffin-embedded block were deparaffinized by xylene, and tumor DNA was extracted by using a manual microdissection method under a microscope (Nikon E600, Tokyo, Japan). Microdissected tumor cells were subjected to Proteinase K treatment in a digestion buffer (50 mmol/L of tris(hydroxymethyl)aminomethane [pH 8.5], 1 mmol/L of EDTA, and 0.5% polysorbate 20) and then incubated overnight at 37°C.

Polymerase chain reaction (PCR) was performed in 20-μL reactions containing 2.0 μL of DNA, 2 μL of commercial PCR buffer (Applied Biosystems, Foster City, CA), 1.0 to -1.5 mmol/L of magnesium chloride, 200 μmol/L of each deoxynucleoside triphosphate, 20 pmol of each primer, and 3 U of AmpliTaq gold polymerase (Applied Biosystems). The PCR reaction was carried out on a Uno II Thermoblock (Biometra, Gottingen, Germany). Initial denaturation at 94°C for 10 minutes was followed by 41 cycles and a final extension step (7 minutes at 72°C). The cycles included denaturation at 95°C for 1 minute, annealing at 55°C to 58°C for 1 minute, and extension at 72°C for 2 minutes. The amplified DNA was electrophoresed on 2% agarose gel for 1 hour at 110 V. The amplification products were then purified by using the MinElute PCR Purification Kit (Qiagen, Hilden, Germany) as indicated by the manufacturer's instructions. PCR products were then sequenced in both directions with the ABI Prism BigDye Terminator v1.1 Cycle Sequencing kit (Applied Biosystems), using the same primers as used for PCR. PCR products were finally purified by Centri-Sep Spin Columns (Applied Biosystems) and subsequently run on the ABI Prism 310 automatic sequencer (Applied Biosystems). The data were analyzed with Sequencing Analysis 5.2 Software (Applied Biosystems). The forward and reverse oligonucleotide primers used to amplify *EGFR* exons 18, 19, 20, and 21; *HER2* exons 19 and 20, and K-*ras* exon 2 are listed in **Table 1**.

Results

The clinicopathologic characteristics of the cases are summarized in **Table 2**. There were 18 cases of adenocarcinoma and AAH-related foci from 13 patients, 8 women and 5 men with a mean age of 59.6 years (range, 43-73 years). Seven patients were current smokers, 3 had no history of smoking, and 3 were former smokers. Three patients (all

DOI: 10.1309/THU13F3JRJVWLM30

Table 1
Oligonucleotide Primers Used

Genes and Exons/Primer	Fragment Size (base pairs)	Annealing Temperature (°C)
K-*ras*		
Exon 2	176	52
Forward; 5'-CAT GTT CTA ATA TAG TCA CA -3'		
Reverse: 5'-AAC AAG ATT TAC CTC TAT TG -3'		
EGFR		
Exon 18	381	65
Forward: 5'-CAA GTG CCG TGT CCT GGC ACC CAA GC-3'		
Reverse: 5'-CCA AAC ACT CAG TGA AAC AAA GAG-3'		
Exon 19	297	57
Forward; 5'-GTG CAT CGC TGG TAA CAT CC-3'		
Reverse; 5'-TGT GGA GAT GAG CAG GGT CT-3'		
Exon 20	372	56
Forward: 5'-ATC GCA TTC ATG CGT CTT CA-3'		
Reverse: 5'-ATC CCC ATG GCA AAC TCT TG-3'		
Exon 21	348	55
Forward: 5'-GCT CAG AGC CTG GCA TGA A-3'		
Reverse; 5'-CAT CCT CCC CTG CAT GTG T-3'		
HER2		
Exon 19	187	67
Forward: 5'-GCC CAC GCT CTT CTC ACT CA-3'		
Reverse: 5'-ATG GGG TCC TTC CTG TCC TC-3'		
Exon 20	342	67
Forward: 5'-GCC ATG GCT GTG GTT TGT GAT GG-3'		
Reverse: 5'-ATC CTA GCC CCT TGT GGA CAT AGG-3'		

EGFR, epidermal growth factor receptor.

women) had multiple tumors (cases 2, 3, and 7). By the end of the study, 8 patients were well without disease, 4 were alive with disease, and 1 died of disease. The mean follow-up was 24.9 months (range, 2-74 months). Among adenocarcinomas, there were 10 classic acinar type, 3 nm-BAC, 2 mixed acinar–mucinous BAC, 2 papillary type, and 1 mucinous BAC. Results of mutational status studies for *EGFR*, *HER2,* and K-*ras* are listed in **Table 3**.

Four cases of adenocarcinoma (22%; 3 nm-BAC and 1 acinar type) harbored *EGFR* mutations, and all consisted of

Table 2
Clinicopathologic Characteristics

Case No./ Sex/Age (y)	Smoking Status	Stage/y	Site	Histologic Type	Therapy	Follow-up (mo)	Other Malignancies/y
1/M/58	Current	pT2N1/2004	RUL	Papillary adc	L	AW (32)	No
2/F/53	No	pT1N0/2003	RLL	Acinar adc G2	L	AW (47)	Breast/1995; thyroid/1999
		pT1N0/2004	LLL	nmBAC	WR + CT		
3/F/68	Former	pT1N0/2004	RUL	mBAC	L	AW (33)	No
		pT1N0/2004	RUL	nmBAC			
4/F/69	Current	pT1N1/2004	LLL	adc-mBAC	L	AW (31)	No
5/M/61	Current	pM1/2004	RP	adc-mBAC	P + CT	DOD (7)	No
6/M/43	Current	pT2N0/2004	LLL	Acinar adc G2	L + CT	AWD (28)	No
7/F/47	No	pT1N0/2001	LLL	Acinar adc G1	L	AWD (74)	Breast/1992; thyroid/2003
		pT1N0/2004	RUL	nmBAC	WR		
		pT1N0/2004	RUL	Papillary adc	WR		
		pT2N1/2006	RLL	Acinar adc G3	WR + CT + erlotinib		
8/F/67	Former	pT2N0/2005	LUL	Acinar adc G2	L	AW (25)	No
9/F/64	Current	pT1N0/2005	RLL	Acinar adc G3	L + CT	AWD (19)	Breast/2006
10/F/44	Current	pT1N0/2005	RUL	Acinar adc G2	L	AW (18)	No
11/F/61	Former	pT2N0/2006	RUL	Acinar adc G2	WR	AW (5)	No
12/M/67	Current	pM1/2007	RLL/RML	Acinar adc G2	WR + CT	AWD (3)	No
13/M/73	No	pT2N0/2007	RUL	Acinar adc G3	L	AW (2)	Liposarcoma/1996; thyroid/2002; GIST/2005

