Mutation in the Tyrosine Kinase Domain of Epidermal Growth Factor Receptor Is a Predictive and Prognostic Factor for Gefitinib Treatment in Patients with Non–Small Cell Lung Cancer

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Abstract

Purpose: Mutations in epidermal growth factor receptor (EGFR) can be used to predict the tumor response of patients receiving gefitinib for non–small cell lung cancer (NSCLC). We investigated the association between mutations in EGFR tyrosine kinase domain and tumor response and survival in gefitinib-treated NSCLC patients.

Experimental Design: EGFR mutations in exons 18 to 21 were analyzed by DNA sequencing of paraffin-embedded tumor tissues from gefitinib-treated NSCLC patients. The results were correlated with clinical variables.

Results: EGFR mutations were found in 61.1% (33 of 54) of cases; response rate and disease control rate were 56.8% and 68.5%, respectively. There was no significant difference in mutation rates between adenocarcinoma (29 of 43) and nonadenocarcinoma (4 of 11; $P = 0.085$). However, all four nonadenocarcinomas with EGFR mutations had no response to gefitinib. Presence of EGFR mutations was the only independent predictor for disease control ($P = 0.003$) and tumor response ($P = 0.017$) in multivariate analysis; positive predictive values were 87.9% and 70.8% and negative predictive values were 61.9% and 69.2%, respectively. In comparison with patients whose tumor was negative for EGFR mutations, patients with EGFR mutations had better progression-free survival (median, 7.6 versus 1.7 months; $P = 0.011$) and overall survival (median, 14.7 versus 4.7 months; $P = 0.046$).

Conclusions: Mutations in EGFR tyrosine kinase correlate with treatment response and survival in gefitinib-treated NSCLC patients and can be used as a predictive and prognostic factor. Thus, analysis of EGFR tyrosine kinase mutations in lung adenocarcinoma is of clinical significance, as it can permit the customization of treatment with EGFR tyrosine kinase inhibitors.

Lung cancer is the leading cause of cancer-related deaths in many countries, including Taiwan (1–4). Only a small proportion of lung cancer patients present with localized disease that can be effectively treated with conventional therapy. The overall 5-year survival rate of non–small cell lung cancer (NSCLC) is <15%, indicating the highly malignant nature of this disease.

Protein tyrosine kinases play important roles in the pathogenesis of many malignant tumors (5). Among them, epidermal growth factor receptor (EGFR) tyrosine kinase, the first receptor protein tyrosine kinase described, has been implicated in the initiation and progression of NSCLC (6–8). Monoclonal antibodies and small molecular weight compounds that inhibit the EGFR signaling pathway have been developed and shown to have antitumor effects (9).

Gefitinib, a selective EGFR tyrosine kinase inhibitor, is an orally active agent for advanced NSCLC. In two phase II trials, IDEAL 1 and IDEAL 2 (10, 11), gefitinib was shown to have substantial effect when used alone as salvage treatment for patients treated previously with chemotherapy. In Taiwan, where Chinese descendants represent a major proportion of the population, we have shown previously a tumor response rate of 33% (12). This outcome was higher than that observed in Caucasian patients but similar to that of Japanese patients (10, 11). These observations suggest ethnic or geographic differences in the response of NSCLC patients to gefitinib treatment. Recently, Lynch et al. (13) and Paez et al. (14) reported that mutations in the tyrosine kinase domain of EGFR play a critical role in determining tumor response in NSCLC patients

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receiving gefitinib. Interestingly, one of these studies also showed a significant difference in the prevalence of EGFR mutations between Caucasian (2-8%) and Japanese (26%) NSCLC patients (14). Although EGFR mutations have been shown to be strongly associated with the response of NSCLC patients to gefitinib treatment, their role in response duration and patient survival is still unclear, especially in a population that has a higher rate of gefitinib response.

Using pretreatment tumor samples, we have conducted a retrospective study to detect mutations in the tyrosine kinase domain of the *EGFR* gene and investigated the relationship between EGFR tyrosine kinase mutations and clinical outcome in 54 Taiwanese NSCLC patients.

