Modern Pathology (2007) 20, 967–973

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Epidermal growth factor receptor gene mutations in atypical adenomatous hyperplasias of the lung

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Activating epidermal growth factor receptor (*EGFR*) gene mutations are frequently detected in lung adenocarcinomas, especially adenocarcinomas with a nonmucinous bronchioloalveolar carcinoma component. *EGFR*-mutated lung adenocarcinomas respond well to EGFR tyrosine kinase inhibitors. We previously found that most (88%) pure nonmucinous bronchioloalveolar carcinomas (adenocarcinoma *in situ*) already harbor *EGFR* mutations, indicating that the mutations are an early genetic event in the pathogenesis. We examined 54 atypical adenomatous hyperplasias, precursor lesions of lung adenocarcinomas, obtained from 28 Japanese patients for the hotspot mutations of *EGFR* exons 19 and 21 and K-*ras* codon 12. *EGFR* mutations were observed in 17 of the 54 (32%) atypical adenomatous hyperplasias examined: Ten and seven atypical adenomatous hyperplasias had deletion mutations at exon 19 or point mutations (L858R) at exon 21, respectively. We did not observe apparent histological differences between atypical adenomatous hyperplasias with and without *EGFR* mutations. K-*ras* mutation (G12S) was detected in only one atypical adenomatous hyperplasia. As *EGFR* mutational frequency of atypical adenomatous hyperplasias was much lower than that of nonmucinous bronchioloalveolar carcinomas, we surmise that *EGFR*-mutated atypical adenomatous hyperplasias, but not atypical adenomatous hyperplasias with wild-type *EGFR*, are likely to progress to nonmucinous bronchioloalveolar carcinomas.
Modern Pathology (2007) **20,** 967–973; doi:10.1038/modpathol.3800929; published online 6 July 2007

Keywords: lung; atypical adenomatous hyperplasia; bronchioloalveolar carcinoma; EGFR; mutation

Epidermal growth factor receptor (*EGFR*) gene mutations have been detected frequently in lung adenocarcinomas, especially adenocarcinomas with a nonmucinous bronchioloalveolar carcinoma component,[1–9] and the mutations have been significantly associated with sensitivity to EGFR tyrosine kinase inhibitors such as gefitinib or erlotinib.[10,11] On the other hand, a subset of lung adenocarcinomas has mutations in the K-*ras* gene, a known downstream signaling molecule in the EGFR signaling pathway.[12,13] K-*ras*-mutated lung adenocarcinomas, as opposed to *EGFR*-mutated adenocarcinoma, often show a histopathological feature of mucinous bronchioloalveolar carcinoma,[12] and K-*ras* mutations are found more frequently in patients who are resistant to therapy with EGFR tyrosine kinase inhibitors.[14] *EGFR* and K-*ras* mutations have been reported to be mutually exclusive in lung adenocarcinomas, and be significantly more associated with never- and ever-smokers, respectively.[4–7,9] Thus, *EGFR*- or K-*ras*-mutated adenocarcinomas are considered to be distinct subsets of lung adenocarcinomas.

Atypical adenomatous hyperplasia is considered to be a precursor lesion of peripheral lung adenocarcinoma; a multistep carcinogenesis where invasive lung adenocarcinoma develops from atypical adenomatous hyperplasia through bronchioloalveolar carcinoma (adenocarcinoma *in situ*) has been postulated.[15] *EGFR* or K-*ras* gene mutation seems to have a critical role in the development of lung adenocarcinomas and to be an initiating event, as

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Received 5 January 2007; revised 16 June 2007; accepted 18 June 2007; published online 6 July 2007

some atypical adenomatous hyperplasias or pure bronchioloalveolar carcinomas have been found to harbor *EGFR* or K-*ras* gene mutation,[7–9,13,16,17] and moreover in transgenic mouse models expressing mutated *EGFR* or K-*ras* gene in type II pneumocyte, development of lung adenocarcinomas through atypical adenomatous hyperplasias has been observed.[18,19] Although it is important to know the exact incidence of *EGFR* and K-*ras* gene mutations in atypical adenomatous hyperplasias to understand pathogenesis of lung adenocarcinoma, the results obtained so far are controversial.[7,8,13,16,17] We have previously reported that most pure nonmucinous bronchioloalveolar carcinomas have *EGFR* mutations, whereas mucinous bronchioloalveolar carcinomas are significantly associated with K-*ras* mutations by means of loop-hybrid mobility shift assay (LH-MSA),[9] which is a rapid and sensitive PCR-based method to detect hot spot mutations.[20,21] In the present study, we evaluated 54 atypical adenomatous hyperplasias of the lung for the hot spot mutations in exons 19 and 21 of *EGFR* gene and in codon 12 of K-*ras* gene using LH-MSA, (1) to elucidate the frequencies of *EGFR* and K-*ras* gene mutations in atypical adenomatous hyperplasias, then (2) to compare those between atypical adenomatous hyperplasias and adenocarcinomas, and finally (3) to discuss whether or not all atypical adenomatous hyperplasias progress to adenocarcinomas with a nonmucinous or mucinous bronchioloalveolar carcinoma component.

