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Intron 1 Polymorphism, Mutation and the Protein Expression of Epidermal Growth Factor Receptor in Relation to the Gefitinib Sensitivity of Korean Lung Cancer Patients

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Background : Epidermal growth factor receptor (EGFR) intron 1 polymorphism in non-small cell lung cancer (NSCLC) has been found to have therapeutic implications for the patients treated with EGFR tyrosine kinase inhibitors. However, its clinical significance as related to gefitinib responsiveness is still controversial. We examined CA repeat polymorphism in intron 1 of the EGFR gene and its relation with the EGFR gene mutation in NSCLC patients who were treated with gefitinib. **Methods :** Sixty seven patients who were treated with gefitinib were analyzed for intron 1 polymorphism in the *EGFR* gene, the *EGFR* mutations and the EGFR protein expression. Two hundred twenty seven samples of NSCLC were analyzed for *EGFR* mutations. **Results :** CA repeat was low in 27 patients (40.3%) and high in 40 (59.7%) patients. The response rate for gefitinib therapy was higher in the patient population with a low number of CA repeats in the EGFR gene (p=0.047) and in the patients with the mutated type of *EGFR* (p=0.048), though these two factors were not related. Thirty four patients (15.0%) harbored *EGFR* mutations. **Conclusions :** This study suggests that the intron 1 CA repeat polymorphism of the EGFR gene may serve as a predictor of the clinical outcome of NSCLC patients treated with gefitinib, and this without regard for *EGFR* mutation. Our data further supports the importance of *EGFR* mutations with regard to a distinct clinical profile and the prognostic implications for NSCLC patients.

Key Words : Receptor, Epidermal growth factor; Mutation; Gefitinib; Polymorphism, Genetic; Lung; Cancer

Lung cancer is the leading cause of cancer-related deaths worldwide and it is one of the most common tumors in humans. Despite the recent advances in multimodality and molecular-targeted therapies for lung cancers, the prognosis still remains poor. Especially, the discovery that mutation of the tyrosine kinase domain of the epidermal growth factor receptor (EGFR) gene occurs in a subset of lung cancers and this can predict the sensitivity to tyrosine kinase inhibitors (TKIs) has generated enormous interest and intense research in many laboratories worldwide.[1,2] All these findings have demonstrated that small molecule EGFR-TKIs, like erlotinib and gefitinib, have favorable efficacy for treating non-small cell lung cancer (NSCLC). Gefitinib (Iressa®) is a targeted agent that inhibits the tyrosine kinase activity of EGFR by competitively blocking the adenosine triphosphate binding site[3] and it has produced response rates of 10-20% for NSCLC patients who have failed on standard therapy.[4] The patients who were women, never smoked, of Asian descent and presented with an adenocarcinoma histology showed an increased response to gefitinib.[4-6] Several retrospective studies have revealed that tumors with *EGFR* mutations are associated with a higher objective response to gefitinib and a prolonged time to progression (TTP) than are those tumors with the wild-type *EGFR*.[7] However, there are some exceptions to the correlation between the mutation status and a response to gefitinib. The variable response rate to TKIs in patients with or without *EGFR* mutation suggests that *EGFR* mutation is not the only predictor for a response to treatment with EGFR-TKIs.[8] Therefore, other factors may play roles in the clinical response to gefitinib. It has recently been shown that genetic polymorphisms can influence the treatment response to medications.[9] Although adenocarcinomas of the lung are associated with EGFR gene mutations as well as sensitivity to EGFR-TKIs, it remains unclear whether or not polymorphisms of *EGFR* affect these associations. CA repeat polymorphism in intron 1 of the EGFR gene is associated with transcription of the EGFR expression. The short alleles are found to be correlated with an increased EGFR protein expression in breast can-

cer[10,11] and to have an increased response to erlotinib in patients with head and neck cancer[12]. However, there is limited information on the role of the CA repeat polymorphism in intron 1 of the EGFR gene as a predictor of the outcome of gefitinib treatment in Korean patients with NSCLC. In this study, our aim was to perform *EGFR* polymorphism analysis in patients who were treated with gefitinib and to correlate the tesults with the treatment outcomes and to analyze the association with *EGFR* mutation and the EGFR protein expression. In addition, we also examined the prevalence of *EGFR* mutation in the NSCLC of a Korean population.

MATERIALS AND METHODS

Patients and the tissue preparation

A total of 227 consecutive samples of NSCLC that included squamous cell carcinomas (n=116) and adenocarcinomas (n=111), were collected from Yeungnam University Medical Center between January 2006 and August 2007. All the specimens were from Korean patients and they were obtained by bronchoscopic or needle biopsy. The clinical information including the patients' age and gender, the tumor histology and the smoking history, were available for all the patients. Among these patients, *EGFR* intron1 polymorphism and the *EGFR* mutation and protein expression were analyzed in 67 patients who had received gefitinib and who had follow-up data. The response to gefitinib treatment was assessed according to the Response Evaluation Criteria Solid Tumors guideline.[13] Treatment efficacy was evaluated by the computed tomography images and this was classified into 4 groups: a complete response (CR), partial response (PR), stable disease (SD), and progressive disease (PD). The cases having a CR, PR or, SD were considered as the gefitinib-sensitive group and the cases with PD were considered as the treatment-failure group. The TTP was calculated from the start date of gefitinib therapy to the date of disease progression or the date of last contact. The tumor specimens were fixed in formalin and then embedded in paraffin wax. Six 6 μm-thick serial sections that contained malignant cells were used for DNA extraction.