adc, adenocarcinoma; AW, alive and well; AWD, alive with disease; CT, chemotherapy; DOD, died of disease; G, grade; GIST, gastrointestinal stromal tumor; L, lobectomy; LLL, left lower lobe; LUL, left upper lobe; mBAC, mucinous bronchioloalveolar carcinoma; nmBAC, nonmucinous bronchioloalveolar carcinoma; P, pneumonectomy; RLL, right lower lobe; RML, right middle lobe; RP, right pleura; RUL, right upper lobe; WR, wedge resection.

DOI: 10.1309/THU13F3JRJVWLM30

Table 3
Distribution of *EGFR, HER2*, and K-*ras* Mutations in Lung Adenocarcinoma and Related AAH

Case No.	Histologic Type	*EGFR*	*HER2*	K-*ras*
1	Papillary adenocarcinoma	wt	wt	G13D
	AAH	wt	wt	wt
2	Acinar adenocarcinoma G2	wt	wt	wt
	AAH	wt	wt	wt
	nmBAC	L858R	wt	wt
	AAH	L858R	wt	wt
3	mBAC	wt	wt	G12D
	AAH	wt	wt	wt
	nmBAC	L858R	wt	wt
	AAH	L858R	wt	wt
4	Adenocarcinoma-mBAC	wt	wt	G12S
	AAH	wt	wt	G12S
5	Adenocarcinoma-mBAC	wt	wt	wt
	AAH	wt	wt	wt
6	Acinar adenocarcinoma G2	wt	wt	wt
	AAH	wt	wt	wt
7	Acinar adenocarcinoma G1	wt	wt	wt
	AAH	wt	wt	wt
	nmBAC	L858R	wt	wt
	AAH	L858R	wt	wt
	Papillary adenocarcinoma	wt	wt	G12C
	AAH	wt	wt	G12C
	Acinar adenocarcinoma G3	wt	wt	wt
	AAH	wt	wt	wt
8	Acinar adenocarcinoma G2	wt	wt	wt
	AAH	wt	wt	wt
9	Acinar adenocarcinoma G3	wt	wt	wt
	AAH	wt	wt	wt
10	Acinar adenocarcinoma G2	wt	wt	wt
	AAH	wt	wt	wt
11	Acinar adenocarcinoma G2	wt	wt	wt
	AAH	wt	wt	wt
12	Acinar adenocarcinoma G2	wt	wt	wt
	AAH	wt	wt	wt
13	Acinar adenocarcinoma G3	L858R	wt	wt
	AAH	wt	wt	wt

AAH, atypical adenomatous hyperplasia; EGFR, epidermal growth factor receptor; G, grade; mBAC, mucinous bronchioloalveolar carcinoma; nmBAC, nonmucinous bronchioloalveolar carcinoma; wt, wild type.

puntiform mutations in exon 21 (L858R) **Image 1A**, **Image 1B**, **Image 1C**, and **Image 1D**. Three of these cases had an identical mutation in AAH-related foci, whereas in the remaining case, AAH-related foci were of the wild type. All of these *EGFR* mutations occurred in women who were never (3 cases) or former smokers. Mutations of the K-*ras* gene were observed in 4 cases. Three cases had mutations at codon 12 (G12C, G12D, G12S) and 1 at codon 13. Half of these cases had an identical mutation also in AAH-related lesions **Image 1E**, **Image 1F**, **Image 1G**, and **Image 1H**, while 2 cases of concomitant AAH were of the wild type. Mutations of K-*ras* were found in 2 current, 1 former, and 1 never smoker (3 women and 1 man). Two patients had papillary type adenocarcinoma, 1 had mucinous BAC, and 1 had mixed acinar-mucinous BAC.

None of the tumors with *EGFR* mutations had K-*ras* mutations, and no mutations of *HER2* were observed in adenocarcinomas or AAH. Among adenocarcinoma and related AAH, concordant mutations of *EGFR* (3 of 4) or K-*ras* (2 of 4) were observed in 5 (63%) of 8 cases.

Multiple tumors were metachronous in 2 cases (cases 2 and 7) and synchronous in 1 (case 3). Of note, none of those cases had an identical mutational setup. In multiple tumors, all *EGFR* mutations occurred in nm-BAC, whereas K-*ras* mutations were found in 1 papillary adenocarcinoma and in 1 mucinous BAC.

Overall, concordant mutational findings between adenocarcinoma and AAH-related foci were observed in 15 cases (83%; 5 cases with *EGFR* or K-*ras* mutations and 10 with wild-type genome), whereas 3 cases had discordant mutations of *EGFR* (1 case) or K-*ras* (2 cases).