Materials and methods

Patients and Tumor Tissues

A total of 54 atypical adenomatous hyperplasias were obtained from 28 patients (Table 1). All the atypical adenomatous hyperplasias were diagnosed by three pathologists (YS, YK, and ET) according to the 2004 WHO classification system; 'atypical adenomatous hyperplasia is a localized proliferation of mild to moderately atypical cells lining alveoli and sometimes respiratory bronchioles'.[15] Mean tumor size, in greatest diameter, was 5 ± 2 mm (range, 2–10 mm). Twenty-eight atypical adenomatous hyperplasias were incidental findings in 13 adenocarcinoma patients and the other atypical adenomatous hyperplasias ($n = 26$) were major findings in the remaining 15 patients. All the 28 patients underwent pulmonary resection at the Kanagawa Cancer Center Hospital, Yokohama, Japan, from July 1996 to January 2006. The 28 patients included 9 men and 19 women with a median age of 59.5 years (range, 42–77 years). There were 20 never-smokers and 8 ever-smokers. Informed consents were obtained for all cases and the institutional review board of the Kanagawa Cancer Center approved this study.

DNA Extraction and Sequencing Analyses of the EGFR and K-ras Genes

Formalin-fixed, paraffin-embedded tissue sections were used for DNA isolation from the all atypical adenomatous hyperplasia lesions as described previously.[20] Mutational analyses of *EGFR* gene exons 19 and 21, and K-*ras* gene codon 12 were performed by LH-MSA and sequencing as described previously.[20]

Immunohistochemistry

Immunohistochemical evaluations were performed using the avidin-biotin-peroxidase complex method with 3 μm-thick sections of the formalin-fixed, paraffin-embedded specimens. A monoclonal antibody against the Ki-67 antigen (MIB-1; MBL, Nagoya, Japan; 1:100 dilution) was used to assess the proportion of proliferating tumor cells. The Ki-67 labeling index (LI) was defined as the ratio of MIB-1-stained tumor cells to all tumor cells counted, multiplied by 100. To evaluate the Ki-67 LI, stained tumor cells were counted in at least three high-power fields that showed the highest positivity for each section.

Statistical Analyses

Values are shown as mean $\pm$ s.d. Fisher's exact test was used to assess the association of *EGFR* mutations with clinical factors including patients' age, sex, and smoking history. Associations between histological subtypes and *EGFR* or K-*ras* mutations were evaluated by Fisher's exact test. Maximum size and Ki-67 LI of the atypical adenomatous hyperplasias with and without *EGFR* mutations were compared by the Mann-Whitney's *U* test. A *P*-value of < 0.05 were considered to be statistically significant.

Results

EGFR Mutations in Atypical Adenomatous Hyperplasias

EGFR mutations were detected in 17 of the 54 atypical adenomatous hyperplasias (32%) examined; 10 tumors had a deletion mutation at exon 19, and the others ($n = 7$) had a point mutation at exon 21 (L858R) (Table 1).

Correlation of Clinicopathological Characteristics with EGFR Mutations in Atypical Adenomatous Hyperplasias

Although atypical adenomatous hyperplasias of younger patients, women, and never-smokers had *EGFR* mutations more frequently, no statistically significant associations were found between the *EGFR* mutation status and either age, sex, or smoking history (Table 2).