Analysis of the CA repeats in intron 1 of the EGFR gene

Sixty-seven patients had CA repeat polymorphism in their tumor samples. The CA-repeat-containing region of intron 1 was amplified by PCR. The sequences of the primers were 5′-CCA ACC AAA ATA TTA AAC CTG TCT T-3′ (forward) and 5′-CCT GAA CCA GGG AGA GCA AT-3′ (reverse). Instrumentation and reagents from Genetic Analyzer were used for the analysis of the repeat allele lengths and the relative ratios. All the samples were prepared for fragment separation on a ABI Prism 3,100 Genetic Analyzer (Applied Biosystems, Foster, CA, USA) with using 0.7 μL of the amplified samples combined with 0.3 μL of the GeneScan 500 Size Standard and 9 μL of HiDi Formamide. The fragment length was determined using the GeneScan Analysis Software (Applied Biosystems). The data was displayed as the plot of the electrophoresis with the base-pair size displayed on the x-axis against the peak signal intensity (peak height), in relative fluorescent units (rfu), on the y-axis. The DNA from the A431 cell line, which has the 16/16 repeat, was used as a control. The patients were classified as having either low CA repeat (any allele $\leq$16) or high CA repeat (16>).

EGFR protein immunohistochemistry

The expression of EGFR protein on the tumor tissues of 67 patients was detected by using the EnVision system kit (DAKO, Carpinteria, CA, USA) and mouse anti-human EGFR monoclonal antibody (1:100, Zymed Laboratories, San Francisco, CA, USA), according to the manufacturer's instructions. Briefly, after the standard de-paraffinization, hydration and blocking of the endogenous peroxidase, 3 μm-sectioned tissue microarray array (TMA) slides were subjected to microwave retrieval, and this was followed by incubation at 121℃ for 10 min. After rinsing the TMA sections with normal goat serum for 6 min, all the prediluted (1:100 dilution) primary antibodies were applied for 60 min at room temperature. The sections were then allowed to react with peroxidase conjugated streptoavidin for 45 min; this was followed by color development using diaminobenzidine, and counterstaining was done with hematoxylin. The immunoreactivity was considered positive if more than 10% of the tumor cells showed complete membranous staining.

EGFR gene mutation

Genomic DNA was extracted from 227 lung tumor samples, including those from the 67 patients who were treated with gefitinib. *EGFR* mutations were detected by PCR based direct sequencing of exon 18-21 of the TK domain of the EGFR gene in the tumors. Details about the *EGFR* mutation types and the methodologies for mutation detection have been published elsewhere.[14] Briefly, DNA extraction from the Formalin-Fixed

Paraffin-Embedded (FFPE) tissue was based on the QIAamp DNA FFPE Tissue kit (Qiagen, Hilden, Germany) as per the manufacturer's protocol. Fifty nanograms of DNA were amplified in a 20 μL reaction solution containing 10 μL of 2 X concentrated HotStarTaq Master Mix (Qiagen), including PCR Buffer with 3 mM $MgCl_2$, 400 μM of each dNTP, and 0.3 μM of each primer pairs (exon 18F: 5′-cca tgt ctg gca ctg ctt t-3′, 18R: 5′-cag ctt gca agg act ctg g-3′, exon 19F: 5′-tgt ggc acc atc tca caa ttg-3′, 19R: 5′-gga ccc cca cac agc aa-3′, exon 20F: 5′-ggt cca tgt gcc cct cct-3′, 20R: 5′-tgg ctc ctt atc tcc cct cc-3′, exon 21F: 5′-cca tga tga tct gtc cct ca-3′, 21R: 5′- aat gct ggc tga cct aaa gc-3′). Amplifications of EGFR (exon 18-21) were performed using a 15-min initial denaturation step at 95 ℃ and this was followed by 35 cycles of 30 s at 94℃, 30 s at 59℃, and 30 s at 72℃, and a 10-min final extension step at 72 ℃. The PCR products were then 2% gel-purified with a Hi-Yield™Gel/PCR DNA extraction Kit (RBC). The DNA templates were processed for the DNA sequencing reaction using the ABI-PRISM BigDye Terminator version 3.1 (Applied Biosystems) with both the forward and reverse sequence-specific primers. The sequence data was generated with the ABI PRISM 3100 DNA Analyzer. The sequences were analyzed by Sequencing Analysis 5.1.1. software to compare the variations.