Materials and Methods

Patient selection and clinical data collection. Advanced NSCLC patients (n = 146) were treated with gefitinib in a clinical study at Taipei Veterans General Hospital (Taipei, Taiwan) from March 2002 to February 2004. Patient eligibility for study participation was histologically or cytologically confirmed, inoperable NSCLC, irrespective of the presence of measurable lesions or good performance status. Selected patients had failed prior platinum-based chemotherapy or had a poor performance status at diagnosis and were considered at high risk for conventional chemotherapy. Patients with a life expectancy of <1 week were excluded. Patients were treated with a 250 mg fixed daily dose of gefitinib [Iressa (ZD1839), AstraZeneca Pharmaceuticals, Wilmington, DE] as monotherapy. Treatment continued until there was intolerable toxicity, disease progression, or death. All patients gave written informed consent. The treatment protocol was approved by the Taipei Veterans General Hospital Institutional Review Board.

Histopathology review and DNA preparation. Histopathology slides of specimens from bronchoscopic, computed tomography–guided, or sonography-guided biopsy, wedge resection, or lobectomy were reviewed. Tumors were classified according to the 1999 WHO Histological Typing (15) and staged according to the American Joint Committee on Cancer *Cancer Staging Manual* (16). Genomic DNA was prepared from formalin-fixed, paraffin-embedded sections of tumor specimens using xylene deparaffinization, proteinase K digestion, and phenol/chloroform extraction followed by ethanol precipitation. An alternative DNA extraction method using QIAamp DNA Mini kit (Qiagen, Hilden, Germany) was used for larger size specimens. Extracted DNA was evaluated by UV spectrophotometry and PCR of *β-globin* gene.

PCR and sequencing of EGFR gene from genomic DNA. Eight pairs of oligonucleotide primers were used to amplify exons 18 to 21 of *EGFR* gene by nested PCR according to the previously described procedures (13). PCR products were sequenced using the ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction kit and the ABI PRISM 3700 Genetic Analyzer (PE Applied Biosystems, Foster City, CA). Sequences of PCR amplicons were compared with the cDNA sequence of EGFR obtained from Genbank (accession no. NM 005228.3) by Mutation Surveyor 2.03 (SoftGenetics, State College, PA), Sequencer 4.14 (Gene Codes, Ann Arbor, MI), and manual review.

Evaluation for tumor response and patient survival. Pretreatment staging, treatment response, and adverse events were evaluated as described previously (12). Clinical results of the first 76 patients from this study have been published. Objective tumor response was assessed by unidimensional method in accordance with Response Evaluation in Solid Tumors criteria (17). Responses of patients with measurable lesions were classified as complete response, partial response, stable disease, or progressive disease; in patients with nonmeasurable lesions, they were classified as nonprogressive disease or progressive disease. Response evaluation was done at the eighth week and every 8 weeks thereafter. In addition, chest X-rays were done on days 8, 15, and 29 and then every 4 weeks. Toxicities were assessed on days 8, 15, and 29 and then every 4 weeks using the WHO toxicity criteria (18), except nail change, which was graded by the modified National Cancer Institute Common Toxicity Criteria version 2.0.

Objective response rate was calculated from patients with measurable lesions and disease control rate was calculated from all patients who had no disease progression at the first response evaluation. Duration of response was calculated from the date of documentation of complete response or partial response to the first instance of disease progression or death. Progression-free survival was calculated from the date of initiation of gefitinib to the date of disease progression or death. Overall survival was defined as the period from the start of gefitinib treatment to the date of death.

Statistical analyses of clinical data and epidermal growth factor receptor mutations. Univariate analysis of patient characteristics and tumor response was done by the χ^2 test and the Fisher's exact test. The considered variables were age (≤65 or >65 years), gender, performance status (Eastern Cooperative Oncology Group performance status 0/1 or ≥2), smoking (ex-smoker/current smoker or nonsmoker), histology (adenocarcinoma or nonadenocarcinoma), number of prior chemotherapy regimens (0, 1, or ≥2), number of metastatic organs (0/1 or ≥2), and EGFR mutation (mutant or nonmutant). Logistic regression model with a backward stepwise procedure was used for multivariate analysis. Survival curves were plotted by the Kaplan-Meier method and compared by log-rank test. For ordinal variables, a log-rank test of trend was applied. Cox regression model with a backward stepwise procedure was done for multivariate survival analysis. The association between the response and toxicity profile was analyzed using the maximal grade of toxicity experienced during the first month of treatment (early toxicity profile). Analyses and figures were carried out with SPSS for Windows version 12.0 (SPSS, Inc., Chicago, IL).