Discussion

AAH was first described in detail by Shimosato et al[53] and Kodama et al[54] as a lesion frequently occurring in lung resections for adenocarcinomas. Basically, this association and the close morphologic resemblance with nmBAC remain the most important evidence supporting the adenoma-carcinoma sequence in the multistep tumor pathway of lung adenocarcinoma.[1-5,55-61]

Several genetic studies have confirmed the neoplastic nature of AAH. In particular, the finding of loss of heterozygosity at the 3p, 9p, and 17p loci[13,16-18] and the K-*ras* mutations in 155 to 50% of such lesions[8,14,62,63] seem to convincingly support the close genetic relationship between adenocarcinoma and concomitant AAH foci.

More recently, Morandi et al[23] showed the lack of a clonal relationship between AAH and associated tumors in 9 of 13 informative cases analyzed by direct sequencing of mitochondrial DNA. However, a close genetic correlation was instead found in a small subset of adenocarcinomas and AAH-related lesions.[23] Based on previous work,[23,64] identical genetic alterations between adenocarcinoma and AAH may suggest the precursor role of AAH in adenocarcinomagenesis but alternatively may represent a spread of tumor cells from the adenocarcinoma, mainly when AAH is of high grade. The finding in our study of mutations in adenocarcinoma or adenocarcinoma and concomitant AAH, but not in AAH alone, seems to favor the role of AAH as a precursor of adenocarcinoma (through the adenoma-carcinoma progression) rather than the result of tumor cells spreading (through the carcinoma-adenoma sequence).

The recent discovery of *EGFR* mutations in lung tumors sensitive to small molecule tyrosine kinase inhibitors (TKI) targeting EGFR (ie, gefitinib and erlotinib) has generated great interest in lung carcinogenesis. *EGFR* mutations are, in fact, more frequent in female nonsmokers with histologic findings of adenocarcinoma and possibly of Asian ethnicity.[33-45]

DOI: 10.1309/THU13F3JRJVWLM30

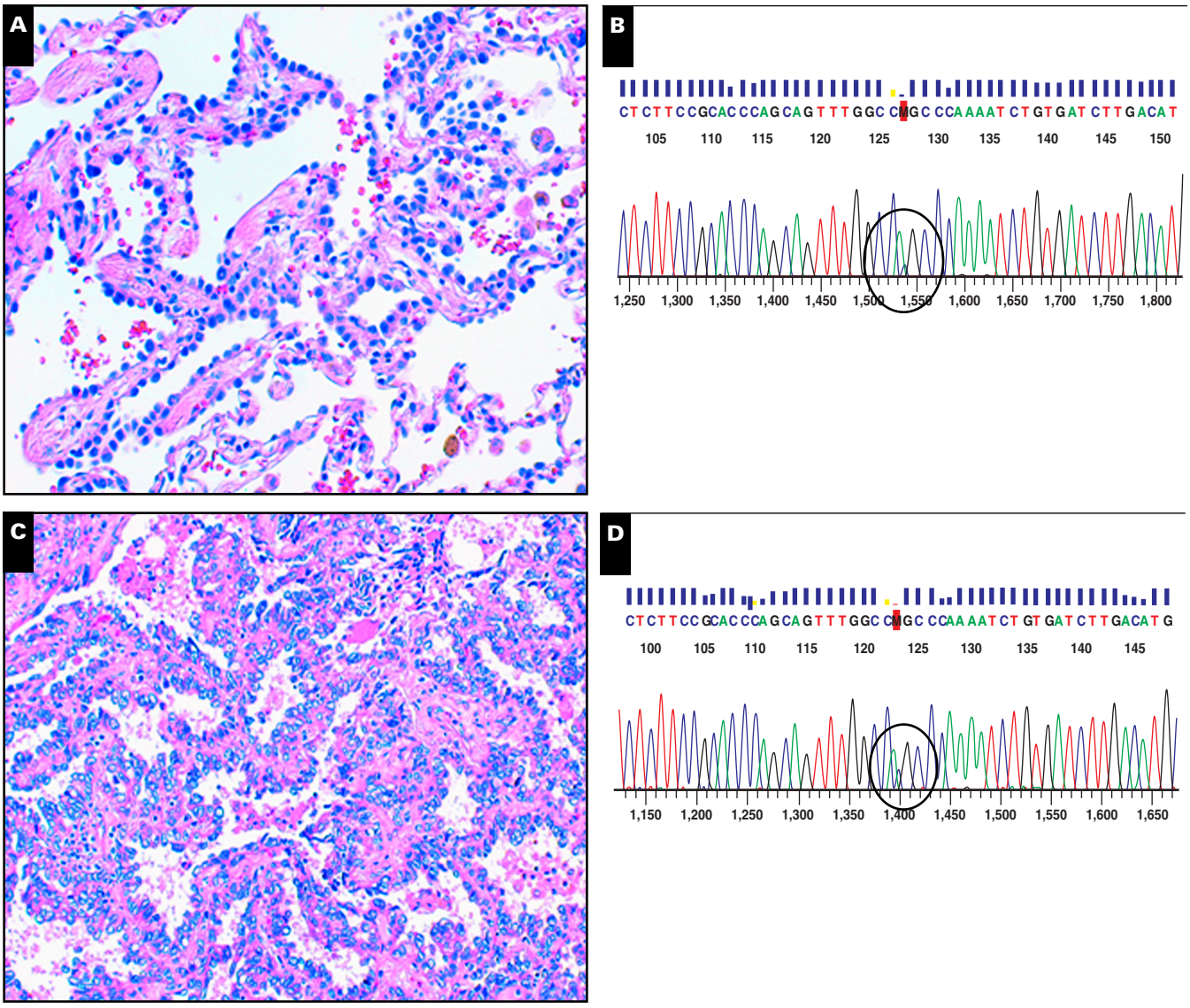


Image 1 **A**, **B**, **C**, and **D** (Case 7), Atypical adenomatous hyperplasia (AAH) (**A**, H&E, ×125) with *EGFR* exon 21 puntiform mutation (**B**, electrophoretogram; circle). The associated nonmucinous-type bronchioloalveolar carcinoma (**C**, H&E, ×125) had an identical *EGFR* mutation (**D**, electrophoretogram; circle).