Statistical analysis

The relationship between the various EGFR abnormalities and gefitinib sensitivity was performed using Pearson's chi-square tests or Fisher's exact tests where appropriate. Comparison between the different groups was done using log-rank tests. The TTPs were calculated using the Kaplan-Meier method. Multivariate analysis was carried out using the stepwise Cox regression model to evaluate the effects of the CA repeat polymorphism in intron 1 of the EGFR gene, mutation of the EGFR gene and the EGFR protein expression on the objective response to gefitinib. A p value less than 0.05 was regarded as statistically significant. All the analyses were performed using SPSS for Windows, version 12.0 (SSPS Inc, Chicago, IL, USA).

Table 1. Clinical features of patients with NSCLC

Characteristics	No (%) of patients (n=227)	*EGFR* mutation (n=34)	p
Age (median years)	67	65	
Sex			
Male	162 (71%)	13	0.005
Female	65 (29%)	21	
Smoking status			
Nonsmoker	60 (26%)	23	0.038
Smoker	167 (74%)	11	
Stage			
I-II	49 (22%)	9	0.109
III-IV	178 (78%)	25	
Histology			
Squamous cell carcinoma	116 (51%)	6	0.000
Adenocarcinoma	111 (49%)	28	

NSCLC, non-small cell lung cancer; EGFR, epidermal growth factor receptor.

RESULTS

The relationship between the clinical characteristics of the patients and the EGFR mutations

The clinical characteristics of the patients are described in Table 1. Thirty four patients (15.0%) harbored *EGFR* mutations which included 28 cases (25.2%) of adenocarcinomas and 6 cases (5.2%) of squamous cell carcinomas (p<0.001). Deletions in exon 19 (19 patients, 55.9%) were most frequent, although the *EGFR* mutations were known to be distributed throughout the kinase domain. There were 17 cases (60.7%) of exon 19 deletion and 10 cases (35.7%) of exon 21 (L858R; 9, H835L; 1) point mutations in adenocarcinoma. There were 2 cases (33.3%) of deletions in exon 19 and 4 cases (66.7%) of exon 21 point mutations (L858R; 3, V786M; 1) in squamous cell carcinoma (p=0.363) (Table 2). *EGFR* mutations were more frequently observed in 21 out of the 65 (32.3%) female patients than in 13 out of the 162 (8.0%) male patients (p=0.005). Twenty-three patients (67.6%) had never smoked while 11 patients (32.4%) were smokers (p=0.038). These results showed that *EGFR* mutation was significantly associated with adenocarcinoma, the female gender and non-smokers, but it was not associated with the patients' age

Table 2. Frequency of *EGFR* mutation types according to the histologic subtype

EGFR mutation	Squamous cell carcinoma (n=116)	Adenocarcinoma (n=111)	p
Negative	110 (94.8%)	83 (74.8%)	0.000
Positive	6 (5.2%)	28 (25.2%)	
Exon 18	0	1 (3.6%)	0.363
Exon 19	2 (33.3%)	17 (60.7%)	
Exon 20	0	0	
Exon 21	4 (66.7%)	10 (35.7%)	

EGFR, epidermal growth factor receptor.

or clinical stage.

Comparison of *EGFR* mutation and protein overexpression

Among the selected 67 patients (41 cases of adenocarcinoma and 26 cases of squamous cell carcinoma) treated with gefitinib, EGFR overexpression was detected in 46 (68.7%) patients; EGFR overexpression was detected in 26 cases (61.0%) of adenocarcinoma and in 21 cases (80.8%) of squamous cell carcinoma according to immunohistochemical analysis. EGFR mutations were detected in 17 patients (25.4%), of which 16 patients had adenocarcinoma (94.1%) and one patient had squamous cell carcinoma (5.9%). *EGFR* mutations were detected in 26.1% (12/46 patients) of the patients with EGFR overexpression and in 23.8% (5/21 patients) of the patients with no EGFR expression. Therefore, EGFR overexpression was not correlated with the *EFGR* mutation status (p=0.923) (Table 3) (Fig. 1).

Table 3. Relationship between EGFR immunohistochemistry and EGFR gene mutation in patients received gefitinib treatment

IHC	Patients (n)	NSCLC (n=67)		SCC (n=26)		ADC (n=41)	
		M (+)	M (−)	M (+)	M (−)	M (+)	M (−)
Positive	46 (68.7%)	12	34	0	21	11	14
Negative	21 (31.3%)	5	16	1	4	5	11
Total	67	17	50	1	25	16	25
p-value		0.923		0.345		0.332	

EGFR, epidermal growth factor receptor; M, mutation; NSCLC, non-small cell lung cancer; IHC, immunohistochemistry, SCC; squamous cell carcinoma, ADC; adenocarcinoma.