Results

Patient characteristics. Of the 146 patients enrolled for gefitinib treatment, 62 had no available tumor tissue samples, 21 had only limited samples that precluded research analysis, and 9 provided specimens that yielded inadequate DNA for analysis. Consequently, tumor tissue samples that were adequate for DNA analysis were available in 54 patients. In this study, there were similar numbers of male and female patients. Most patients were nonsmokers (66.7%) and had adenocarcinoma (79.6%). Patient characteristics are summarized in Table 1. The median follow-up was 16.1 months (range, 5.1-24.5 months); 42 patients had progressive disease and 33 patients died.

Epidermal growth factor receptor mutations. Thirty-three of the 54 patients (61.1%) had mutations in exons 18 to 21 of *EGFR* gene. As summarized in Table 2, 12 patients had deletion mutations, 19 had substitution mutations, 1 had combined deletion and substitution mutations, and 1 had double substitution mutations. All but one deletion mutation were found in exon 19 and all substitution mutations were found in exons other than 19. The frequency of mutations in exons 18 to 21 was 9%, 22%, 9%, and 24%, respectively. Deletions in exon 19 (n = 11) and substitution mutation of L858R (n = 9) in exon 21 were the two most common types of mutations, representing 61% of total cases. Other types of mutations were found in one patient each and, with the exception of L861Q and G719S (13, 14), were unreported previously. We identified one deletion mutation and four substitution mutations but no duplication/insertion mutations in exon 20.

Association between epidermal growth factor receptor mutation and patient characteristics. In the univariate analysis, there was no statistically significant association between EGFR mutations and age (≤65 versus >65 years, 60.0% versus

Table 1. Patient characteristics ($N = 54$)

Characteristics	No. patients (%)
Gender	
Male	29 (53.7)
Female	25 (46.3)
Age (y)	
Median	64.0
Range	29-80
Eastern Cooperative Oncology Group performance status	
0-1	26 (48.1)
2	18 (33.3)
3-4	10 (18.5)
History of smoking	
Nonsmoker	36 (66.7)
Ex-smoker	12 (22.2)
Current smoker	6 (11.1)
Prior chemotherapy regimens	
0	9 (16.7)
1	19 (35.2)
2	14 (25.9)
3	9 (16.7)
≥4	3 (5.6)
Histologic subtype	
Adenocarcinoma	43 (79.6)
Squamous cell carcinoma	10 (18.5)
NSCLC, unspecified	1 (1.9)
Stage	
III	3 (5.6)
IV	51 (94.4)
No. organs with metastases	
0	3 (5.6)
1	20 (37.0)
2	17 (31.5)
≥3	14 (25.9)

62.5%; $P = 1.000$), gender (male versus female, 51.7% versus 72.0%; $P = 0.213$), smoking status (nonsmoker versus ex-smoker/current smoker, 69.4% versus 44.4%; $P = 0.139$), and histologic subtype (adenocarcinoma versus nonadenocarcinoma, 67.4% versus 36.4%; $P = 0.085$). None of the patients fulfilled the criteria of bronchioloalveolar carcinoma (BAC) by the 1999 WHO Histological Typing. We estimated the proportion of bronchioloalveolar growth pattern in each adenocarcinoma specimen with a 10% increment scale. Accordingly, we did a subgroup analysis and found that neither the presence nor the proportion of BAC pattern of adenocarcinoma correlated with EGFR mutations (data not shown).

There was no statistically significant difference in demographic characteristics between patients with EGFR deletion mutations and patients with EGFR substitution mutations (data not shown).

Association between epidermal growth factor receptor mutation and tumor response. A total of 37 patients had measurable lesions and 17 had nonmeasurable lesions. Of the 37 patients with measurable lesions, 21 had partial response, 7 had stable disease, and 9 had progressive disease. The objective response rate was 56.8% (95% confidence interval, 40.0-73.5%). In univariate analysis, histologic subtype (adenocarcinoma versus nonadenocarcinoma, 70.0% versus 0%; $P = 0.001$) and EGFR mutations (mutant versus nonmutant, 70.8% versus 30.8%; $P = 0.045$) were significantly associated with tumor response. In multivariate analysis, presence of EGFR mutations was the only independent variable in predicting tumor response ($P = 0.017$; Table 3).