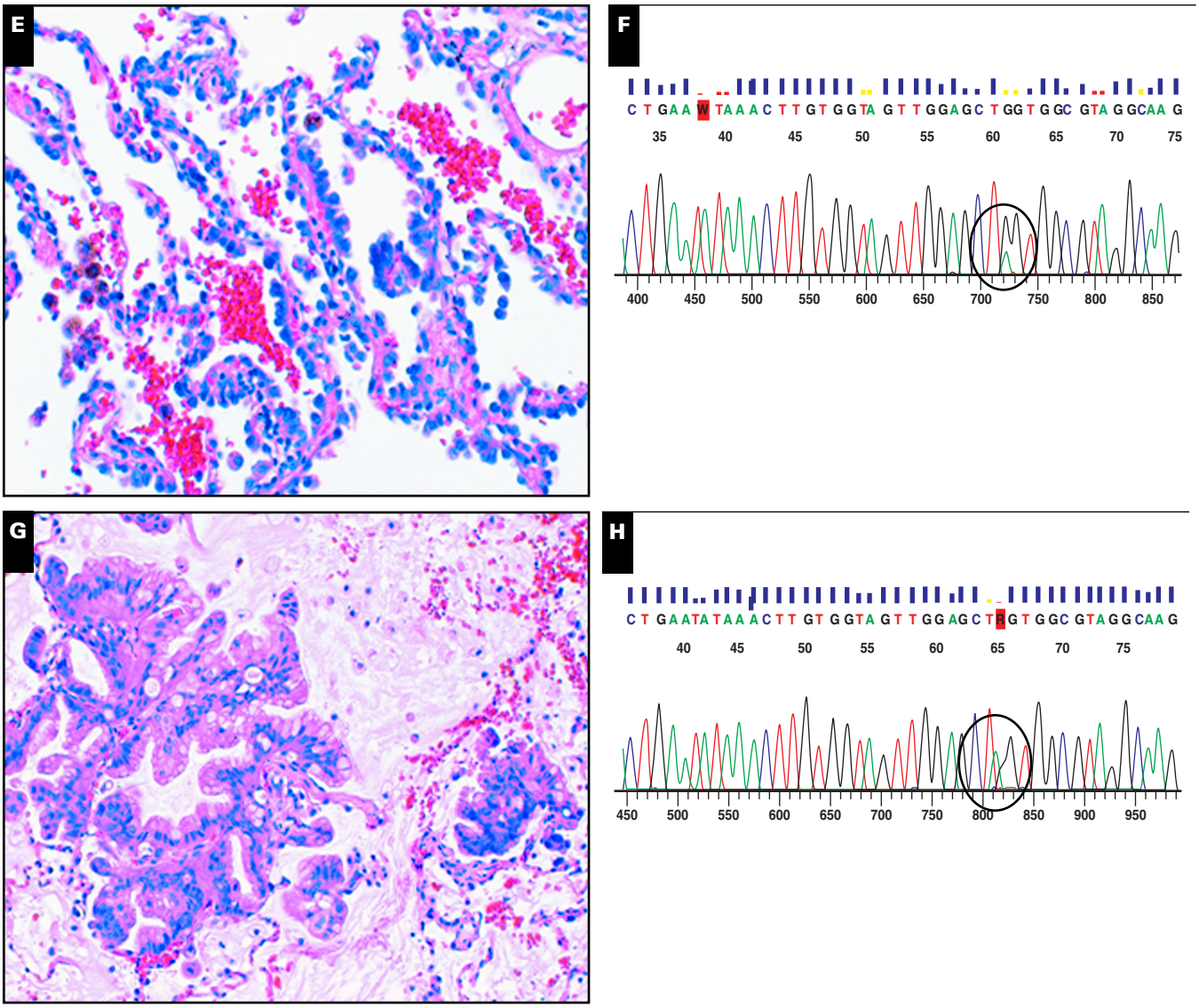
In addition, occurrence of *EGFR* and K-*ras* mutations in lung cancer seems to be mutually exclusive, K-*ras* mutations being more frequently observed in smokers and representing a sign of primary resistance to TKI.[46-49,65,66]

Mutational events involving *HER2/neu* have been observed in a minor subset of NSCLCs in patients with the same clinicopathologic characteristics as patients with *EGFR* mutations. *HER2* mutations were mutually exclusive with *EGFR* and K-*ras*.[51,52]

When altered, all of these genes are thought to be involved in the early stage of pathogenesis of pulmonary adenocarcinoma, but only a few studies have investigated mutations of *EGFR*, *HER2*, and K-*ras* in adenocarcinoma and related AAH, and they have controversial results.[67-69] Yatabe et al[67] found *EGFR* mutations in 2 of 7 AAH cases, 1 of which was identical to the concomitant adenocarcinoma. By contrast, Haneda et al[68] found no *EGFR* mutations in the 5 tested AAH lesions, and Yoshida et al[69] found *EGFR* mutations in 1 (3%) of 35 AAH lesions and K-*ras* mutations in 8 (27%) of 30 AAH lesions among patients with multiple lung tumors. However, identical mutations of neither *EGFR* nor K-*ras* were observed in AAH and related tumors.[69]

In this study, we analyzed 18 cases of AAH and concomitant adenocarcinoma (obtained from 13 patients) for *EGFR*, *HER2*, and K-*ras* mutations. A mutational event was found in 8 cases (44%), 4 cases each involving *EGFR* and K-*ras*, whereas *HER2* was of the wild type in all cases. Mutations were mutually exclusive. An identical *EGFR* mutation on