The relationship between EGFR abnormalities and gefitinib efficacy

The response rate to gefitinib in the patients with EGFR protein overexpression was 71.7%, and this was slightly lower than that in the wild-type EFGR group (76.2%). There was no dif-

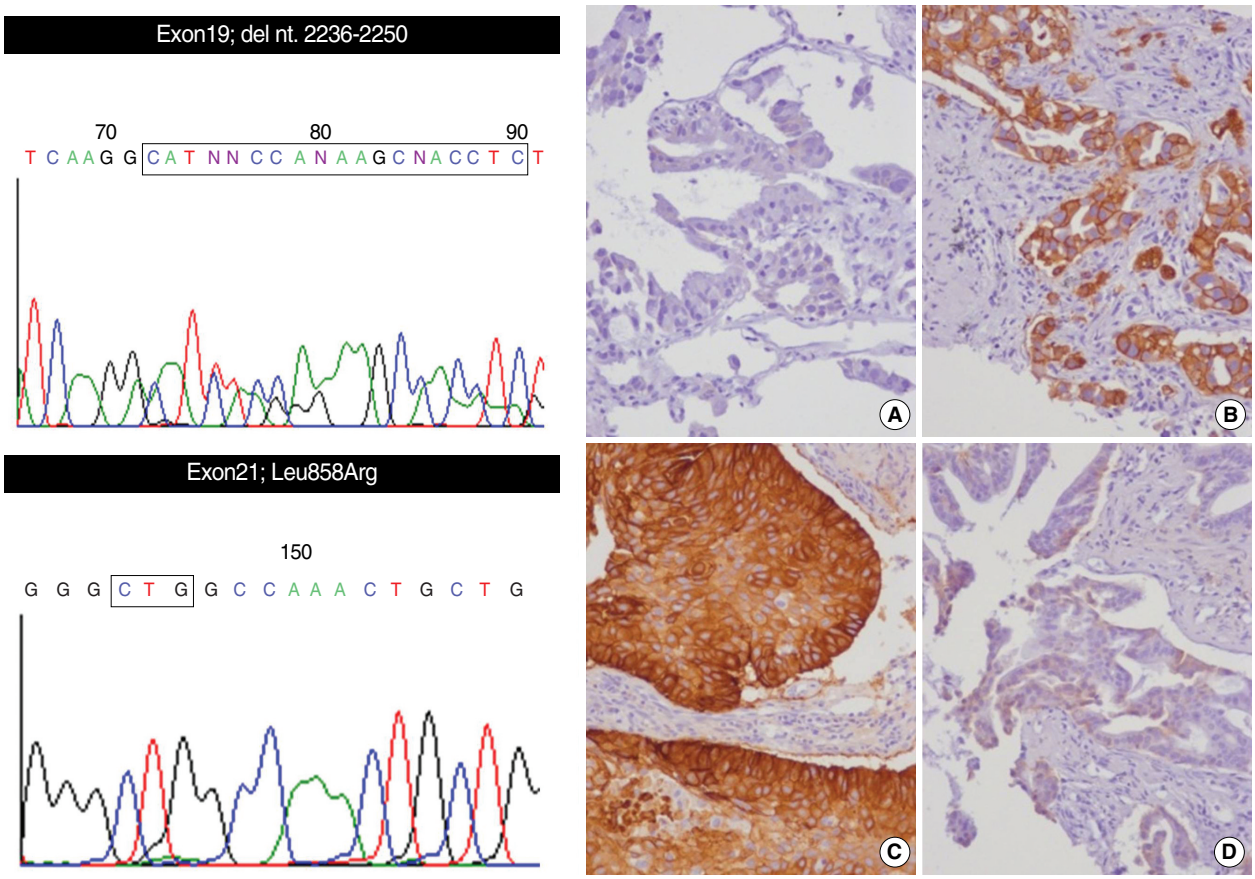

Fig. 1. Deletion in *EGFR* exon 19 is mainly found in adenocarcinoma patients, of whom negative (A) or positive (B) staining on EGFR immunohistochemistry is observed. p. L858R mutation in EGFR exon 21 is frequently detected in squamous cell carcinomas with intense membranous positive expression (C), while, some of adenocarcinoma have negative (1+) staining (D).

ferences in the response rate (p=0.703) and TTP (p=0.220) between the patients with EGFR over expression and the patients with no expression to a low expression. Fifteen patients with *EGFR* mutations achieved objective responses (8 CR and 7 SD), resulting in an overall response rate of 88.2% (95% confidence interval) (p=0.048). Compared to patients with no EGFR TK domain mutations, those with deletions at exon 19 (11/17 cases) were associated poor responsiveness (3 PRs, 6 SDs and 2 PDs) to gefitinib treatment. On the other hand, *EGFR* mutations at exon 21 with the L858R substitution (6/17 cases) had a better responsiveness (5 PRs and 1 SD), but in neither case was the difference statistically significant. The median TTP was 5.1 months (95% confidence interval) for the patients with *EGFR* mutations (Table 4).