In further analysis of the tumor response in patients with different patterns of EGFR mutations, patients with mutations in exon 18 (4 of 4, 100%) and exon 19 (6 of 7, 85.7%) seemed to have better tumor response rates in comparison with patients with mutations in exon 20 (2 of 4, 50%) and exon 21 (5 of 8, 62.5%; Table 4).

Of the 17 patients with nonmeasurable lesions, 9 had nonprogressive disease and 8 had progressive disease. Therefore, 37 of the total 54 patients had their disease controlled at the first response evaluation and the disease control rate was 68.5% (95% confidence interval, 55.7-81.3%). Gender (female versus male, 88.0% versus 51.7%; $P = 0.010$), histologic subtype (adenocarcinoma versus nonadenocarcinoma, 79.1% versus 27.3%; $P = 0.002$), and EGFR mutations (mutant versus nonmutant, 87.9% versus 38.1%; $P < 0.001$) were all correlated with disease control. In multivariate analysis, presence of EGFR mutations ($P = 0.003$) was the only independent predictor again (Table 3).

Of the 33 patients with tumors positive for mutant EGFR, 4 patients had disease progression within 2 months of gefitinib treatment. All these tumors were squamous cell carcinoma and the patterns of mutation were delA767_V769 (exon 20), A763V mutation (exon 20), L858R mutation (exon 21), and a double substitution mutation, G719S and L861Q, in exons 18 and 21.

Table 4 shows the tumor responses of patients with or without EGFR mutations. With respect to EGFR mutations in exons 18 to 21, the positive predictive values were 88% (29 of 33) and 71% (17 of 24) and the negative predictive values were 62% (13 of 21) and 69% (9 of 13) for disease control and tumor response, respectively.

Association between epidermal growth factor receptor mutation and patient survival and response duration. Using Kaplan-Meier method and log-rank test, patients with EGFR mutation had a significantly longer progression-free survival (median, 7.6 versus 1.7 months; $P = 0.001$; Fig. 1A). Additionally, female gender ($P = 0.035$), old age ($P = 0.035$), and adenocarcinoma ($P < 0.001$) all favored a longer progression-free survival. In Cox regression model with a backward stepwise procedure, histologic subtype ($P < 0.001$), EGFR status ($P = 0.013$), age ($P = 0.007$), and performance status ($P = 0.008$) were all independent predictors for a longer progression-free survival. In univariate analysis of overall survival, patients with good performance status ($P = 0.002$), adenocarcinoma ($P = 0.012$), or mutated EGFR ($P = 0.046$) had a longer survival (Fig. 1B). In multivariate analysis, only performance status ($P = 0.001$) and histologic subtype ($P = 0.002$) were independent predictors (Table 3).

In 21 patients with objective tumor responses, the median duration of response was 5.8 months. Although there was no statistically significant difference between patients with and without EGFR mutations, there was a trend favoring a longer duration of response in patients with mutations (Fig. 1C). However, duration of response of patients with mutations in

Table 2. Clinical features and mutation patterns in 33 NSCLC patients with mutated *EGFR* gene