DOI: 10.1309/THU13F3JRJVWLM30



E, **F**, **G**, and **H**, (Case 4), AAH (**E**, H&E, ×125) and concurrent adenocarcinoma (**G**, H&E, ×125) with K-*ras* mutation at codon 12 (**F** and **H**, electrophoretogram, respectively; circles).

exon 21 (L858R) among AAH and concomitant adenocarcinoma was observed in 3 of 4 cases; all of these patients were women with nmBAC (2 never and 1 former smoker). Four adenocarcinomas had K-*ras* mutations on exon 2 (3 at codon 12 and 1 at codon 13), 2 of which showed an identical mutation with the concurrent AAH. Mutations occurring only in AAH were not observed.

Our results support the data by Yatabe et al,[67] suggesting the early role of *EGFR* in the pathogenesis of a subset of lung adenocarcinomas possibly originating from the terminal respiratory unit because all *EGFR*-mutated AAH lesions were associated with nmBAC, the prototype of such peripheral type of adenocarcinoma. Although our case series is too limited to make conclusive remarks, the finding of all *EGFR* mutations characterized by the leucine to arginine substitution at codon 858 (L858R) on exon 21 and mainly involving nmBAC is in agreement with a previous study on 860 NSCLCs by Marchetti et al.[65] In this large study of Italian patients, the authors found that the L858R *EGFR* mutation was the most frequently observed and more than two thirds of *EGFR* mutations were of the nmBAC histologic type.

The finding of an identical K-*ras* mutation in 2 of 4 adenocarcinoma and AAH lesions (both women, 1 never smoker and 1 current smoker) further suggest the role of K-*ras* as another gene altered early in lung adenocarcinoma, not only in current smokers, but also in a minority of never smokers.[70-72]

Interestingly, Kim et al[73] recently underscored the significant relationship between the papillary subtype of adenocarcinoma and the response to gefitinib. However, we identified K-*ras* mutations, a biologic predictor of resistance to TKI, in both

DOI: 10.1309/THU13F3JRJVWLM30

cases of papillary adenocarcinoma present in our series (1 man, a current smoker, and 1 woman, a never smoker). The papillary variant of adenocarcinoma may result in a challenging and poorly reproducible diagnosis. The true papillary variant of adenocarcinoma is uncommon and originally was associated with men who were current smokers and with a poor prognosis.[74,75] But papillary structures are frequently found in nmBAC, with which there is striking morphologic overlap,[76,77] and more recent studies on papillary adenocarcinoma found a close relationship with nonsmoking women.[78] Of note, all of these features (nmBAC, female sex, and nonsmoking history) are significantly related to *EGFR* mutations and responsiveness to TKI.[28-43]

One patient (case 7) had 4 lung adenocarcinomas during a period of 6 years. All the tumors were associated with AAH in the adjacent normal pulmonary parenchyma, and an identical mutation involving *EGFR* and K-*ras* was observed in AAH and adenocarcinoma in 2 of 4 lesions. Similarly, case 3 experienced the occurrence of 2 lung tumors with various mutational setups for *EGFR* and K-*ras*, and case 2 had an *EGFR*-mutated nmBAC and a wild-type acinar adenocarcinoma. In both tumors in case 2, the AAH mutational setup was concordant with that of the adenocarcinoma. These findings further highlight the genetic heterogeneity of multiple lung tumors occurring in the same patient.[79] In addition, one of these patients (case 7) was the only one undergoing treatment with EGFR TKI (erlotinib), but without clinical response. The patient started erlotinib treatment after the third tumor occurrence when molecular analysis showed a K-*ras* mutation in the lung tumor. Because patients with adenocarcinoma tend to have multiple tumors at a significant rate,[1,4,5,57] the finding of different mutations in multiple tumors ("field effect" cancerization) underscores the importance of testing the biologic characteristics of each synchronous or metachronous multiple lung tumor in order to provide molecular therapies when the neoplasm is more sensitive to EGFR TKI.[80,81]

We have shown the presence of concordant mutations involving *EGFR* and K-*ras* in a subgroup of adenocarcinoma and concomitant AAH lesions. This finding further supports the role of these genes in the early stage of pathogenesis of lung adenocarcinoma and suggests that AAH may be a precursor lesion of lung adenocarcinoma. Lack of *HER2* mutations is consistent with the involvement of this gene in a limited number of NSCLCs. The finding of different mutations in multiple tumors reinforces the idea that it is crucial to study each independently occurring lung tumor and to start TKI when neoplastic molecular characteristics may predict drug sensitivity; the preliminary comparable clinical results between chemotherapy and EGFR inhibitors in advanced NSCLC also reinforce use of these measures.[82,83]

From the Sections of [1]Pathologic Anatomy and [2]Oncology, Azienda Policlinico, Modena, Italy; [3]Oncology Division, Hospital "Ramazzini," Carpi, Italy; [4]Operative Unit of Pulmonology, Civic Hospital of Mirandola, Mirandola, Italy; and [5]Operative Unit of Pathologic Anatomy, Azienda Santa Maria Nuova, Reggio Emilia, Italy.

Address reprint requests to Dr Rossi: Section of Pathologic Anatomy, Azienda Policlinico, Via del Pozzo, 71- 41100- Modena, Italy.