The relationship between the repeat length of *EGFR* intron 1 and the clinical responsiveness to gefitinib

We were able to obtain the complete clinical treatment data on 67 patients with NSCLC and who received treatment with gefitinib. The median follow up was 10.5 months and the median TTP was 5.1 months. The median duration of gefitinib treatment was 3.5 months. Of these patients, the most frequent CA repeat genotype was 20/20 repeats (25 patients, 37.3%) followed by 16/20 (16 patients, 23.9%), and 19/20 (8 patients, 11.9%) (Fig. 2). CA repeat was low in 27/67 patients (40.3%) and high in 40/67 (59.7%) patients. Low repeat polymorphism was encountered in 18 adenocarcinoma patients (66.7%) and 9 squamous cell carcinoma patients (33.3%) whereas 22 squamous cell carcinoma patients (55.0%) and 18 adenocarcinoma patients (45.0%) had high repeat polymorphism. Statistical analysis showed no difference of *EGFR* intron 1 polymorphism and the clinical characteristics, including age, gender, the smoking status and histological differentiation. *EGFR* intron 1 polymorphism had no relationship with the *EGFR* mutation status (25.9% (7/27) in the low vs. 25.0% (10/40) in the high (p=0.850). The low repeat patients showed better clinical outcomes than that of the high repeat group (p=0.047) and a trend of a longer TTP rather than high CA repeat, but this was not statistically significant (p=0.413) (Table 4). There was no significant difference of the repeat length of the *EGFR* intron 1 with respect to the EGFR protein expression level (p=0.350) (Table 5) (Fig. 3).

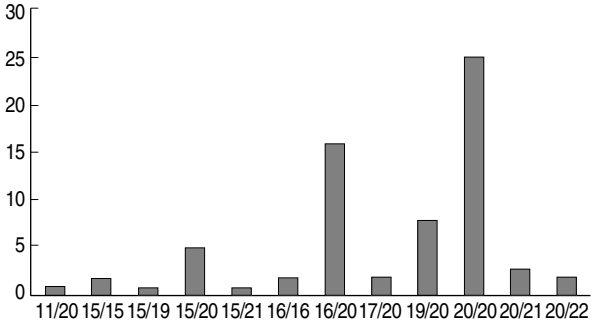

Fig. 2. Distribution of *EGFR* intron 1 (CA) genotypes in cohort.

DISCUSSION

The recent findings that mutations in the EGFR gene in lung cancers can predict, if somewhat imprecisely, the response to EGFR TKIs has caused others to suggest that other factors may play a role in predicting the response to therapy.[8] In this study, we examined the ability of the polymorphism in the EGFR gene

Table 5. Association between *EGFR* mutation, IHC, and polymorphism of *EGFR* intron 1

Polymorphisms	No.	EGFR expression		p	*EGFR* mutation		p
		Negative	Positive		Negative	Positive	
Intron 1	67	21	46	0.350	50	17	0.850
Low CA repeat	27	10	16		20	7	
High CA repeat	40	11	29		30	10	

EGFR, epidermal growth factor receptor; IHC, immunohistochemistry.

Table 4. Relation of EGFR overexpression, mutation and polymorphism with gefitinib responsiveness

EGFR abnormalities		No.	Responsiveness (%CI)	p	Median TTP (months)	p
EGFR protein expression	Positive	46	33 (71.7%)	0.703	4.5	0.220
	Negative	21	16 (76.2%)		3.3	
EGFR gene mutation status	Positive	17	15 (88.2%)	0.048	5.1	0.173
	Negative	50	31 (62.0%)		3.8	
Polymorphism of *EGFR* intron 1	Any allele ≤16CA	27	22 (81.5%)	0.047	4.4	0.413
	Any allele >16CA	40	26 (65.0%)		3.7	

EGFR, epidermal growth factor receptor; TTP, time to progression.

