The Relationship between Epidermal Growth Factor Receptor Mutations and Clinicopathologic Features in Non–Small Cell Lung Cancers

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ABSTRACT

Purpose: **Recent studies reported that clinical responsiveness to gefitinib was associated with somatic mutation of epidermal growth factor receptor (*EGFR*) gene in non–small cell lung cancers (NSCLC). Here, we investigated the relationship between *EGFR* mutation and clinicopathologic features.**

Experimental Design: EGFR **mutational status of 120 NSCLCs was determined mainly in *EGFR* exons 18 to 21 by direct sequence and correlated with clinicopathologic parameters.**

Results: EGFR **mutations were present in 38 cases (32%) and the majority of mutations were in-frame deletions of exon 19 (19 cases) and a missense mutation in exon 21 (18 cases). *EGFR* mutations were frequently associated with adenocarcinoma ($P < 0.0001$), never smoker ($P < 0.0001$), and female gender ($P = 0.0001$). Of interest, increasing smoke exposure was inversely related to the rate of *EGFR* mutation ($P < 0.0001$). Multivariate analysis showed that smoking and histology were independent variables. Furthermore, gender difference was observed for the mutational location ($P = 0.01$) dominance of exon 19 for males and exon 21 for females. Twenty-one cases were treated with gefitinib and found that *EGFR* mutation was significantly related to gefitinib responsiveness ($P = 0.002$). In addition, median survival times of patients with and without *EGFR* mutations treated with gefitinib were 25.1 and 14.0 months, respectively. Patients with *EGFR* mutations had approximately 2-fold survival advantage; however, the difference was not significant.**

Conclusions: **We show that *EGFR* mutations were significantly related to histology and smoke exposure and were a strong predictive factor for gefitinib responsiveness in NSCLC.**

INTRODUCTION

Lung cancer is one of the major causes of cancer deaths in the world with over 1 million cases diagnosed every year (1). Human lung cancers are classified into two major types, small cell lung cancer (SCLC) and non–small cell lung cancer (NSCLC), the latter consisting of several types (2), mainly adenocarcinoma and squamous cell carcinoma. Previously, squamous cell carcinoma was the predominant form of NSCLC, but in the last few decades it has been replaced by adenocarcinoma (3, 4). Tobacco smoking is a widely recognized risk factor for lung cancer, especially for squamous cell carcinoma and SCLC, but smoke exposure seems to be a less potent oncogenic factor for adenocarcinoma.

NSCLC is generally less sensitive to chemotherapy than SCLC and curative intent surgical resection is the treatment of first choice (5). However, chemotherapy and/or radiotherapy are often used for advanced or recurrent cases. With the accumulation of knowledge of molecular biology of lung cancer, several genetic changes including *TP53* mutation were reported to be related to response to chemotherapy (6). Epidermal growth factor receptor (*EGFR*) is a receptor tyrosine kinase identified as being highly expressed in cancer cells including lung cancers (7). EGFR is a transmembrane protein consisting of an extracellular ligand-binding domain, a transmembrane domain, an intracellular tyrosine kinase (TK) domain and a regulatory region (8). After ligand binding, specific tyrosine residues of the intracellular domain are autophosphorylated, which results in initiation of the intracellular signaling cascade, including the Ras/Raf/MAPK, JAK/STAT and PI3K-Akt pathways, leading to a multitude of effects including cell proliferation, cell differentiation, angiogenesis, metastasis, and antiapoptosis (9). Gefitinib is an orally active EGFR TK inhibitor and has been widely used in clinical trials and is approved for the treatment of advanced NSCLC (10–12).

The mechanism of antitumor effect or drug sensitivity has not been clearly understood (12); recently, however, Lynch et al. and Paez et al. reported that clinical responsiveness to gefitinib was associated with somatic mutations in the TK domain of *EGFR* gene in NSCLCs (13, 14). These mutations occurred near the ATP cleft of the TK domain in which

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Table 1 Patient characteristics and *EGFR* mutation