Table 1 Clinicopathological findings and *EGFR* and K-*ras* mutational analyses of 54 atypical adenomatous hyperplasias obtained from 28 patients

No.	*Age/sex*	*Smoking index*[a]	*Site*	*Size*[b]	*Ki-67LI*	EGFR *mutation*		*K*-ras *mutation*
						Exon 19	*Exon 21*	
1a	70/F	Ever (600)	RU	2	1.9	N	N	ND
1b			RU	5	1.2	N	N	ND
1c			RU	4	0.9	Del (G3)	ND	ND
2	46/F	Never	RU	4	2.5	N	N	N
3a	66/M	Ever (400)	RU	3	1.4	N	N	G12S
3b			RU	3	1.6	N	N	N
3c			RU	7	1.0	N	N	N
3d			RU	4	2.3	N	N	N
3e			RU	4	3.1	N	N	N
3f			RL	6	1.8	N	N	N
3g			RL	8	1.9	N	N	N
3h			RL	7	0.9	N	N	N
3i			RL	6	1.5	N	N	ND
3j			RL	2	1.7	Del (G3)	N	N
4	61/M	Ever (620)	LU	4	0.4	Del (G3)	N	N
5	55/F	Never	LU	3	0.9	Del (G2)	ND	N
6a	46/F	Never	RU	7	1.1	Del (G2)	N	N
6b			RU	8	0.8	N	N	N
7a	46/M	Ever (780)	RM	6	2.6	Del (G2)	N	N
7b			RM	4	1.6	N	N	N
8	77/M	Never	RU	9	1.1	N	N	N
9	59/F	Never	RM	5	1.4	Del (G2)	N	N
10	67/F	Never	RU	7	2.8	Del (G2)	N	ND
11	43/M	Ever (540)	LL	5	1.8	N	N	N
12	60/F	Never	RU	8	1.5	N	L858R	N
13	71/M	Ever (2.5)	LU	5	1.9	N	N	N
14	52/F	Never	RU	8	1.7	N	N	N
15	60/F	Never	LU	8	2.5	N	N	N
16a	77/M	Ever (1180)	LU	5	1.6	N	N	N
16b			LU	5	1.1	N	N	N
16c			LU	5	1.2	N	N	N
17	69/F	Never	RU	6	2.1	N	N	N
18	59/F	Never	RU	5	0.9	N	L858R	N
19	49/M	Ever (500)	RU	3	1.0	N	N	N
20a	61/F	Never	LU	7	1.1	N	L858R	N
20b			LU	2	2.5	N	N	N
20c			LU	2	0.7	N	N	N
20d			LU	3	0.9	N	N	N
20e			LU	2	1.0	N	N	N
20f			LU	4	1.6	ND	L858R	N
21	57/F	Never	LU	7	1.8	N	N	N
22	66/F	Never	RL	5	2.4	N	N	N
23a	71/F	Never	LL	3	2.4	N	N	N
23b			LL	4	2.3	N	N	N
23c			LL	5	2.7	N	N	N
23d			LL	6	1.3	N	N	N
24a	68/F	Never	LU	5	3.3	N	L858R	N
24b			LU	5	2.8	N	L858R	N
25	42/F	Never	RM	5	2.6	N	L858R	N
26	57/M	Never	RM	7	1.5	Del (G2)	N	N
27a	56/F	Never	RU	3	1.5	N	N	N
27b			RU	5	5.0	N	N	N
27c			RU	7	1.6	Del (G4)	N	N
28	51/F	Never	RU	10	1.8	N	N	N

Del, deletion; F, female; G2, deletion of nucleotides 2235–2249 resulted in deletion of amino acids 746–750; G3, deletion of nucleotides 2236–2250 resulted in deletion of amino acids 746–750; G4, deletion of nucleotides 2240–2254 resulted in deletion of amino acids 747–751; LI, labeling index (%); LL, left lower lobe; LU, left upper lobe; M, male; N, normal; ND, not done; RL, right lower lobe; RM, right middle lobe; RU, right upper lobe.

[a]Cigarettes smoked per day × years of smoking.

[b]Maximum size of greatest diameter in mm.

Both mean sizes of *EGFR*-mutated and wild-type atypical adenomatous hyperplasias were ~5 mm; no statistically significant association between *EGFR* mutation and tumor size was observed. Mean Ki-67 LI of *EGFR*-mutated atypical adenomatous hyperplasias was 1.7%, whereas that of atypical

Table 2 Relationship between clinicopathological characteristics and frequency of *EGFR* mutations in the 28 atypical adenomatous hyperplasia patients

Variables	*Total no. of AAH*	EGFR *mutation (%)*	P-*value*
Age, years			
≤59 ($n=14$)	18	8 (44%)	NS
≥60 ($n=14$)	36	9 (25%)	
Sex			
Male ($n=9$)	21	4 (19%)	NS
Female ($n=19$)	33	13 (39%)	
Smoking history			
Never smoker ($n=20$)	32	13 (41%)	NS
Ever smoker ($n=8$)	22	4 (18%)	

AAH, atypical adenomatous hyperplasia; NS, not significant.