References

1. Travis WD, Brambilla E, Muller-Hermelink HK, et al, eds. *Pathology and Genetics of Tumours of the Lung, Pleura, Thymus and Heart*. Lyon, France: IARC Press; 2004. *World Health Organization Classification of Tumours*.
2. Kerr KM. Pulmonary preinvasive neoplasia. *J Clin Pathol*. 2001;54:257-271.
3. Kerr KM. Morphology and genetics of pre-invasive pulmonary disease. *Curr Diagn Pathol*. 2004;10:259-268.
4. Colby TV, Wistuba II, Gazdar AF. Precursors to pulmonary neoplasia. *Adv Anat Pathol*. 1998;4:205-215.
5. Lopez JI, Colby TV, Gazdar AF. Current status of small peripheral adenocarcinomas of the lung and their importance to pathologists. *Ann Diagn Pathol*. 2005;9:115-122.
6. Park CM, Goo JM, Lee HJ, et al. CT findings of atypical adenomatous hyperplasia in the lung. *Korean J Radiol*. 2006;7:80-86.
7. Kerr KM, Carey FA, King G, et al. Atypical alveolar hyperplasia: relationship with pulmonary adenocarcinoma, p53, and cerbB2 expression. *J Pathol*. 1994;174:249-256.
8. Westra WH, Baas IO, Hruban RH, et al. K-ras oncogene activation in atypical alveolar hyperplasias of the human lung. *Cancer Res*. 1996;56:2224-2228.
9. Mori M, Tezuka F, Chiba R, et al. Atypical adenomatous hyperplasia and adenocarcinoma of the human lung: their heterology in form and analogy in immunohistochemical characteristics. *Cancer*. 1996;77:665-674.
10. Kitamura H, Kameda Y, Ito T, et al. Cytodifferentiation of atypical adenomatous hyperplasia and bronchioloalveolar lung carcinoma: immunohistochemical and ultrastructural studies. *Virchows Arch*. 1997;431:415-424.
11. Kurasono Y, Ito T, Kameda Y, et al. Expression of cyclin D1, retinoblastoma gene protein, and p16MTS1 protein in atypical adenomatous hyperplasia and adenocarcinoma of the lung: an immunohistochemical analysis. *Virchows Arch*. 1998;432:207-215.
12. Slebos RJ, Baas IO, Clement MJ, et al. p53 alterations in atypical alveolar hyperplasia of the human lung. *Hum Pathol*. 1998;29:801-808.
13. Niho S, Yokose T, Suzuki K, et al. Monoclonality of atypical adenomatous hyperplasias of the lung. *Am J Pathol*. 1999;154:249-254.
14. Kitamura H, Kameda Y, Ito T, et al. Atypical adenomatous hyperplasia of the lung; implications for the pathogenesis of peripheral lung adenocarcinoma. *Am J Clin Pathol*. 1999;111:610-622.
15. Chapman AD, Kerr KM. The association between atypical adenomatous hyperplasia and primary lung cancer. *Br J Cancer*. 2000;83:632-636.
16. Yamasaki M, Takeshima Y, Fujii S, et al. Correlation between genetic alterations and histopathological subtypes in bronchiolo-alveolar carcinoma and atypical adenomatous hyperplasia of the lung. *Pathol Int*. 2000;50:778-785.