Variables	No.	*EGFR* mutation (%)	*P*
A. In NSCLCs			
Age (y)			
≤67	60	21 (35%)	
>67	60	17 (28%)	
Gender			
Male	83	17 (20%)	0.0001
Female	37	21 (57%)	
Smoking history			
Never smoker	36	25 (69%)	<0.0001
Ever smoker	84	13 (15%)	
Histology			
Adenocarcinoma	82	37 (45%)	<0.0001*
Squamous cell carcinoma	35	0	
Adenosquamous cell carcinoma	1	1 (100%)	
Large cell carcinoma	2	0	
B. In adenocarcinomas			
Age (y)			
≤67	42	21 (50%)	
>67	40	16 (40%)	
Gender			
Male	52	17 (33%)	0.003
Female	30	20 (67%)	
Smoking history			
Never smoker	33	24 (73%)	<0.0001
≤30 pack-years	16	5 (31%)	
>30 pack-year	33	8 (24%)	
Smoking status			
Former smoker	21	5 (24%)	
Current smoker	28	8 (29%)	

*Histological difference was examined between adenocarcinoma and other types of NSCLCs. *P* values are stated when there were significant differences between groups.

4-anilinoquinazoline compounds such as gefitinib compete with ATP for binding (13). In addition, Paez et al. found that the mutations were more frequent in cases of adenocarcinoma histology, female gender, and Japanese origin (14).

In this study, we examined the *EGFR* mutational status in 120 NSCLC specimens including 21 cases treated with gefitinib and analyzed the relationship between the *EGFR* status and clinicopathologic features to investigate the clinical importance of *EGFR* mutation in NSCLCs.

MATERIALS AND METHODS

Clinical Samples. Surgically resected specimens of 120 NSCLCs consisting of 82 adenocarcinomas, 35 squamous cell carcinomas, 2 large cell carcinomas, 1 adenosquamous cell carcinoma, and corresponding 10 nonmalignant peripheral lung tissues were obtained from Okayama University Hospital, Okayama, Japan, after acquiring informed consent from each patient, between 1994 and 2003. Institutional review board permission and informed consent were obtained for all cases. The NSCLC patients consisted of 83 males and 37 females and their median age was 67. Seventy patients had stage I disease, 20 stage II, 24 stage III, and 3 stage IV. Disease stages were not known in 3 patients because systemic dissection for lymph node was not done for these cases. Eighty-four cases were from ever smokers with a median smoke exposure of 45.5 pack-years (50 cases were current smokers and 34 were former smokers) and 36 cases were never smokers. The patient characteristics are shown in Table 1. Twenty-one cases were treated with gefitinib for recurrent disease and clinical responsiveness was recorded. These cases had been previously treated with some chemotherapy that failed to respond to disease. For evaluation of response, all patients underwent complete blood counts, blood chemistry, urinalysis, plain chest radiograph at least weekly for a month from the beginning of gefitinib treatment and at least monthly thereafter, and serum carcinoembryonic antigen monthly; computed tomography scan of the chest (and abdomen if tumor had spread to intraabdominal organs) was taken at least every 3 months for 2 years. Response was assessed using Eastern Cooperative Oncology Group criteria (15). Cases of complete or partial response were considered as responsive cases and that of no change or progressive disease as nonresponsive cases. Clinicopathologic staging was determined according to International Union Against Cancer tumor-node-metastasis classification of malignant tumors (16).

DNA Extraction and Sequencing Analysis. Genomic DNA was isolated by digestion with proteinase K followed by phenol-chloroform (1:1) extraction and ethanol precipitation from frozen specimen (17) and by DEXPAT (TaKaRa, Shiga, Japan) from paraffin embedded tissues following the manufacturer's instructions. *EGFR* mutations were detected using PCR-based direct sequencing of the seven exons of the TK domain (exons 18-24). PCR amplification was done in 20-μL volume containing genomic DNA using HotStarTaq DNA polymerase (Qiagen Inc., Valencia, CA). DNA was amplified for 40 cycles at 94°C for 20 seconds, 56°C to 65°C for 30 seconds, and 72°C for 20 seconds, followed by 7 minutes extension at 72°C. The primers and condition of PCR amplification are shown in Table 2. PCR products were incubated using ExoSAP-IT (Amersham Biosciences Corp., Piscataway, NJ) and sequenced directly using Applied Biosystems PRISM dye terminator cycle sequencing method (Perkin-Elmer Corp., Foster City, CA) with ABI PRISM 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA). Several samples, whose mutation status were difficult to determine by direct sequence, were amplified and cloned into pBluescript after sequencing with M4 and RV6 primer (Fig. 1).