	EGFR mutation									
	Exon	Patterns	Sex/Age	Smoking status	Pathology	Performance status	Prior regimen	Response	Progression-free survival (mo)	Overall survival (mo)
1	18	V689M	M/62	Ex-smoker	Adenocarcinoma	2	3	PR	3.9	10.6
2	18	N700D	M/65	Ex-smoker	Adenocarcinoma	2	2	PR	7.9	15.9
3	18	E709Q	F/55	Nonsmoker	Adenocarcinoma	1	2	PR	6.0	10.6
4	18	S720P	F/79	Nonsmoker	Adenocarcinoma	2	4	PR	13.2	20.5
5	19	delE746.A750	M/64	Ex-smoker	Adenocarcinoma	0	2	PR	11.1	20.2
6	19	delE746.A750	M/52	Ex-smoker	Adenocarcinoma	1	3	PR	22.3	22.3
7	19	delE746.A750	M/80	Nonsmoker	Adenocarcinoma	2	3	NPD	1.9	3.0
8	19	delE746.A750	F/57	Nonsmoker	Adenocarcinoma	1	1	PR	2.6	12.0
9	19	delE746.A750	F/49	Nonsmoker	Adenocarcinoma	1	0	PR	7.8	9.1
10	19	delE746.A750	F/55	Nonsmoker	Adenocarcinoma	2	0	NPD	3.4	6.0
11	19	delE746.A750	F/40	Nonsmoker	Adenocarcinoma	4	3	PR	5.0	9.9
12	19	delE746.T751insA	F/69	Nonsmoker	Adenocarcinoma	1	0	NPD	13.5	18.6
13	19	delL747.T751	F/73	Nonsmoker	Adenocarcinoma	4	1	NPD	22.4	22.4
14	19	delL747.P753insS	M/71	Ex-smoker	Adenocarcinoma	1	1	PR	22.3	22.3
15	19	delL747.P753insS	M/56	Nonsmoker	Squamous cell carcinoma	1	2	SD	2.4	2.5
16	20	A763V	F/80	Nonsmoker	Squamous cell carcinoma	3	0	PD	1.9	1.9
17	20	V765A	F/52	Nonsmoker	Adenocarcinoma	1	1	PR	3.9	14.7
18	20	T783A	F/42	Nonsmoker	Adenocarcinoma	1	1	PR	6.0	20.0
19	20	delA767.V769	M/54	Nonsmoker	Adenocarcinoma	1	2	PD	0.3	0.9
20	21	N826S	M/57	Ex-smoker	Squamous cell carcinoma	1	1	SD	6.7	9.0
21	21	L858R	M/29	Current-smoker	Squamous cell carcinoma	3	2	PD	2.0	2.5
22	21	L858R	M/72	Ex-smoker	Adenocarcinoma	1	6	NPD	6.6	6.6
23	21	L858R	M/72	Nonsmoker	Adenocarcinoma	2	3	PR	4.8	8.5
24	21	L858R	M/57	Nonsmoker	Adenocarcinoma	2	1	PR	2.9	4.3
25	21	L858R	F/62	Nonsmoker	Adenocarcinoma	1	3	PR	20.4	20.4
26	21	L858R	F/77	Nonsmoker	Adenocarcinoma	1	2	NPD	23.7	23.7
27	21	L858R	F/74	Nonsmoker	Adenocarcinoma	2	2	NPD	22.0	22.0
28	21	L858R	F/54	Nonsmoker	Adenocarcinoma	3	2	NPD	7.6	10.9
29	21	L858R	F/68	Nonsmoker	Adenocarcinoma	4	2	SD	2.7	3.7
30	21	L861Q	M/68	Nonsmoker	Adenocarcinoma	2	4	PR	18.3	21.9
31	21	G863D	M/73	Nonsmoker	Adenocarcinoma	1	0	PR	6.5	9.1
32	18+21	G719S + L861Q	F/65	Nonsmoker	Adenocarcinoma	2	1	PD	0.5	2.0
33	19+20	delE746.T751insVA + R803W	F/79	Nonsmoker	Adenocarcinoma	1	1	NPD	7.7	7.7

Abbreviations: PR, partial response; SD, stable disease; NPD, nonprogressive disease; PD, progressive disease.

exon 20 was relatively worse and very similar to that of patients without EGFR mutation (Fig. 1D). No other variables had a correlation with response duration.

Discussion

In this study, we showed a high EGFR mutation rate in our NSCLC patient population, with mutation patterns very similar to those reported previously (13, 14). We documented that EGFR mutations played a crucial role in determining gefitinib response in NSCLC patients, confirming findings from two previous landmark studies of <20 gefitinib-treated patients (13, 14). For the first time, we have also shown that mutations in EGFR could confer longer survival in gefitinib-treated NSCLC patients. Furthermore, of those patients achieving partial response, patients with mutations in EGFR had a trend of longer response durations in comparison with patients lacking EGFR mutations.

Gefitinib has shown antitumor activity in pretreated NSCLC patients with response rates of 10% to 20% (10, 11). From these two phase II clinical studies (10, 11), gefitinib seemed to be more effective in females and patients with adenocarcinoma and a good performance status. In a subsequent Expanded Access Program study, nonsmokers