Table 3 Comparison of clinicopathological data between atypical adenomatous hyperplasias with and without *EGFR* gene mutations

Variable	*Mutation (+) (n = 17)*	*Mutation (−) (n = 37)*	P-*value*
Tumor size (mm)	5.4 ± 1.7	5.1 ± 2.1	NS
Ki-67 LI (%)	1.7 ± 0.8	1.8 ± 0.8	NS

LI, labeling index; NS, not significant.

adenomatous hyperplasias with wild-type *EGFR* was 1.8%; no statistically significant relationship between *EGFR* mutation and Ki-67 LI was observed (Table 3). We observed no apparent histological differences between atypical adenomatous hyperplasias with and without *EGFR* mutations (Figure 1).

Characteristics of the Patient having a K-*ras*-Mutated Atypical Adenomatous Hyperplasia

Genomic DNAs of K-*ras* in five atypical adenomatous hyperplasias were not amplified sufficiently by PCR. Thus, we evaluated the remaining 49 atypical adenomatous hyperplasias for K-*ras* mutations. K-*ras* mutation was observed in only 1 of the 49 atypical adenomatous hyperplasias (2%) examined; the mutation is G12S (GGT > AGT). The patient having K-*ras*-mutated atypical adenomatous hyperplasia (Case No. 3) was a 66-year-old man and had smoking history. Interestingly, he had 10 atypical adenomatous hyperplasias; no mutation was observed in 8 of the 10 atypical adenomatous hyperplasias, and the other 2 had a K-*ras* or *EGFR* (deletion at exon 19) mutation.

Discussion

We have shown that 17 of the 54 (32%) atypical adenomatous hyperplasias examined had a hot spot mutation (deletion mutation at exon 19 or L858R at exon 21) of the *EGFR* gene. To date, *EGFR* mutations in atypical adenomatous hyperplasias have been reported in three previous studies where it was observed that 2 of 7, 0 of 5, or 1 of 35 atypical adenomatous hyperplasias had *EGFR* mutations.[7,8,17] One of these groups reported that 1 of 35 (3%) atypical adenomatous hyperplasias and 4 of 37 (11%) nonmucinous bronchioloalveolar carcinomas examined had *EGFR* mutations by PCR-direct sequencing of formalin-fixed materials.[17] Our results that *EGFR* mutations were detected in 32% of atypical adenomatous hyperplasias (in this study) and 88% of nonmucinous bronchioloalveolar carcinomas (in a previous study[9]) using LH-MSA sharply contrasts to their study in terms of *EGFR* mutational frequency. These discrepancies may be due to the methodology used in studies. As LH-MSA, which is a modified heteroduplex technique, is able to detect mutations in samples containing as little as 5% mutated DNA,[20] whereas direct sequencing has been reported to require at least 30% of mutated DNA in the sample,[22] LH-MSA is considered to be more sensitive than direct sequencing[20] and to have an advantage especially in analyzing tissues such as atypical adenomatous hyperplasias, which often contains not so many tumor cells as compared with the normal counterparts.

As atypical adenomatous hyperplasias and nonmucinous bronchioloalveolar carcinomas probably represent continuum of progression of alveolar intraepithelial neoplasia, differential diagnosis between atypical adenomatous hyperplasias and small-sized nonmucinous bronchioloalveolar carcinomas is often difficult.[15] The possibilities that some atypical adenomatous hyperplasias in this study may be diagnosed as 'nonmucinous bronchioloalveolar carcinomas' by other pathologists cannot be ruled out. However, we consider that *EGFR* mutations were not observed only in atypical adenomatous hyperplasias with relatively high cellularity and severe cytological atypia, which may correspond to BACs for some pathologists, from our following observations: (1) both atypical adenomatous hyperplasias with and without *EGFR* mutations were almost the same in maximum size and proliferation index (Table 3) and (2) variability in cellularity and cytological atypia was similarly observed in *EGFR*-mutated atypical adenomatous hyperplasias as well as atypical adenomatous hyperplasias without the mutation (Figure 1). These results lead us to conclude that *EGFR* mutations are already present in a subset of atypical adenomatous hyperplasias with relatively low cellularity and mild cytological atypia, which would be diagnosed as atypical adenomatous hyperplasias by any pathologists. Our measurement that *EGFR* mutations were observed in about 30% of atypical adenomatous hyperplasias examined would be reasonable, although further studies are needed to validate the mutational frequency.