Statistical Analyses. The rates of *EGFR* mutation between two groups were compared using the χ^2 or Fisher's exact test. Fisher's exact test was done if there were five or fewer observations in a group. Logistic regression models were used to further explore observed differences and to identify baseline factors that may independently predict for *EGFR* mutation. The Cochran-Armitage test for trend was used to examine the dose effect of smoke exposure on *EGFR* mutation. Univariate analysis of overall survival was carried out by the Kaplan-Meier method using the log rank test and generalized Wilcoxon test for the two groups. Probability values <0.05 were defined as being statistically significant. All statistical tests were two-sided.

RESULTS

***EGFR* Mutation in Non–Small Cell Lung Cancers.** Because results of sequencing for *EGFR* TK domain (exons 18-24) in unselected 45 cases indicated that nucleotide change

compared with original sequence (Genebank accession no. AL365274) were limited to the first four exons (exons 18-21), further analyses were limited to these four exons. We examined the *EGFR* status in 120 cases of NSCLCs and found mutations in 38 cases (32%). Mutation was detected in one case of point mutation in exon 18, 19 cases of in-frame deletion in exon 19 involving 3 to 8 codons around the uniformly deleted codons 747 to 749 (Leu-Arg-Glu sequence), and 18 cases of point mutation (L858R) in exon 21. Representative nucleotide sequences of the *EGFR* gene mutation were shown in Fig. 1. There were eight types of deletion variants in exon 19 (Fig. 2) and new types of deletion were variants 4, 5, 7, and 8. In addition, the third letter of codon 787 (CAG) in exon 20 was changed in eight cases homozygously (CAA) and 14 cases heterozygously (CAG and CAA) resulting in no amino acid change. Thus, this change was considered to be a polymorphism or silent mutation. We also examined 10 nonmalignant lung tissues of cases in which the corresponding tumors harbored mutation and found no mutations, indicating that these mutations were somatic in origin.

***EGFR* Mutation and Clinicopathologic Correlations.** We analyzed the relationship between the *EGFR* status and clinicopathologic factors (Table 1, A and B). *EGFR* mutations were frequently present in adenocarcinoma ($P < 0.0001$), never smokers ($P = 0.0001$), and female gender ($P = 0.003$) in NSCLCs (Table 1A). Logistic regression models showed that smoking status ($P = 0.005$) and histology ($P < 0.0001$) were independent variables. Because adenocarcinoma is the dominant histology for mutation, further analyses were limited to adenocarcinoma. *EGFR* mutations were significantly associated with female gender (67%, $P = 0.003$) and never smoker status (73%, $P < 0.0001$) compared with male gender (33%) and ever smoker status (27%), respectively, by univariate analysis (Table 1B). Logistic regression models showed that smoking status was the only independent variable in adenocarcinomas ($P = 0.01$). In addition, the cases were divided into three groups based on smoke dose: (*a*) never smokers ($n = 33$), (*b*) smokers with exposure of ≤30 pack-years ($n = 16$), and (*c*) smokers with exposure of >30 pack-years ($n = 33$). The Cochran-Armitage test for trend was done to examine the dose effect of smoke exposure on *EGFR* mutation. We found that there was an inverse trend between smoking dose and mutation ($P < 0.0001$; Table 1B). We also examined the relationship between *EGFR* mutations and detailed smoking status (current and former smokers); however, there was no significant difference between these two groups (Table 1B). Exon 19 deletions were significantly more frequent in males, and exon 21 mutations were more frequent in females ($P = 0.049$). To exclude the possible effect of smoke exposure on the difference of mutation location and gender, the same analysis was done in the never-smoking adenocarcinoma group showing significant difference as well ($P = 0.008$). *EGFR* mutations were not related to other clinicopathologic factors including disease stage and patient age.

***EGFR* Mutation and Gefitinib Responsiveness.** Among 120 NSCLC patients, 21 cases were treated with gefitinib and clinical responsiveness was recorded. These cases consisted of 8 (38%) cases of females, 8 (38%) of never smokers, and 15 (71%) of adenocarcinomas. Detailed characteristics of these cases are shown in Table 3. In 21 treated cases, mutations were present in 8 of 10 cases with gefitinib responsiveness and in 1 of 11 cases with nonresponsiveness, showing that *EGFR* mutations were significantly more frequent in gefitinib response cases ($P = 0.002$). However, gender, smoke exposure, and

Table 2 Summary of primer sequences and annealing temperatures for direct sequence