Table 3. Multivariate regression model in tumor response and patient survival

Response	Odds ratio	95% Confidence interval	*P*
Objective tumor response			
EGFR mutation	8.5, in favor of mutant EGFR	1.5-50.0	0.017
Disease control			
EGFR mutation	10.8, in favor of mutant EGFR	2.3-51.3	0.003
Survival	**Hazard ratio**	**95% Confidence interval**	***P***
Progression-free survival			
Histologic subtype	4.7, in favor of adenocarcinoma	2.1-10.6	<0.001
EGFR mutation	2.3, in favor of mutant EGFR	1.2-4.3	0.013
Age	2.5, in favor of age >65 y	1.3-5.0	0.007
Performance status (Eastern Cooperative Oncology Group)	2.5, in favor of performance status 0/1	1.3-4.9	0.008
Overall survival			
Performance status (Eastern Cooperative Oncology Group)	3.9, in favor of performance status 0/1	1.8-8.6	0.001
Histologic subtype	3.9, in favor of adenocarcinoma	1.6-9.3	0.002

and BAC subtype were also considered as promising predictors of gefitinib response (19). The issue, such as Japanese or non-Japanese patients, was initially considered to be irrelevant by the multivariate regression model (10). However, accumulated clinical evidence strongly suggests that ethnic/geographic factors play an important role in the response of patients to gefitinib treatment. This is based on the high response rates reported exclusively from countries in the Asia Pacific region, including Taiwan (12, 20, 21). Although the similarity of high response rates (~30%) among each independent group in this region was quite convincing, most of these data came from observational studies with no supporting molecular analyses. However, two studies recently showed a strong association between EGFR mutations and gefitinib response (13, 14). Additionally, the prevalence of EGFR mutations in NSCLC was strikingly higher in Japanese patients than in Caucasian patients, and the figures were close to the previously reported gefitinib response rates in these two patient populations (10). This was the first genetic evidence supporting the theory of ethnic/geographic differences in the response of NSCLC patients to treatment with gefitinib.

All patients in our study were Taiwanese and the majority (83%) had been heavily pretreated with chemotherapy. The mutation rate (61.1%) in our study was not only higher than that observed in Caucasian NSCLC patients (~2%) but also higher than that usually noted in Japanese and Taiwanese (~40%) treatment-naive NSCLC patients (13, 14, 22, 23) and was most likely due to the skewed population. In comparison to the lung cancer patient population demographics of Taiwan, there was a high proportion of female (46% versus 30%) patients and patients with no previous history of smoking (67% versus 40%) in this study—both of these factors could be associated with a higher EGFR mutation rate (14, 19, 24). The most likely reason for the skewed demographics was the

Table 4. Objective tumor response in patients with EGFR mutations in different exons

	No. patients			
	Complete response*	**Partial response***	**Stable disease*/ Nonprogressive disease†**	**Progressive disease*/ Progressive disease†**
EGFR mutation (*n* = 33)	0	17	3/9	4/0
Exon 18 (*n* = 4)	0	4	0/0	0/0
Exon 19 (*n* = 11)	0	6	1/4	0/0
Exon 20 (*n* = 4)	0	2	0/0	2/0
Exon 21 (*n* = 12)	0	5	2/4	1/0
Exon 18 + 21 (*n* = 1)	0	0	0/0	1/0
Exon 19 + 20 (*n* = 1)	0	0	0/1	0/0
Non-EGFR mutation (*n* = 21)	0	4	4/0	5/8

*Patients with measurable lesions.
†Patients with nonmeasurable lesions.