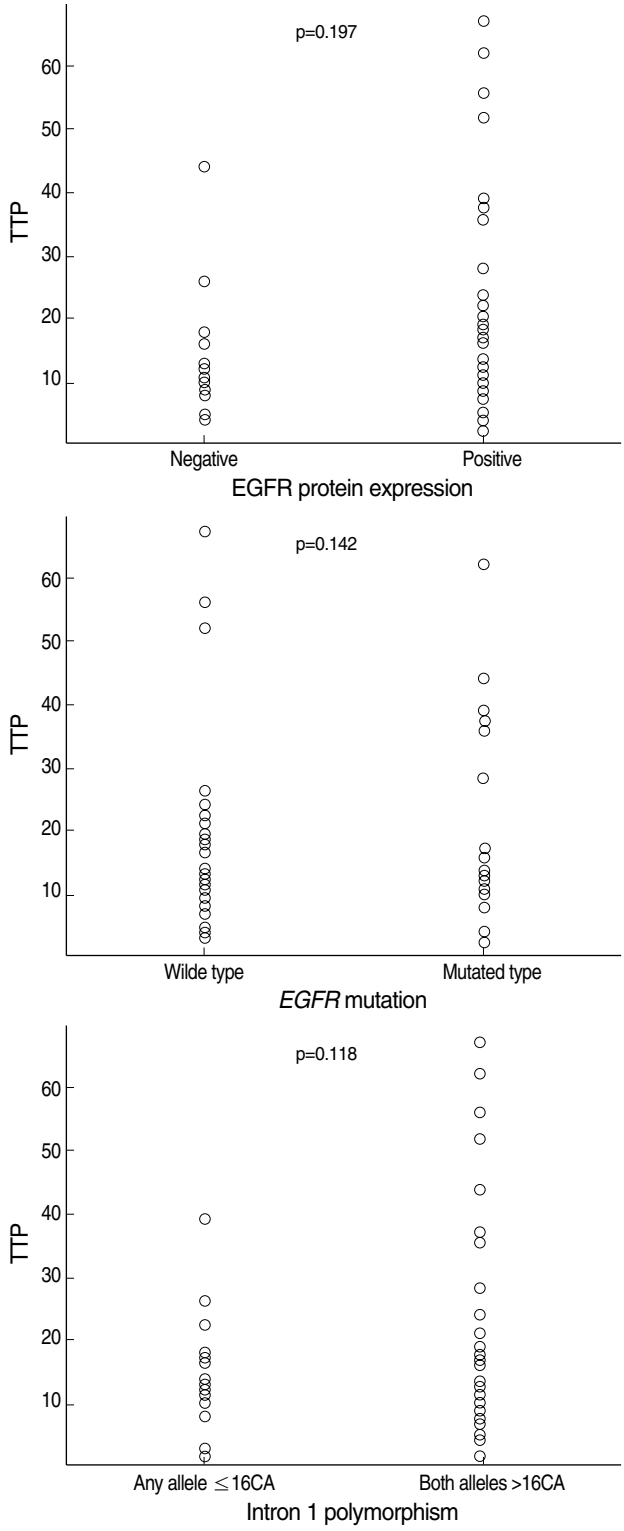

Fig. 3. Distribution of TTP according to EGFR protein expression, *EGFR* mutation, and intron 1 polymorphism of EGFR genes.

to predict the sensitivity to gefitinib and its relationship to EGFR gene mutation and the EGFR protein expression in NSCLC patients. Alleles 20 (37.3%) and 16 (23.9%) were the two most common allele genotypes in our study. This result is in agreement with that of other previous studies[15-17] for Asian populations, including Koreans. Our results showed a lower frequency in the 20 repeats and a slightly higher frequency in the 16 repeats, as ccompared to other studies in which the frequency was 45% to 63% in allele 20 and 16% to 21% in allele 16. Liu *et al.*[17] revealed a reversed distribution in the Caucasian population (43% for allele 16 and 21% for allele 20), as compared to the Asian population. Therefore, our study demonstrates that *EGFR* CA repeat polymorphism also causes variations in the responsiveness to TKIs treatment according to the ethnic group. We found that polymorphism of the EGFR gene was significantly associated with gefitinib responsiveness. In addition, the low number of CA repeats in intron 1 of *EGFR* was related to a better clinical response to gefitinib and a tendency toward a longer TTP. These results are consistent with those of previous studies[16,18] indicating that polymorphism of the EGFR gene is associated with the prognosis of NSCLC patients after gefitinib treatment. Polymorphism of the EGFR gene has been identified in colorectal cancer, breast cancer and oral cancer, as well as lung cancer, and this has been shown to be associated with sensitivity to erlotinib. In our study, the polymorphism status did not correlate with the *EGFR* mutations. Although there was no statistical significance, it has been reported that *EGFR* mutations at exon 19 were closely associated with a shorter CA-repeat length.[19] Recent studies have showed that *EGFR* polymorphism of lung cancers seemed to be less frequent in Asians but it had higher repeats as compared to that of Caucasians.[17,20] Our results are consistent with those of a study by Han *et al.*[16] about Korean people and those of a study by Nie *et al.*[18] about the Chinese, but they are not same as those results reported by Nomura *et al.*[21] for the Japanese. Furthermore, the CA repeat status had no relationship with the EGFR protein expression, and this stands in contrast to previous reports showing an increased EGFR expression in low CA repeats.[21,22] Therefore, the CA repeat length may independently serve as a predictive marker for EGFR TKIs responsiveness.

In addition to CA-repeat polymorphism, we also examined the association between *EGFR* mutations and gefitinib sensitivity. There have been many studies concerned with EGFR abnormalities, such as the *EGFR* mutations, increases in the gene copy number and an altered protein expression via immunohistochemical analysis, and their relationship with The gefitinib responsiveness and all these factors have been extensively studied.[1,2,6,8,16,23] In the present study, *EGFR* mutations were also significantly

