RESEARCH ARTICLE

EGFR Mutation Genotype Impact on the Efficacy of Pemetrexed in Patients with Non-squamous Non-small Cell Lung Cancer

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Abstract

Background: Pemetrexed monotherapy has come to be recognized as one of the standard second-line therapies for advanced non-squamous non-small cell lung cancer (NSCLC). However, there have been no reports of studies that have evaluated the efficacy of pemetrexed according to type of active EGFR mutation, i.e., an exon 19 deletion or an L858R point mutation. Materials and Methods: The records of non-squamous NSCLC patients harboring an EGFR mutation who received pemetrexed monotherapy as a second or later line of chemotherapy at Kitasato University Hospital between March 2010 and October 2015 were retrospectively reviewed, and the treatment outcomes were evaluated. Results: The overall response rate and progression-free survival time (PFS) of the 53 patients with non-squamous NSCLC were 15.1% and 2.3 months, respectively. There were significant differences between the disease control rate (37.5% vs. 76.2%) and PFS time (1.8 months vs. 3.3 months) of the exon 19 deletion group and the L858R point mutation group, and a multivariate analysis identified type of EGFR mutation as well as performance status (PS) as independent predictors of PFS. Conclusions: The clinical data obtained in this study provided a valuable rationale for considering type of EGFR mutation as well as non-squamous histology as predictors of the efficacy of pemetrexed monotherapy.

Keywords: Non-squamous non-small cell lung cancer, Pemetrexed, Predictor, EGFR mutation

Asian Pac J Cancer Prev, **17 (7)**, 3249-3253

Introduction

Non-small cell lung cancer (NSCLC) is a major cause of cancer-related deaths worldwide. Although advanced NSCLC is still incurable, various antineoplastic agents are now available to treat it. Platinum-based chemotherapy has been considered the standard first-line therapy for advanced NSCLC worldwide (Ohe et al., 2007; Schiller et al., 2001; Schiller et al., 2002). A randomized phase III trial and revealed that a treatment with pemetrexed monotherapy resulted in clinically equivalent efficacy outcomes, but with significantly fewer side effects compared with docetaxel in the second-line treatment of patients with advanced NSCLC (Hanna et al., 2004; Di et al., 2014; Huang et al., 2014). The results of several clinical trials have shown that pemetrexed efficacy was limited to patients with non-squamous NSCLC (Ciuleanu et al., 2009; Kubota al., 2009; Ohe et al., 2008; Solomon B et al., 2005; Joerger et al., 2010).

Three pivotal studies revealed strong correlations between the presence of somatic mutations in the kinase domain of the epidermal growth factor receptor (EGFR) and responsiveness to gefitinib (Lynch et al., 2004; Paez et al., 2004; Pao et al., 2004) and several subsequent phase III studies have demonstrated promising efficacy of individualized treatment for advanced NSCLC patients with EGFR tyrosine kinase inhibitors (EGFR-TKIs) on the basis of their EGFR gene mutation status (Fukihara et al., 2014; Okami et al., 2007; Maemondo et al., 2010; Mitsudomi et al., 2010; Yang et al., 2015; Matam et al., 2015; Alharbi KK et al., 2015). Two meta-analyses have clearly indicated improved progression-free survival (PFS) and response rates to EGFR-TKI therapy in comparison with chemotherapy in patients with EGFR mutations (Hasegawa et al., 2015; Lee et al., 2015). On the other hand, treatment with a cytotoxic agent of pemetrexed has been reported to result in a higher response among patients with a EGFR mutation and to extend their PFS in comparison with "wild-type" patients (Wu et al., 2011). However, there have been no reports that have evaluated the efficacy of pemetrexed according to the type of EGFR mutation, i.e., according to exon 19 deletion or L858R point mutation. The purpose of this study was to evaluate the efficacy of pemetrexed in patients with a

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non-squamous NSCLC harboring a major EGFR mutation according to the type of the mutation.