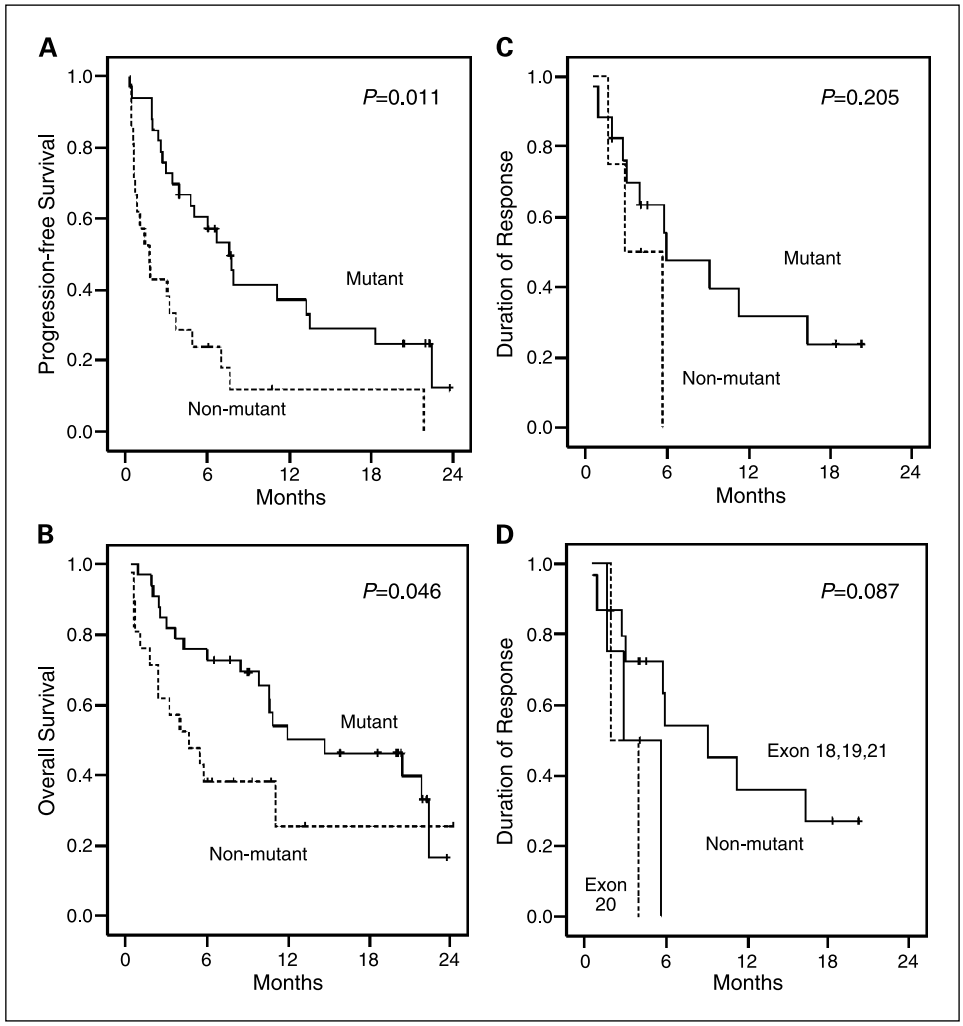

Fig. 1. Kaplan-Meier plots of progression-free survival (*A*), overall survival (*B*), and duration of response (*C*) in patients with mutant versus nonmutant EGFR. Kaplan-Meier plots of duration of response in patients with EGFR mutation in exons 18, 19, and 21 versus exon 20 versus nonmutant (*D*).

"selection effect" of prior chemotherapy. It was well documented that females and nonsmokers have better prognosis translating to improved survival when treated by systemic chemotherapy (25, 26). This would thus result in a tendency to include more females and nonsmokers in studies evaluating the second-line and post-second-line treatment.

A retrospective study has shown better response rates in patients with BAC (19) and some groups have implied that BAC might have a different biology (27). In this study, none of the patients fulfilled the criteria of BAC by the 1999 WHO Histological Typing. Nevertheless, we calculated the BAC proportion in each adenocarcinoma specimen and did a subgroup analysis in these patients. We did not find any statistical difference of BAC proportion in the frequency of EGFR mutations, objective tumor response rate, or disease control rate. Accordingly, at least in our Taiwanese patient population, we did not observe evidence supporting a difference in gefitinib response among tumors with varied BAC components (data not shown).

In our 33 patients with EGFR mutations, 11 had deletions in exon 19, 9 had a substitution mutation at L858R in exon 21, 10 had variable types of single-nucleotide substitution mutations in exon 18, 20, or 21, 1 had deletion in exon 20, 1 had double substitution mutations in exons 18 and 21, and 1 had combined deletion in exon 19 and substitution mutation in exon 20. The patterns of EGFR mutations were quite complex and this finding was similar to a previous report from this area (23).

To the best of our knowledge, in a total of 212 published cases with EGFR mutation (13, 14, 22–24), only 3 cases were nonadenocarcinoma: 2 were adenosquamous cell carcinomas and 1 was large cell carcinoma. None of these three cases had received gefitinib treatment; therefore, the clinical significance was unknown. In this study, we found four nonadenocarcinomas with EGFR mutations and all were squamous cell carcinomas. The mutation patterns were A763V, L858R, N826S, and delL747_P753insS in one each. Patients with the former two mutations had progressive disease after gefitinib treatment; the latter two had stable disease with a relatively shorter progression-free survival (6.7 and 2.4 months, respectively). In 29 patients with adenocarcinoma bearing EGFR mutation, 17 had partial response, 9 had nonprogressive disease, 1 had stable disease, and only 2 had progressive disease. In contrast, none of our seven patients with nonadenocarcinoma had gefitinib response, irrespective of their tumor EGFR mutation status. This finding of the lack of predictive value of EGFR mutation in lung squamous cell carcinoma needs to be confirmed.