Exon	Primer sequences	T_m (°C)	Product size (bp)
For DNA templates from frozen samples			
18	F, 5′-GAGGTGACCCTTGTCT CTGTGT-3′ R, 5′-AGCCCAGAGGCCTG TGCCA-3′	57	189
19	F, 5′-CCAGATCACTGGGCAGCAT GTGGCACC-3′ R, 5′-AGCAGGGTCTAGAGCAGA GCAGCTGCC-3′	65	265
20	F, 5′-ACTGACGTGCCTCTCC CTCC-3′ R, 5′-CCGTATCTCCCTTCCCT GATT-3′	57	235
21	F, 5′-ATCTGTCCCTCACAGCA GGGTC-3′ R, 5′-GGCTGACCTAAAGCC ACCT-3′	57	210
22	F, 5′-AATTAGGTCCAGAGTGAG TTAAC-3′ R, 5′-ACTTGCATGTCAGAGGAT ATAATG-3′	65	251
23	F, 5′-CATCAAGAAACAGTAACC AGTAATG-3′ R, 5′-AAGGCCTCAGCTGTTTG GCTAAG-3′	65	320
24	F, 5′-TTGACTGGAAGTGTCGCA TCACC-3′ R, 5′-CATGTGACAGAACACAGT GACATG-3′	65	279
For DNA templates from paraffin-embedded samples			
18a*	F, 5′-GAGGTGACCCTTGTCTCGTGT-3′ R, 5′-ATTCAGTTTCCTTCAAGA TCCTC-3′	56	110
18b**	F, 5′-AGTGGAGAAGCTCCCAAC CAAGC-3′ R, 5′-AGCCCAGAGGCCTGTGCCA-3′	65	131
19	F, 5′-AACGTCTTCCTTCTCTCTC TGTCAT-3′ R, 5′-CCACACAGCAAAGCAGA AACTC-3′	56	150
20a*	F, 5′-ACTGACGTGCCTCTCC CTCC-3′ R, 5′-AAGGGCATGAGCTGCG TGA-3′	56	127
20b**	F, 5′-TGCCTCACCTCCACCGT-3′ R, 5′-CCGTATCTCCCTTCCCT GATT-3′	56	152
21a*	F, 5′-ATCTGTCCCTCACAGCAG GGTC-3′ R, 5′-TGATCTTGACATGCTGCG GTGTT-3′	56	126
21b**	F, 5′-AGCCAGGAACGTACTGGTGA-3′ R, 5′-GGCTGACCTAAAGCCACCT-3′	56	134

NOTE. a* and b**, exon 18, 20, and 21 were divided into two parts for PCR amplification and sequenced separately.

Abbreviations: T_m, annealing temperature; *F*, forward primer; *R*, reverse primer.