DOI: 10.1309/THU13F3JRJVWLM30

17. Yamasaki M, Takeshima Y, Fujii S, et al. Correlation between morphological heterogeneity and genetic alteration within one tumor in adenocarcinomas of the lung. *Pathol Int*. 2000;50:891-896.
18. Takamochi K, Ogura T, Suzuki K, et al. Loss of heterozygosity on chromosome 9q and 16p in atypical adenomatous hyperplasia concomitant with adenocarcinoma of the lung. *Am J Pathol*. 2001;159:1941-1948.
19. Kayser K, Nwoye JO, Kosjerina Z, et al. Atypical adenomatous hyperplasia of lung: its incidence and analysis of clinical, glycohistochemical and structural features including newly defined growth regulators and vascularization. *Lung Cancer*. 2003;42:171-182.
20. Kawai T, Hiroi S, Nakanishi K, et al. Telomere length and telomerase expression in atypical adenomatous hyperplasia and small bronchioloalveolar carcinoma of the lung. *Am J Clin Pathol*. 2007;127:254-262.
21. Nakanishi K, Matsuo H, Kanai Y, et al. LAT1 expression in normal lung and in atypical adenomatous hyperplasia and adenocarcinoma of the lung. *Virchows Arch*. 2006;448:142-150.
22. Sano T, Kitayama Y, Igarashi H, et al. Chromosomal numerical abnormalities in early stage lung adenocarcinoma. *Pathol Int*. 2006;56:117-125.
23. Morandi L, Asioli S, Cavazza A, et al. Genetic relationship among atypical adenomatous hyperplasia, bronchioloalveolar carcinoma and adenocarcinoma of the lung. *Lung Cancer*. 2007;56:35-42.
24. Tomida S, Yatabe Y, Yanagisawa K, et al. Throwing new light on lung cancer pathogenesis: updates on three recent topics. *Cancer Sci*. 2005;96:63-68.
25. 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) [published correction appears in *J Clin Oncol*. 2004;22:4811]. *J Clin Oncol*. 2003;21:2237-2246.
26. 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.
27. Shepherd FA, Rodrigues PJ, Ciuleanu T, et al. Erlotinib in previously treated non–small cell lung cancer. *N Engl J Med*. 2005;353:123-132.
28. Sridhar SS, Seymour L, Shepherd FA. Inhibitors of EGFRs: a review of clinical research with a focus on non–small cell lung cancer. *Lancet Oncol*. 2003;4:397-406.
29. Prudkin L, Wistuba II. EGFR abnormalities in lung cancer: pathogenetic and clinical implications. *Ann Diagn Pathol*. 2006;10:306-315.
30. Pao W, Miller VA. EGFR mutations, small-molecule kinase inhibitors, and non–small cell lung cancer: current knowledge and future directions. *J Clin Oncol*. 2005;23:2556-2568.
31. Yatabe Y, Mitsudomi T. Epidermal growth factor receptor mutations in lung cancer. *Pathol Int*. 2007;57:233-244.
32. Miller VA, Kris MG, Shah N, et al. Bronchioloalveolar pathologic subtype and smoking history predict sensitivity to gefitinib in advanced non–small cell lung cancer. *J Clin Oncol*. 2004;22:1103-1109.
33. Lynch TJ, Bell DW, Sordella R, et al. Activating mutations in epidermal growth factor receptor underlying responsiveness of non–small-cell lung cancer to gefitinib. *N Engl J Med*. 2004;350:2129-2139.
34. Paez JG, Janne PA, Lee J, et al. EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. *Science*. 2004;304:1497-1500.
35. Pao W, Miller VA, Zakowski M, et al. EGFR 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.
36. Dowell JE, Caplan NM, Palmer BF. EGFR mutations in non–small cell lung cancer: a basic science discovery with immediate clinical impact. *Am J Med Sci*. 2006;331:139-149.
37. Mitsudomi T, Kosaka T, Yatabe Y. Biological and clinical implications of EGFR mutations in lung cancer. *Int J Clin Oncol*. 2006;11:190-198.
38. Taron M, Ichinose Y, Rosell R, et al. Activating mutations in the tyrosine kinase domain of the EGFR are associated with improved survival in gefitinib-treated chemorefractory lung adenocarcinoma. *Clin Cancer Res*. 2005;11:5878-5885.
39. Johnson BE, Jänne PA. Epidermal growth factor receptor mutations in patients with non–small cell lung cancer. *Cancer Res*. 2005;65:7525-7529.
40. Chan SK, Gullick WJ, Hill ME. Mutations of the EGFR in non–small cell lung cancer: search and destroy. *Eur J Cancer*. 2006;42:17-23.
41. Murray S, Timotheadou E, Linardou H, et al. Mutations of the EGFR tyrosine kinase domain and associations with clinicopathologic features in non–small cell lung cancer patients. *Lung Cancer*. 2006;52:225-233.
42. Shigematsu H, Kin L, Takahashi T, et al. Clinical and biological features associated with EGFR gene mutations in lung cancers. *J Natl Cancer Inst*. 2005;97:339-346.
43. Gazdar AF, Shigematsu H, Herz J, et al. Mutations and addiction to EGFR: the Achilles "heal" of lung cancers? *Trends Mol Med*. 2004;10:481-486.
44. Riely GJ, Pao W, Pham DK, et al. Clinical course of patients with non–small cell lung cancer and EGFR exon 19 and exon 21 mutations treated with gefitinib or erlotinib. *Clin Cancer Res*. 2006;12:839-844.
45. Sequist LV, Joshi VA, Janne PA, et al. Epidermal growth factor receptor mutation testing in the care of lung cancer patients. *Clin Cancer Res*. 2006;12(14 pt 2):4403s-4408s.
46. 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. doi:10.1371/journal.pmed.0020017.
47. Eberhard DA, Johnson BE, Amler LC, et al. Mutations in the EGFR and in KRAS are predictive and prognostic indicators in patients with NSCLC treated with chemotherapy alone or in combination with erlotinib. *J Clin Oncol*. 2005;23:5900-5909.
48. Tam IY, Chung LP, Suen WS, et al. Distinct epidermal growth factor receptor and KRAS mutation patterns in non–small-cell lung cancer patients with different tobacco exposure and clinicopathologic features. *Clin Cancer Res*. 2006;12:1647-1653.
49. Han SW, Kim TY, Jeon YK, et al. Optimization of patient selection for gefitinib in non–small-cell lung cancer by combined analysis of epidermal growth factor receptor mutation, K-ras mutation, and Akt phosphorylation. *Clin Cancer Res*. 2006;12:2538-2544.
50. Stephens P, Hunter C, Bignell G, et al. Lung cancer: intragenic ERBB2 kinase mutations in tumours. *Nature*. 2004;431:525-526.
51. Shigematsu H, Takahashi T, Nomura M, et al. Somatic mutations of the HER2 kinase domain in lung adenocarcinomas. *Cancer Res*. 2005;65:1642-1646.
52. Buttitta F, Barassi F, Fresu G, et al. Mutational analysis of the HER2 gene in lung tumors from Caucasian patients: mutations are mainly present in adenocarcinomas with bronchioloalveolar features. *Int J Cancer*. 2006;119:2586-2591.