Among 212 published cases with EGFR mutation, 33 patients who had EGFR mutations in exons 18, 19, and 21 had been treated with gefitinib or erlotinib and only 1 case showed no tumor response. The mutation in that case resulted in a stop codon and therefore truncated EGFR protein (23), thus explaining the failure of gefitinib treatment. In our study, 4 of 33 patients with mutated EGFR had progressive disease after gefitinib treatment. The patterns of EGFR mutation included delA767_V769, double substitution mutation (G719S and L861Q), A763V, and L858R in one each. Both delA767_V769 and A763V were in exon 20 and these two patterns are unreported previously. Substitution mutations at L858R and L861Q have both been associated with gefitinib response in previous reports (13, 23). Substitution mutation of G719S has been reported but not in gefitinib-treated patient (14, 22). Although Lynch et al. (13) had reported one gefitinib responder with G719C mutation, the association between G719S and gefitinib response is unknown. Further study is required to clarify the association between the sites and patterns of mutation and the treatment response, especially in exon 20.

In the present study, we used formalin-fixed, paraffin-embedded tumor tissue for mutational analysis of *EGFR* gene. The fragmentation of DNA may lower the yield of the PCR reactions but does not compromise the accuracy of sequencing. In a recent study from Taiwan (23), the authors analyzed 16 paraffin-embedded tumor tissues and 50% of the specimens had EGFR mutations. They also did mutation analyses on freshly frozen specimens of 69 adenocarcinoma cases and found a similar mutation rate (55.1%). We consider DNA extracted from formalin-fixed, paraffin-embedded tumor tissues to be as adequate and suitable for mutational analysis as that extracted from freshly frozen tissues.

In this study, there were two findings that were somewhat contradictory to previous reports. First, a high mutation rate (40%) was found in squamous cell carcinomas. Although the histologic typing of these tumors had been blindly reviewed and confirmed by two other pathologists, this result should be carefully interpreted due to the limited number of cases. Second, there was no difference in EGFR mutation rates between patients who were current smokers or ex-smokers and those who had no previous history of smoking. However, this finding concurred with a recent report of EGFR mutational analysis in 101 unselected, non-gefitinib-treated NSCLC in Taiwan (23). Ongoing investigations will determine if factors other than a history of smoking, for example, exposure to viruses or fumes from cooking oils (28, 29), play a role in EGFR mutations and are involved in the pathogenesis of lung adenocarcinomas.

Gene amplification plays an important role in the pathogenesis in several types of cancer and frequently precedes and may favor the occurrence of mutations (30). However, the association between *EGFR* gene amplification and gefitinib response is unknown. *EGFR* gene amplification was observed in 34% of glioblastoma; however, no objective tumor response was noted in a phase II trial of gefitinib (31). Similarly, increased *EGFR* gene copy number was evident in 60% of NSCLC, but the response rate to gefitinib was only 10% to 20% in the corresponding group of patients (32). *EGFR* gene amplification may create a predisposition to EGFR mutations but, as a single factor, it seems to be unrelated to the gefitinib tumor response.

Although the frequency of EGFR mutations in our selected group of patients was relatively higher at 61.1%, we have confirmed that EGFR mutations correlated with a better response to gefitinib treatment as illustrated by response rate (56.8%) and patient survival.

Cell type, mutation status of EGFR, and performance status but not the number of prior chemotherapy regimen was associated with gefitinib response of NCSLC patients (12). When compared with currently available front-line chemotherapy, gefitinib showed much higher treatment efficacy in adenocarcinomas with EGFR mutations (85%) and comparable efficacy in adenocarcinomas with no EGFR mutations ($\sim$40%). EGFR mutations had no predictive value in patients with lung squamous cell carcinoma.

Based on the above findings, we suggest that sequencing of EGFR tyrosine kinase domain in lung adenocarcinoma patients, at least those of East Asian origin, would be of clinical significance and permit the customization of treatment using EGFR tyrosine kinase inhibitors as front-line treatment in neoadjuvant, adjuvant, or metastatic settings.

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