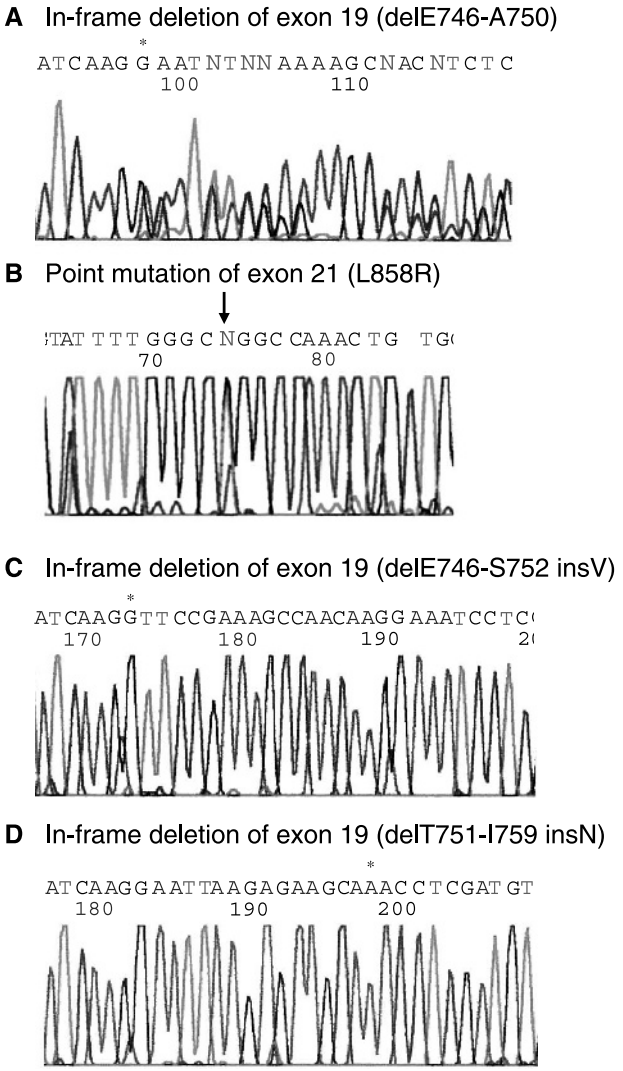

Fig. 1 Representative nucleotide sequence of the *EGFR* gene in tumor specimens *A,* the nucleotide sequence of heterozygous in-frame deletions in exon 19 by direct sequencing (*double peaks*). *B,* heterozygous point mutation in exon 21 (L858R, CTG to CGG). *C* and *D,* the nucleotide sequence of in-frame deletions in exon 19 of cloned sequencing. Amplified sample DNAs were cloned into pBluescript and sequenced. *, the break point of in-frame deletion in exon 19. The vertical arrow indicates the site of point mutation in exon 21.

histology were not related to gefitinib responsiveness. There was no relationship between mutational type and clinical responsiveness to gefitinib therapy in our study. One case with exon 21 mutations (L858R) but no responsiveness to gefitinib was a case of adenosquamous cell carcinoma in a female. In two gefitinib-responsive cases, mutations were not present, and these consisted of an adenocarcinoma and a squamous cell carcinoma in a male. A case of squamous cell carcinoma with complete response was previously reported (18), and the status of partial response has been continuing for 2 years. A case of adenocarcinoma is a 70-year-old Japanese man with partial response and the disease has been controlled for 22 months. Both cases have a smoking history. We also analyzed the relationship between patient survival and *EGFR* mutation in gefitinib-treated cases. Overall, survival curves are shown in Fig. 3 using the Kaplan-Meier method. Survival rate (% $\pm$ SE) at 1 and 2 years for patients with mutations were 87.5 $\pm$ 11.7% and 87.5 $\pm$ 11.7%, and those for patients without mutations 62.5 $\pm$ 15.1% and 37.5 $\pm$ 16.4%, respectively. Median survival times of patients with and without *EGFR* mutations were 25.1 and 14.0 months, respectively. Although patients with *EGFR* mutations had approximately 2-fold survival advantage, there was no significant difference in the overall survival between two groups (generalized Wilcoxon test, $P = 0.132$; log rank test, $P = 0.153$).

DISCUSSION

In this study, we searched *EGFR* mutations and explored the relationship between *EGFR* mutational status and clinicopathologic features in NSCLCs. The mutations were limited to the three exons (exons 18, 19, and 21) of the TK domain. These affected codons have already been reported to be sites for *EGFR* mutations, although some of the mutations reported herein are novel. All mutation target structures around the ATP binding cleft probably resulted in repositioning of amino acid residues, stabilizing their interaction with both ATP and its competitive inhibitor gefitinib (13). Previous studies indicated that adenocarcinoma histology, never smoker status, and female gender were factors associated with *EGFR* mutations. Our findings confirmed and extended these observations. First, we show that the degree of smoke exposure was inversely related to *EGFR* mutations in adenocarcinoma. Smoke exposure is a well-established risk factor for lung cancer and can cause specific mutational spectrum in *TP53* gene and epigenetic alterations, including the DNA methylation pattern (4, 19). We previously showed that smoking dose was closely related to the rate of methylation in adenocarcinoma (20). Taken together, these facts strongly suggest that the mechanisms for tumorigenesis

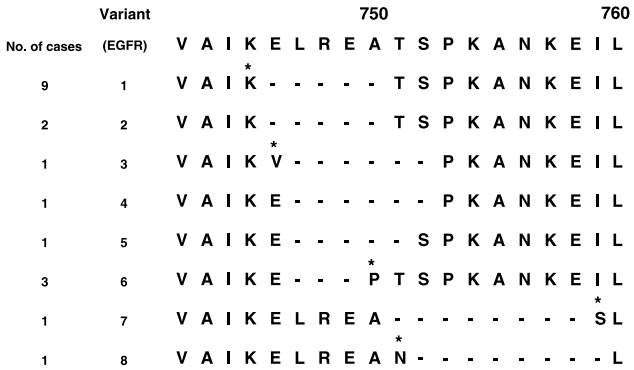

Fig. 2 Summary of in-frame deletion mutations in exon 19. The variation of amino acid residue sequences due to in-frame deletion. The number of cases are described at the left side of each variant. Variants 1 and 2 are the same amino acid sequences; however, nucleotide sequences are different. Deleted nucleotide site of variant 1 is from 2,235 to 2,249; on the other hand, that of variant 2 is from 2,236 to 2,250.