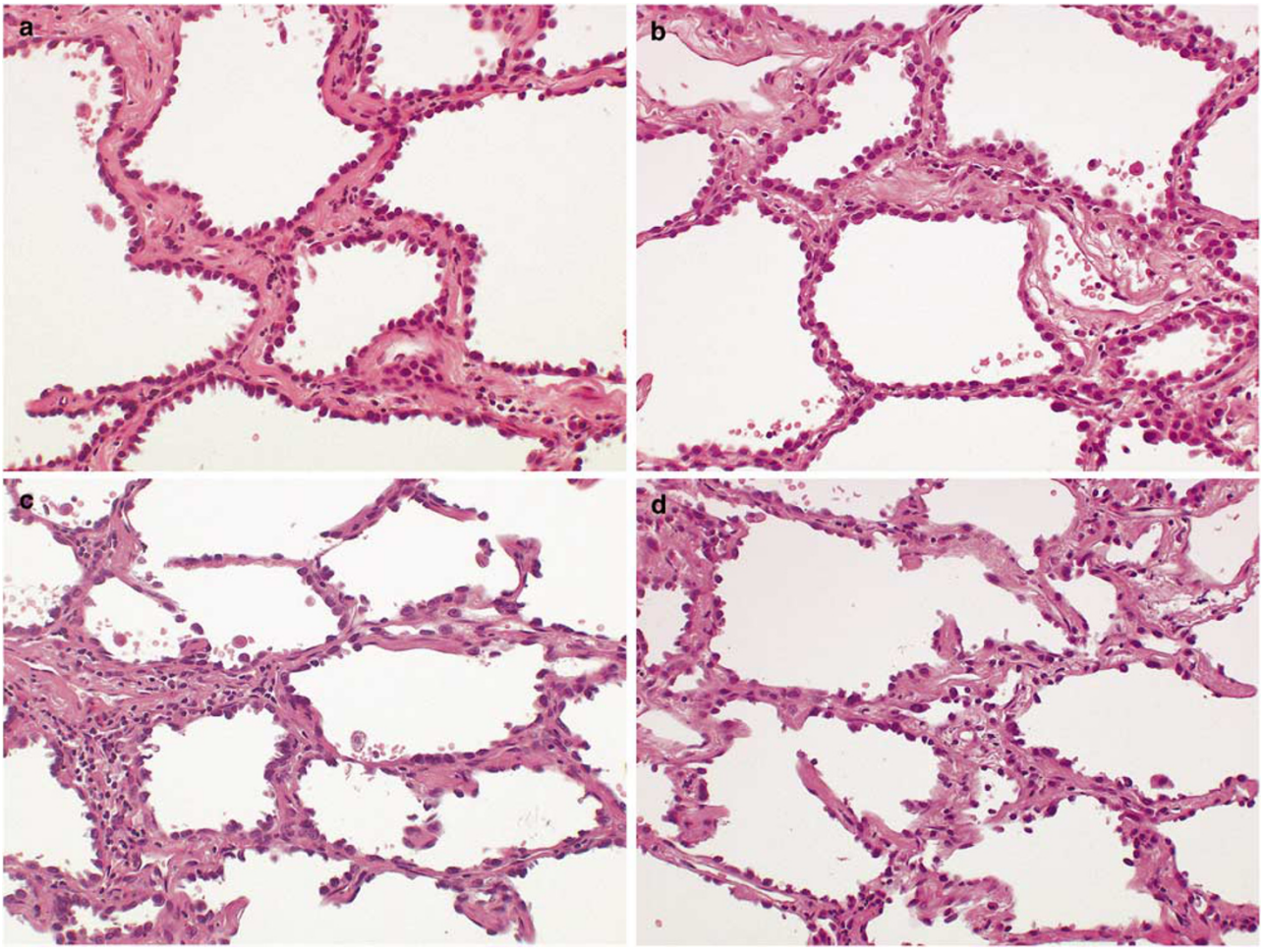

Figure 1 Histopathology of atypical adenomatous hyperplasias with and without *EGFR* mutations. All of these tumors were found in female patients who had never smoked. Both (**a**) atypical adenomatous hyperplasias with *EGFR* mutation (No. 18) and (**b**) without *EGFR* mutation (No. 27b) have relatively high cellularity and show almost the same histology. There is no obvious histological difference between atypical adenomatous hyperplasias with relatively low cellularity (**c** and **d**), but (**c** and **d**) show atypical adenomatous hyperplasias with *EGFR* mutation (No. 10) and without *EGFR* mutation (No. 15), respectively.