DOI: 10.1309/THU13F3JRJVWLM30

53. Shimosato Y, Kodama T, Kameya T. Morphogenesis of peripheral type adenocarcinoma of the lung. In: Shimosato Y, Melamed MR, Nettesheim P, eds. *Morphogenesis of Lung Cancer.* Boca Raton, FL: CRC Press; 1982.
54. Kodama T, Biyajima S, Watanabe S, et al. Morphometric study of adenocarcinomas and hyperplastic epithelial lesions in the peripheral lung. *Am J Clin Pathol.* 1986;85:146-151.
55. Weng SY, Tsuchiya E, Kasuga T, et al. Incidence of atypical bronchioloalveolar cell hyperplasia of the lung: relation to histological subtypes of lung cancer. *Virchows Arch.* 1992;420:463-471.
56. Carey FA, Wallace WAH, Fergusson RJ, et al. Alveolar atypical adenomatous hyperplasia in association with primary pulmonary adenocarcinoma: a clinicopathological study of 10 cases. *Thorax.* 1992;47:1041-1043.
57. Colby TV, Koss M, Travis WD. *Tumors of the Lower Respiratory Tract.* Washington, DC: Armed Forces Institute of Pathology; 1995. *Atlas of Tumor Pathology*; Third series, Fascicle 13.
58. Suzuki K, Nagai K, Yoshida J, et al. The prognosis of resected lung carcinoma associated with atypical adenomatous hyperplasia: a comparison of the prognosis of well-differentiated adenocarcinoma associated with atypical adenomatous hyperplasia and intrapulmonary metastasis. *Cancer.* 1997;79:1521-1526.
59. Takigawa N, Segawa Y, Nakata M, et al. Clinical investigation of atypical adenomatous hyperplasia of the lung. *Lung Cancer.* 1999;25:115-121.
60. Mori M, Rao SK, Popper HH, et al. Atypical adenomatous hyperplasia of the lung: a probable forerunner in the development of adenocarcinoma of the lung. *Mod Pathol.* 2001;14:72-84.
61. Greenberg AK, Yee H, Rom WN. Preneoplastic lesions of the lung. *Respir Res.* 2002;3:20-30.
62. Ohshima S, Shimizu Y, Takahama M. Detection of c-Ki-ras gene mutation in paraffin sections of adenocarcinoma and atypical bronchioloalveolar cell hyperplasia of human lung. *Virchows Arch.* 1994;424:129-134.
63. Cooper CA, Carby FA, Bubb VJ, et al. The pattern of K-ras mutation in pulmonary adenocarcinoma defines a new pathway of tumour development in the human lung. *J Pathol.* 1997;181:401-404.
64. Ullman R, Bongiovanni M, Halbwedl I, et al. Is high-grade adenomatous hyperplasia an early bronchioloalveolar carcinoma? *J Pathol.* 2003;201:371-376.
65. Marchetti A, Martella C, Felicioni L, et al. EGFR mutations in non–small-cell lung cancer: analysis of a large series of cases and development of a rapid and sensitive method for diagnostic screening with potential implications on pharmacologic treatment. *J Clin Oncol.* 2005;23:857-865.
66. Toyooka S, Tokumo M, Shigematsu H, et al. Mutational and epigenetic evidence for independent pathways for lung adenocarcinomas arising in smokers and never smokers. *Cancer Res.* 2006;66:1371-1375.
67. Yatabe Y, Kosaka T, Takahashi T, et al. EGFR mutation is specific for terminal respiratory unit type adenocarcinoma. *Am J Surg Pathol.* 2005;29:633-639.
68. Haneda H, Sasaki H, Shimizu S, et al. Epidermal growth factor receptor gene mutation defines distinct subsets among small adenocarcinomas of the lung. *Lung Cancer.* 2005;52:47-52.
69. Yoshida Y, Shibata T, Kokubu A, et al. Mutations of the epidermal growth factor receptor gene in atypical adenomatous hyperplasia and bronchioloalveolar carcinoma of the lung. *Lung Cancer.* 2005;50:1-8.
70. Rodenhuis S, Slebos RJC, Evers SG, et al. K-ras oncogene activation on adenocarcinoma of the lung: frequency and possible clinical significance. *Cancer Res.* 1998;48:5738-5741.
71. Sagawa M, Saito Y, Fujimura S, et al. K-ras point mutation occurs in the early stage of carcinogenesis in lung cancer. *Br J Cancer.* 1998;77:720-723.
72. Nelson HH, Cristiani DC, Mark EJ, et al. Implications and prognostic value of k-ras mutation for early-stage lung cancer in women. *J Natl Cancer Inst.* 1999;91:2032-2038.
73. Kim YH, Ishii G, Goto K, et al. Dominant papillary subtype is a significant predictor of the response to gefitinib in adenocarcinoma of the lung. *Clin Cancer Res.* 2004;10:7311-7317.
74. Silver SA, Askin FB. True papillary carcinoma of the lung: a distinct clinicopathologic entity. *Am J Surg Pathol.* 1997;21:43-51.
75. Miyoshi T, Satoh Y, Okumura S, et al. Early-stage lung adenocarcinoma with a micropapillary pattern: a distinct pathological marker for a significantly poor prognosis. *Am J Surg Pathol.* 2003;27:101-109.
76. Aida S, Shimazaki H, Sato M, et al. Prognostic analysis of pulmonary adenocarcinoma subclassification with special consideration of papillary and bronchioloalveolar types. *Histopathology.* 2004;45:468-476.
77. Costa DB, Schumer ST. Three-year survival in metastatic non–small cell lung cancer treated with gefitinib [letter]. *Lung Cancer.* 2006;53:123-124.
78. Jian Z, Tomizawa Y, Yanagitani N, et al. Papillary adenocarcinoma of the lung is a more advanced adenocarcinoma than bronchioloalveolar carcinoma that is composed of two distinct histological subtypes. *Pathol Int.* 2005;55:619-625.
79. Ruiz MI, van Cruijsen H, Smit EF, et al. Genetic heterogeneity in patients with multiple neoplastic lung lesions: a report of three cases. *J Thorac Oncol.* 2007;2:12-21.
80. Milton DT, Riely GJ, Pao W, et al. Molecular on/off switch. *J Clin Oncol.* 2006;24:4940-4942.
81. Ryoo BY, Na II, Yang SH, et al. Synchronous multiple primary lung cancers with different responses to gefitinib. *Lung Cancer.* 2006;53:245-248.
82. Giaccone G, Gallegos Ruiz M, Le Chevalier T, et al. Erlotinib for frontline treatment of advanced non–small cell lung cancer: a phase II study. *Clin Cancer Res.* 2006;12:6049-6055.
83. Inoue A, Suzuki T, Fukuhara T, et al. Prospective phase II study of gefitinib for chemotherapy-naive patients with advanced non–small cell lung cancer with epidermal growth factor receptor gene mutations. *J Clin Oncol.* 2006;24:3340-3346.

DOI: 10.1309/THU13F3JRJVWLM30