Table 3 Clinicopathological characteristics of 21 NSCLC cases treated with gefitinib

A

No.	Gender	Age (y)	Smoking status	Histology	ECOG performance status	No. of prior chemotherapy	Radiotherapy	Period from surgery to gefitinib therapy (mo)	*EGFR* mutation (exon)	Response to gefitinib
1	F	60	Never	Adenocarcinoma	2	2	+	19.6	19	CR
2	M	64	Never	Adenocarcinoma	1	2	+	5.4	19	PR
3	M	65	Smoker	Adenocarcinoma	2	2	–	28.4	19	PR
4	M	57	Smoker	Adenocarcinoma	1	2	–	43	19	PR
5	M	40	Never	Adenocarcinoma	1	2	+	21.1	19	PR
6	F	59	Never	Adenocarcinoma	1	1	+	21.5	21	PR
7	F	60	Never	Adenocarcinoma	0	3	+	16.7	21	PR
8	F	68	Never	Adenocarcinoma	2	1	–	35.2	21	PR
9	M	70	Smoker	Adenocarcinoma	2	1	–	63.9	—	PR
10	M	77	Smoker	Squamous cell carcinoma	2	2	+	18.8	—	PR
11	F	77	Never	Adenosquamous cell carcinoma	1	1	+	10	21	NC
12	F	75	Never	Adenocarcinoma	0	3	+	51.4	—	NC
13	F	73	Smoker	Squamous cell carcinoma	2	1	–	17.6	—	NC
14	M	77	Smoker	Squamous cell carcinoma	1	2	+	11.8	—	PD
15	M	70	Smoker	Large cell carcinoma	1	3	+	14.3	—	PD
16	M	68	Smoker	Adenocarcinoma	0	1	–	37.8	—	NC
17	M	63	Smoker	Adenocarcinoma	1	3	–	17	—	NC
18	M	58	Smoker	Adenocarcinoma	3	2	+	17.9	—	NC
19	F	55	Smoker	Squamous cell carcinoma	1	3	+	105	—	PD
20	M	49	Smoker	Adenocarcinoma	1	2	+	14.3	—	NC
21	M	62	Smoker	Adenocarcinoma	1	2	–	87.1	—	NC

B

	Responder (n = 10)	Nonresponder (n = 11)
EGFR mutation	8	1
Median age (range), y	62 (40-77)	68 (49-77)
Gender		
Male	6	7
Female	4	4
Smoking history		
Never	6	2
Smoker	4	9
Histology		
Adenocarcinoma	9	6
Squamous cell carcinoma	1	3
Adenosquamous cell carcinoma	0	1
Large cell carcinoma	0	1
ECOG performance status		
0-1	5	9
2	5	1
3	0	1

NOTE. The case that achieved complete response or partial response based on ECOG criteria was considered as responder and no change or progressive disease was considered as no-responder.

Abbreviations: CR, complete response; PR, partial response; NC, no change; PD, progressive disease; ECOG, Eastern Cooperative Oncology Group.

in adenocarcinoma vary according to smoking status. Second, our results show a gender difference based on mutational location/type. The in-frame deletions of exon 19 were significantly more frequent in male gender and point mutations of exon 21 in females. The reasons for these gender differences are not known, but there is a possibility that some factors derived from gender difference would be present for the causes of *EGFR* mutation. Of interest, gender difference has been shown in the *TP53* mutational spectrum in adenocarcinoma arising in never smokers (19). Third, Paez et al. indicated that female gender was one of the factors for *EGFR* mutation in univariate analysis (14). Indeed, it was reported to be one of the predictive factors for gefitinib-responsive cases (11). In our analysis, female gender was also the factor for *EGFR* mutation in univariate analysis; however, it was not an independent factor in multivariate analysis. Our findings are in agreement with those of Miller et al. (21) who showed that female gender was not an independent predictive factor for gefitinib responsive cases, in contrast to those of Fukuoka et al. (11).