related to gefitinib responsiveness while the EGFR protein expression revealed no correlation with the clinical outcome after gefitinib therapy. As with the previous studies, we found there is a distinct clinical profile for NSCLC patients with *EGFR* mutations. These mutations were more frequently detected in females, patients with adenocarcinoma and non-smokers. Furthermore, the present study demonstrated that one fourth of the Korean patients with NSCLC harbor *EGFR* mutations, and especially mutations causing in-frame deletions in exon 19 and a missense point mutation composed of a single nucleotide substitution c.2573 T>G (p. L858R) in exon 21, as shown by other studies.[14,24] Interestingly, for squamous cell carcinoma, EGFR gene mutation in exon 21 was more frequently detected than EGFR gene mutation in exon 19, although there is an inverse relationship between EGFR gene mutation in exon 19 and exon 21 in adenocarcinoma. These preliminary results need to be confirmed in future studies with larger cohorts, although mutation of the EGFR gene in squamous cell carcinoma is very rare. All the samples examined in our study were biopsy specimens and the results were compared with those results from previous studies in which surgical specimens were used. Sone *et al.*[25] studied *EGFR* mutations and gene amplification using biopsy specimens and they showed that gefitinib efficacy can only be predicted by detecting *EGFR* mutations. Our data supports the importance of *EGFR* mutations in relation to NSCLC tumor's responsiveness to gefinitib, even though the follow-up time was short and overall survival was not evaluated. A recent study demonstrated that *KRAS* and *EGFR* mutations were found to be mutually exclusive and *KRAS* mutations are associated with a lack of TKIs.[26] A relationship between an increased copy number by FISH analysis and mutation has also been described.[22] Furthermore, a correlation between the copy numbers of the EGFR gene and the response to TKIs has been demonstrated.[22-24] A close relationship between the repeat length and EGFR gene amplification was reported to be associated with EGFR protein production.[21]

EGFR overexpression in NSCLC is commonly seen in squamous cell carcinoma and to a lesser extent in adenocarcinoma, and this is commonly coupled with an increased copy number and possibly more aggressive behavior.[27] EGFR protein was overexpressed in 68.7% of the NSCLCs, and this was detected in 80.8% of the squamous cell carcinomas and this was detected in 61.0% of the adenocarcinomas in our study. However, there was no correlation with gefitinib responsiveness or with the *EGFR* mutational status. An increased EGFR expression has been detected in bronchial squamous dysplasia, suggesting that EGFR signaling may play an important role in the early pathogenesis of lung cancer. A low repeat length was found to be correlated with increased EGFR transcription and an increased EGFR protein expression and hence, this was able to determine the outcome of treatment with EGFR TKIs.[23,28] However, there are reports that an EGFR expression is not a significant predictive factor for responsiveness to gefitinib.[29] The prognostic significance of an increased EGFR protein expression in NSCLC is still controversial.

Currently, the best marker to predict responsiveness and better survival for EGFR-TKIs treatment of NSCLC has still not been determined although a tremendous amount of knowledge with regard to EGFR abnormalities in lung cancers has been accumulated.[14,25] Our data suggest that low intron 1 CA repeat polymorphism of the EGFR gene was associated with a better clinical outcome for the NSCLC patients who were treated with gefitinib and a better prognosis for NSCLC patients regardless of the *EGFR* mutation status. This study further supports the importance of *EGFR* mutation with regard to gefitinib sensitivity. Further studies are warranted to investigate the role of CA repeat polymorphism of the EGFR gene in the carcinogenesis of lung cancer. In addition, comprehensive studies that will include assessing polymorphism of the EGFR gene in a larger cohort, EGFR gene amplification, *KRAS* mutations and angiogenesis inhibitors (bevacizumab) to VEGF, which have recently come into the spotlight, need to be done to elucidate other factors that have an influence on the responsiveness to treatment with gefitinib.

REFERENCES

1. Paez JG, Janne PA, Lee JC, *et al. EGFR* mutations in lung cancer: correlation with clinical response to gefitinib therapy. Science 2004; 304: 1497-500.
2. 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: 2129-39.
3. Ranson M, Hammond LA, Ferry D, *et al.* ZD1839, a selective oral epidermal growth factor receptor-tyrosine kinase inhibitor, is well tolerated and active in patients with solid, malignant tumors: results of phase I trial. J Clin Oncol 2002; 20: 2240-50.
4. 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. J Clin Oncol 2003; 21: 2237-46.
5. Miller VA, Kris MG, Shah N, *et al.* Bronchioloalveolar pathologic subtype and smoking history predict sensitivity to gefitinib in ad-

vanced non-small-cell lung cancer (NSCLC). J Cilin Oncol 2004; 22: 1103-9.