Materials and Methods

Patient selection and data collection

A total of 53 patients with advanced non-squamous NSCLC who harbored an active EGFR mutation, i.e., an exon 19 deletion or L858R point mutation and received pemetrexed monotherapy as a second or later line of chemotherapy at Kitasato University Hospital between March 2010 and October 2015 were the subjects of this retrospective cohort study. Patients who were receiving pemetrexed monotherapy in the maintenance setting were excluded. Patients with histologically or cytologically confirmed disease stage IV NSCLC, or post-operative recurrence according to the criteria of the Union for International Cancer Control, version 7 and patients with the disease not amenable to curative therapy were assessed for their eligibility. Consecutive patients who met the following criteria were included as subjects of this retrospective study: a measurable target lesion on chest X-ray or computed tomography (CT) images of the chest and abdomen, or by other diagnostic imaging methods as indicated, including MRI of the head, positron emission tomography (PET), or combined PET/CT; histologically confirmed non-squamous NSCLC; Eastern Cooperative Oncology Group Performance Scale status (ECOG PS) of 3 or less. The subjects were categorized according to smoking status as current smokers, former light smokers (defined as patients who had stopped smoking at least 15 years previously, with a total of ≤10 pack-years of smoking), and never smokers (defined as patients who had smoked <100 cigarettes in their lifetime). The research ethics committee of our institute has approved this retrospective study.

Treatment methods

The NSCLC patients with a non-squamous histology had received pemetrexed at a dose of 500 mg/m^2 as a 10-minute intravenous infusion on day 1 of each cycle. Cycles were repeated until either detection of disease progression or the development of unacceptable toxicity. The patients had been instructed to take folic acid (1000 μg, orally) daily from approximately 1 to 2 weeks before the first dose of pemetrexed until 3 weeks after the final dose. Vitamin B12 (1000 μg, intramuscularly) had been administered approximately 1 to 2 weeks before the first dose of pemetrexed and approximately every 9 weeks thereafter.

Response Analysis

Tumor response was classified according to the Response Evaluation Criteria in Solid Tumors (version 1.1). Patients were evaluated to determine the stage of their disease before treatment and again when their disease progressed or recurred based on the results of chest radiography, CT of the chest and abdomen, and other staging diagnostic imaging examinations, such as MRI of the head and PET.

Statistical analysis

PFS was measured from the start of pemetrexed therapy to the date of documentation of treatment failure (death or disease progression) or the date of censoring at the final follow-up examination. Overall survival time (OS) was measured from the start of pemetrexed therapy to death from any cause or date of censoring. We plotted a survival curve by the Kaplan-Meier method. Differences in PFS were analyzed by the log-rank test. The variables age, gender, smoking status, PS, type of EGFR mutation, presence/absence of brain metastasis, and stage were entered into Cox's proportional hazard models to predict the hazard ratios for PFS. Differences in response rates and disease control rates according to the type of EGFR mutation were compared by the chi-squared test. The statistical significance level was set at $p<0.05$. All of the statistical analyses were performed using the SPSS software program, version 23.0 (SPSS Inc., Chicago, Illinois), for Windows.

Results

Patient characteristics

The main clinical characteristics of the patients are summarized in Table 1. The median age of the 53 patients was 65 years (range: 43-86); there were 18 (34%) men and 35 (66%) women; 45 patients (85%) had a good PS of 0-1, and 24 patients (45%) were current smokers. The histological diagnosis was adenocarcinoma in every patient. The EGFR mutation was the exon 19 deletion in 32 patients (60%) and the L858R point mutation in 21 patients (40%). The median number of cycles of pemetrexed monotherapy was 4 (range: 1-16). An EGFR-TKI had been used to treat 49 (91%) of the 53 patient before the start of pemetrexed monotherapy.

Table 1. Patient Characteristics

Characteristics of the patients	n	%
Gender		
Male	18	34
Female	35	66
Age (years)	65 (43-86)	
Median (range)		
< 75	46	87
≥ 75	7	13
PS		
0-1	45	85
2-3	8	15
Smoking status		
Current smoker	24	45
Never or former light smoker	29	55
EGFR genotype		
Exon 19 deletion	32	60
L858R point mutation	21	40
Stage		
IV	47	89
Recurrence	6	11
Histology		
Adenocarcinoma	53	100
Brain metastasis		
Positive	11	21
Negative	42	79

Table 2. Overall Response According to the Type of EGFR Mutation

	All	EGFR mutation		
	patients (n=53)	Exon 19 deletion (n=32)	L858R point mutation (n=21)	
Partial response	8	4	4	
Stable disease	20	8	12	
Progressive disease	25	20	5	
Response rate (%)	15.1	12.5	19	P= 0.51 *
Disease control rate (%)	52.8	37.5	76.2	P < 0.01*

*Chi squire test

Table 3. Predictors of Progression-Free Survival Identified by Means of Cox Regression Models