We also confirmed previous reports that *EGFR* mutations were significantly associated with gefitinib-responsive cases (13, 14) except some cases. According to Eastern Cooperative Oncology Group criteria (15), one adenosquamous cell carcinoma case with exon 19 mutation was classified as no change. This is the first reported patient with mutation in

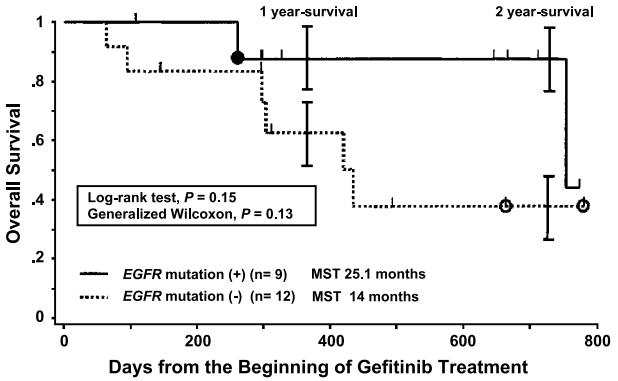

Fig. 3 Correlation of *EGFR* mutational status and patient survival in 21 gefitinib-treated cases by the Kaplan-Meier method. Survival rate (% ± SE) at 1 and 2 years for patients with mutations were 87.5 ± 11.7% and 87.5 ± 11.7%, and those for patients without mutations 62.5 ± 15.1% and 37.5 ± 16.4%, respectively. There was no significant difference in the overall survival between two groups (generalized Wilcoxon test, $P = 0.132$; log rank test, $P = 0.153$). Tics indicate at risk. Bars at 1 and 2 years from the beginning of gefitinib treatment indicate standard errors of cumulative survival rate. ●, patients with no change having *EGFR* mutations. ○, patients with complete or partial response not having *EGFR* mutations. MST, median survival time.

whom gefitinib did not work. Two cases showed marked clinical responses but lacked mutations, as Lynch et al. reported one of nine responders without *EGFR* mutation. These results suggested that there are other mechanisms to determine gefitinib responsiveness in NSCLCs.

Previous study reported that adenocarcinoma histology, never-smoking status, and female gender were predictive factors for gefitinib responsiveness (11). Our result showed that only *EGFR* mutation was significantly associated with gefitinib responsiveness. This discrepancy may be derived from the small number of our treated cases for analysis. Regarding the response rate, the IDEAL 1 trial reported 27.5% in 102 Japanese and 10.4% in 106 non-Japanese patients (11). Our study showed 48% responsiveness in 21 cases treated with gefitinib. We compared the patient characteristics of 21 treated cases with the total 120 cases. Rates of female cases were 38% and 31%, never smoker cases 38% and 30%, and adenocarcinoma cases 71% and 68% in treated and total cases, respectively. The rates of these factors were slightly higher in the treated population than in the total population with no statistical differences, indicating that the 21 cases represented the total population. Takano et al. (22) reported that response rates in female gender, adenocarcinoma, and never smoker were 53%, 38%, and 63%, respectively, suggesting that our response rate was an acceptable value. Further analysis with additional cases is important to discuss the issue of responsiveness.

The overall survival data showed no significant difference in the limited number of cases. However, we showed better survival in patients having *EGFR* mutations than in those without mutations at 1 and 2 years after the beginning of gefitinib treatment. Data from the IDEAL 1 study, which included Japanese patients, showed the median survival time of gefitinib responders (complete or partial response) was 13.3 months, contrasting with the overall survival of 7.6 months (11). Thus, a survival advantage at 1 and 2 years in patients with *EGFR* mutations seems to be probable. Two gefitinib responders without *EGFR* mutations also live longer. Our present study of survival, which was limited to 21 cases, showed an advantage (although not significant) of gefitinib treatment for *EGFR* mutant cases. Further accumulation of treated cases with gefitinib should be necessary to estimate the effect of *EGFR* mutation gefitinib therapy for patient survival.

Our work confirms and extends the previously reported findings regarding *EGFR* mutations, clinicopathologic features, and response to targeted therapy. In addition, our findings strongly suggest that *EGFR* mutation can be one of the main factors to determine the strategy of chemotherapy and indicate the importance of molecular biological analysis of tumor specimens to establish the appropriate molecular-targeted treatment.

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