We had previously reported that 15 of 17 (88%) pure nonmucinous bronchioloalveolar carcinomas (adenocarcinoma *in situ*) and 49 of 65 (75%) invasive adenocarcinomas with a nonmucinous bronchioloalveolar carcinoma component had *EGFR* mutations.[9] In addition to these high frequencies of *EGFR* mutations in lung adenocarcinomas with a nonmucinous bronchioloalveolar carcinoma feature, it is known that (1) most human lung adenocarcinomas expressing mutated EGFR respond well to EGFR tyrosine kinase inhibitors[10,11] and (2) transgenic mice expressing mutated EGFR in type II pneumocytes develop atypical adenomatous hyperplasias, bronchioloalveolar carcinomas, and invasive adenocarcinomas with a nonmucinous bronchioloalveolar carcinoma component in the lung in a time-dependent manner.[19] These observations imply that persistent EGFR signaling due to activating *EGFR* mutations would be essential for development and maintenance in lung adenocarcinomas with a nonmucinous bronchioloalveolar carcinoma component. Therefore, we started the present study with a working hypothesis that the *EGFR* mutational frequency in atypical adenomatous hyperplasias is similar to those of more advanced lesions. However, the frequency of *EGFR* mutations in atypical adenomatous hyperplasias (32%) is significantly lower (Fisher's exact test, $P<0.0001$) than those in nonmucinous bronchioloalveolar carcinomas (88%) and invasive adenocarcinomas with a nonmucinous bronchioloalveolar carcinoma component (75%) (hereafter we call these tumors adenocarcinomas with a nonmucinous bronchioloalveolar carcinoma feature) (Figure 2).[9] Assuming that all adenocarcinomas with a nonmucinous bronchioloalveolar carcinoma feature come from atypical adenomatous hyperplasias, atypical adenomatous hyperplasias with *EGFR* mutations are likely to progress to adenocarcinomas much more frequently than those without the mutations. In other words, atypical adenomatous hyperplasias without *EGFR* mutations are unlikely to progress

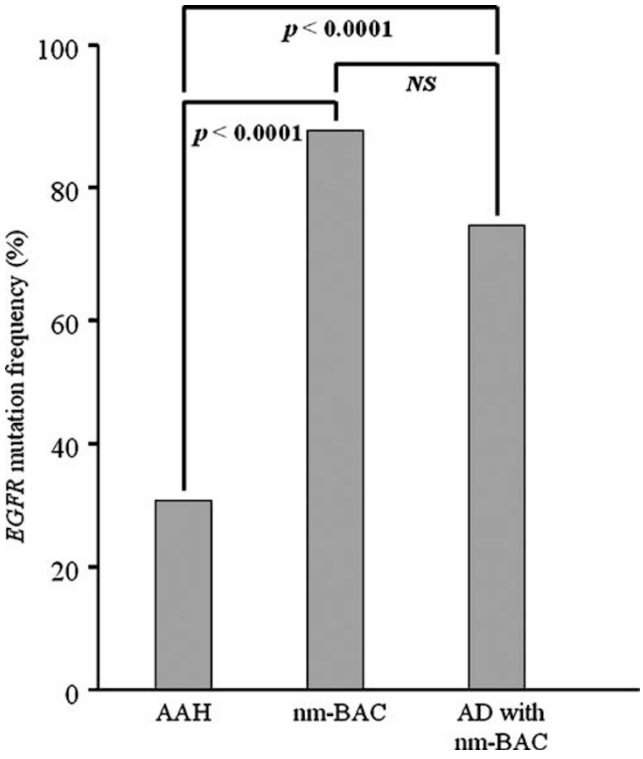

Figure 2 *EGFR* mutational frequencies in atypical adenomatous hyperplasias, nonmucinous bronchioloalveolar carcinomas, and invasive adenocarcinomas with a nonmucinous bronchioloalveolar carcinoma component. AAH, atypical adenomatous hyperplasia; nm-BAC, nonmucinous bronchioloalveolar carcinoma; AD with nm-BAC, invasive adenocarcinomas with a nonmucinous bronchioloalveolar carcinoma component.