6. Pao W, Miller V, Zakowski M, *et al.* EGF receptor gene mutations are common in lung cancer from never smokers and are associated with sensitivity of tumor to gefitinib and erlotinib. Proc Natl Acad Sci USA 2004; 101: 13306-11.
7. Han SH, Kim TY, Hwang PG, *et al.* Predictive and prognostic impact of *epidermal growth factor receptor* mutation in non-small-cell lung cancer patients treated with gefitinib. J Clin Oncol 2005; 23: 1-9.
8. Huang SF, Liu HP, Li LH, *et al.* High frequency of *epidermal growth factor receptor* mutations with complex patterns in non-small cell lung cancers related to gefitinib responsiveness in Taiwan. Clin Cancer Res 2004; 10: 8195-203.
9. Henrion D, Amant C, Benessiano J, *et al.* Angiotensin II type 1 receptor gene polymorphism is associated with an increased vascular reactivity in the human mammary artery in vitro. J Vasc Res 1998; 35: 356-62.
10. Tidow N, Bocker A, Achmidt H, *et al.* Distinct amplification of an untranslated regulatory sequence in the EGFR gene contributes to early steps in breast cancer development. Cancer Res 2003; 63: 1172-8.
11. Buerger H, Packeisen J, Boecker A, *et al.* Allelic length of a CA dinucleotide repeat in EGFR gene correlates with the frequency of amplification of this sequence-first results of an interethnic breast cancer study. J Pathol 2004; 203: 545-50.
12. Amador ML, Oppenheimer D, Perea S, *et al.* An epidermal growth factor receptor intron I polymorphism mediates response to epidermal growth factor receptor inhibitors. Cancer Res 2004; 64: 9139-43.
13. Therasse P, Arbuck SG, Eisenhauer EA, *et al.* New guideline to evaluate the response to treatment in solid tumors. J Natl Cancer Inst 2000; 92: 205-16.
14. Shigematsu H, Lin L, Takahashi T, *et al.* Clinical and biological features associated with *epidermal growth factor receptor* mutations in lung cancers. J Natl Cancer Inst 2005; 97: 339-46.
15. Lee SJ, Kim KM, Chae MH, *et al.* No association between dinucleotide repeat polymorphism in intron 1 of the epidermal growth factor receptor gene *EGFR* and risk of lung cancer. Cancer Genet Cytogenet 2007; 172: 29-32.
16. Han SW, Jeon YK, Lee KH, *et al.* Intron 1 CA dinucleotide repeat polymorphism and mutations of *epidermal growth factor receptor* and gefinitib responsiveness in non-small cell lung cancer. Pharmacogenet Genomics 2007; 17: 313-9.
17. Liu W, Innocenti F, Chen P, Das S, Cook EH, Ratain MJ. Interethnic difference in the allelic distribution of human epidermal growth factor receptor intron 1 polymorphism. Clin Cancer Res 2003; 9: 1009-12.
18. Nie Q, Wang Z, Zhang GC, *et al.* The *epidermal growth factor receptor* intron 1 (CA) microsatellite polymorphism is a potential predictor of treatment outcome in patients with advanced lung cancer treated with gefitinib. Eur J Pharmacol 2007; 10: 1-7.
19. Sueoka-Aragane N, Imai K, Komya K, *et al.* Exon 19 of *EGFR* mutation in relation to CA-repeat polymorphism in intron 1. Cancer Sci 2008; 99: 1180-7.
20. Buerger H, Gebhardt F, Schmidt H, *et al.* Length and loss of heterozygosity of an intron 1 polymorphic sequence of *EGFR* is related to cytogenetic alteration and epithelial growth factor receptor expression. Cancer Res 2000; 60: 854-7.
21. Nomura M, Shigematsu H, Li Lin, *et al.* Polymorphism, mutations and amplification of the EGFR gene in non-small cell lung cancers. PLoS Medicine 2007; 4: 715-27.
22. Etienne-grimaldi MC, Pereira S, Magne N, *et al.* Analysis of the dinucleotide repeat polymorphism in the EGFR gene in head and neck cancer patients. Ann Oncol 2005; 16: 934-41.
23. Cappuzo F, Hirsch FR, Rossi E, *et al.* Epidermal growth factor receptor gene and protein and gefitinib sensitivity in non-small cell lung cancer. J Natl Cancer Inst 2005; 97: 643-55.
24. Prudkin L, Wistuba II. Epidermal growth factor receptor abnormalities in lung cancer. Pathogenetic and clinical implications. Ann Diag Pathol 2006; 10: 306-15.
25. Sone T, Kasahara K, Kimura H, *et al.* Comparative analysis of *epidermal growth factor receptor* mutations and gene amplification as predictors of gefitinib efficacy in Japanese patients with nonsmall cell lung cancer. Cancer 2007; 109: 1836-44.
26. Pao W, Wang TY, Riely GJ, *et al. KRAS* mutations and primary resistance of lung adenocarcinomas to gefitinib or erotinib. PLoS Med 2005; 2: e17.
27. Hirsch FR, Varelia-Garcia M, Bunn Jr PA, *et al.* Epidermal growth factor receptor in non-small cell lung carcinomas: correlation between gene copy number and protein expression and impact on prognosis. J Clin Oncol 2003; 21: 3798-807.
28. Gebhardt F, Zanker KS, Brandt B. Modulation of epidermal growth factor receptor gene transcription by a polymorphic dinucleotide repeat in intron 1. J Biol Chem 1999; 274: 13176-80.
29. Parra HS, Cavina R, Latteri F, *et al.* Analysis of epidermal growth factor receptor expression as a predictive factor expression for response to gefitinib ("Irresa", ZD1839) in non-small-cell lung cancer. Br J Cancer 2004; 91: 208-12.