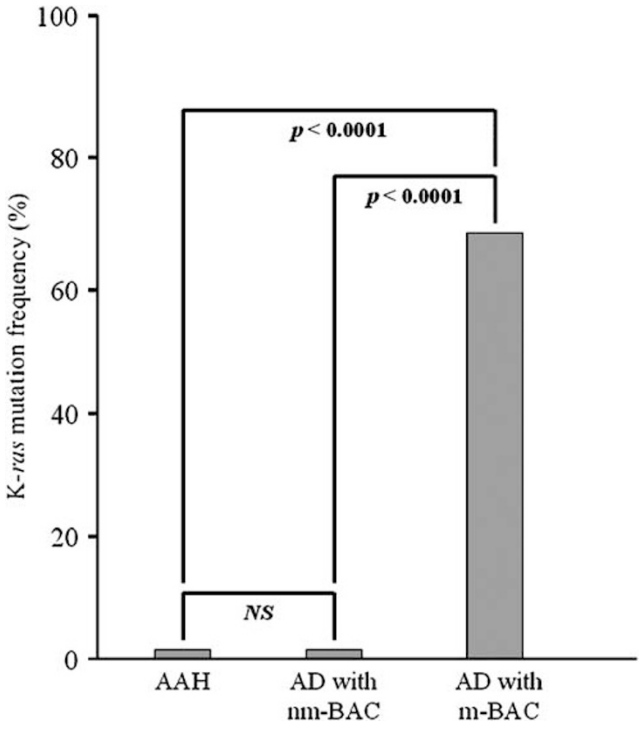

Figure 3 K-*ras* mutational frequencies in atypical adenomatous hyperplasias, adenocarcinoma with a nonmucinous bronchioloalveolar carcinoma feature, and adenocarcinoma with a mucinous bronchioloalveolar carcinoma component. AAH, atypical adenomatous hyperplasia; AD with nm-BAC, adenocarcinoma with a nonmucinous bronchioloalveolar carcinoma feature; AD with m-BAC, adenocarcinoma with a mucinous bronchioloalveolar carcinoma component.

to adenocarcinomas. This speculation matches the clinical observation that about half of pure ground-glass opacities, identified on high-resolution computed tomography and usually diagnosed as atypical adenomatous hyperplasia or nonmucinous bronchioloalveolar carcinoma on histopathological examination, do not increase in size and density for more than 2 years.[23] However, we did not observed significant differences in histology, tumor maximum size, and proliferation activity (estimated by Ki-67 LI) between atypical adenomatous hyperplasias with and without *EGFR* mutations (Figure 1; Table 3). Further studies are needed to determine the biological differences between atypical adenomatous hyperplasias with and without *EGFR* mutations.

We identified only one K-*ras* mutation in the 49 atypical adenomatous hyperplasias (2%) in this study. This frequency is considerably lower than those (15–39%) of previous studies analyzing atypical adenomatous hyperplasias.[13,16,24] Although the reason for this marked discrepancy is unclear, we consider that our methodology for mutational analyses is reliable based on the facts as follows: (1) several prior reports, examining DNAs extracted from fresh tumor tissues in Japan, have clarified that about 10% of Japanese lung adenocarcinomas have K-*ras* mutation,[4,12] and (2) our previous study identified the K-*ras* mutation of almost the same frequency (9%) in 118 Japanese adenocarcinomas by using LH-MSA.[9] In addition, we compared the K-*ras* mutational frequencies between atypical adenomatous hyperplasias (2%) in this study and adenocarcinomas with a nonmucinous bronchioloalveolar carcinoma feature (2 of 82, 2%) or a mucinous bronchioloalveolar carcinoma component (6 of 9, 67%) in our previous study[9] and found that the K-*ras* mutations in atypical adenomatous hyperplasias or in adenocarcinomas with a nonmucinous bronchioloalveolar carcinoma feature were much less frequent (Fisher's exact test, $P<0.0001$) than in adenocarcinomas with a mucinous bronchioloalveolar carcinoma component (Figure 3). As atypical adenomatous hyperplasias are strikingly similar to nonmucinous bronchioloalveolar carcinomas, but quite different from mucinous bronchioloalveolar carcinomas in K-*ras* mutational frequency as well as histopathology, we surmise that atypical adenomatous hyperplasias do progress sequentially to nonmucinous bronchioloalveolar carcinomas, but not to mucinous bronchioloalveolar carcinomas.

In conclusion, about one-third atypical adenomatous hyperplasias examined in this study had *EGFR* mutations, but few atypical adenomatous hyperplasias had K-*ras* mutations. We surmise that atypical adenomatous hyperplasias with *EGFR* mutation

would more frequently progress to invasive adenocarcinomas through nonmucinous bronchioloalveolar carcinomas than atypical adenomatous hyperplasias without *EGFR* mutations. Genetic alterations other than *EGFR* gene mutations seem to be needed for the development of *EGFR*-mutated atypical adenomatous hyperplasias to adenocarcinomas with a nonmucinous bronchioloalveolar carcinoma feature. Clarification of the molecular mechanisms involved in the progression process would enable us to discover new targeting molecules, other than EGFR, in lung adenocarcinoma therapy.

Acknowledgements

This work was supported in part by Public Trust Haraguchi Memorial Cancer Research Fund (to YS) and by Kanagawa Cancer Research Fund (to YM).

References

1 Lynch TJ, Bell DW, Sordella R, *et al.* Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. N Engl J Med 2004;350:2129–2139.
2 Paez JG, Janne PA, Lee JC, *et al.* EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. Science 2004;304:1497–1500.
3 Pao W, Miller V, Zakowski M, *et al.* EGF receptor gene mutations are common in lung cancers from 'never smokers' and are associated with sensitivity of tumors to gefitinib and erlotinib. Proc Natl Acad Sci USA 2004;101:13306–13311.
4 Kosaka T, Yatabe Y, Endoh H, *et al.* Mutations of the epidermal growth factor receptor gene in lung cancer: biological and clinical implications. Cancer Res 2004;64:8919–8923.
5 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.
6 Shigematsu H, Lin L, Takahashi T, *et al.* Clinical and biological features associated with epidermal growth factor receptor gene mutations in lung cancers. J Natl Cancer Inst 2005;97:339–346.
7 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.
8 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 2006;52:47–52.
9 Sakuma Y, Matsukuma S, Yoshihara M, *et al.* Distinctive evaluation of non-mucinous and mucinous subtypes of bronchioloalveolar carcinomas in *EGFR* and *K-ras* gene-mutation analyses for Japanese lung adenocarcinomas: confirmation of the correlations with histological subtypes and gene mutations. Am J Clin Pathol 2007;128:100–108.
10 Takano T, Ohe Y, Sakamoto H, *et al.* Epidermal growth factor receptor gene mutations and increased copy numbers predict gefitinib sensitivity in patients with recurrent non-small-cell lung cancer. J Clin Oncol 2005;23:6829–6837.
11 Taron M, Ichinose Y, Rosell R, *et al.* Activating mutations in the tyrosine kinase domain of the epidermal growth factor receptor are associated with improved survival in gefitinib-treated chemorefractory lung adenocarcinomas. Clin Cancer Res 2005;11:5878–5885.
12 Tsuchiya E, Furuta R, Wada N, *et al.* High K-ras mutation rates in goblet-cell-type adenocarcinomas of the lungs. J Cancer Res Clin Oncol 1995;121:577–581.
13 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.
14 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.
15 Travis WD, Brambilla E, Müller-Hermelink HK, *et al.* Pathology and Genetics: Tumours of the Lung, Pleura, Thymus and Heart. IARC: Lyon, 2004.
16 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.
17 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.
18 Jackson EL, Willis N, Mercer K, *et al.* Analysis of lung tumor initiation and progression using conditional expression of oncogenic K-ras. Genes Dev 2001;15:3243–3248.
19 Ji H, Li D, Chen L, *et al.* The impact of human EGFR kinase domain mutations on lung tumorigenesis and *in vivo* sensitivity to EGFR-targeted therapies. Cancer Cell 2006;9:485–495.
20 Matsukuma S, Yoshihara M, Kasai F, *et al.* Rapid and simple detection of hot spot point mutations of epidermal growth factor receptor, BRAF, and NRAS in cancers using the Loop-hybrid mobility shift assay. J Mol Diagn 2006;8:504–512.
21 Oshita F, Matsukuma S, Yoshihara M, *et al.* Novel heteroduplex method using small cytology specimens with a remarkably high success rate for analysing EGFR gene mutations with a significant correlation to gefitinib efficacy in non-small-cell lung cancer. Br J Cancer 2006;95:1070–1075.
22 Fan X, Furnari FB, Cavenee WK, *et al.* Non-isotopic silver-stained SSCP is more sensitive than automated direct sequencing for the detection of PTEN mutations in a mixture of DNA extracted from normal and tumor cells. Int J Oncol 2001;18:1023–1026.
23 Kodama K, Higashiyama M, Yokouchi H, *et al.* Natural history of pure ground-glass opacity after long-term follow-up of more than 2 years. Ann Thorac Surg 2002;73:386–392.
24 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.