PFS	Univariate analysis	P value	Multivariate analysis	P value
Variable	Hazard ratio (95% CI)		Hazard ratio (95% CI)	
Age (years)	1.20 (0.54-2.71)	0.64		
< 75				
≥ 75				
Gender	1.18 (0.66-2.11)	0.57		
Male				
Female				
PS	3.99 (1.59-9.99)	0.003	3.26 (1.29-8.23)	0.012
0-1				
2-3				
Smoking status	1.21 (0.70-2.12)	0.5		
Current smoker				
Never or former light smoker				
Brain metastasis	0.61 (0.30-1.23)	0.17		
Positive				
Negative				
Type of EGFR mutation	0.45 (0.24-0.83)	0.011	0.49 (0.26-0.91)	0.025
Exon 19 deletion				
L858R point mutation				
Stage	2.03 (0.84-4.90)	0.12		
IV				
Recurrence				

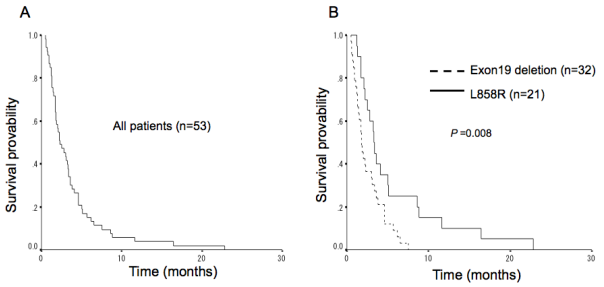

Figure 1. Progression-Free Survival. A) Group of patients as a whole. B) According to type of EGFR mutation

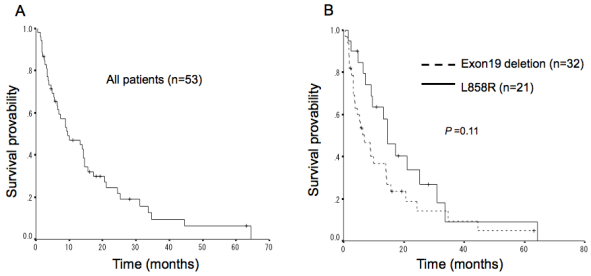

Figure 2. Overall Survival. A) Group of patients as a whole. B) According to type of EGFR mutation

Response to pemetrexed

The objective tumor responses are shown in Table 2. A partial response (PR) was confirmed in 8 of the 53 patients, corresponding to an overall response rate of 15.1% (95% CI, 3.7-26.5%) and disease control rate of 52.8% (95% CI, 36.9-68.7%). Of the 32 patients with exon 19 deletion, 4 patients showed PR, corresponding to an objective response rate of 12.5%. Of the 21 patients with L858R point mutation, 4 showed a PR, corresponding to an objective response rate of 19.0%. The difference in response rate according to type of EGFR mutation was not statistically significant (p=0.51), but the disease control rates of the exon 19 deletion group and L858R point mutation group were 37.5% and 76.2%, respectively, and the difference was significant (p<0.01).

PFS and OS

The overall PFS curve is shown in Figure 1A, and the median PFS time was 2.3 months (95% CI, 1.3-3.3 months). At the time of the data cutoff for the primary analysis on April 2016, the median follow-up time was 8.9 months. The PFS of the exon 19 deletion group and L858R point mutation group were 1.8 months (95% CI: 1.3-2.3 months) and 3.3 months (95% CI: 2.9-3.7 months), respectively (p=0.008, Figure 1B). The univariate analysis of PFS identified PS and type of EGFR mutation as significantly predictive of the PFS. The multivariate analysis identified PS and type of EGFR mutation as independent predictors of PFS (Table 3). The median survival time (MST) of the group of 53 patients as a whole was 9.5 months (95% CI, 3.5-15.5 months,

Figure 2A). The MST of the exon 19 deletion group and L858R point mutation group were 6.9 months (95% CI: 2.4-11.4 months) and 14.6 months (95% CI: 9.4-19.8 months), respectively (p=0.11, Figure 2B), and although the difference was not significant, the MST of the L858R group was twice as long as in the exon 19 deletion group.

Discussion

Needless to say, EGFR-TKIs are indispensable to the treatment of NSCLC patients with an EGFR mutation. Pemetrexed is also a necessary anticancer agent in the treatment progress of not only wild-type of patients with non-squamous NSCLC but also patients with an EGFR mutation (Inoue et al., 2013). Tumor histology has been recognized as a crucial predictor of efficacy of pemetrexed-based chemotherapy (Ciuleanu et al., 2009; Kubota al., 2009; Ohe et al., 2008; Solomon et al., 2005; Joerger et al., 2010). Pemetrexed based chemotherapy has been reported to result in a higher response in patients with an EGFR mutation and to extend their PFS time in comparison with wild-type patients (Wu et al., 2011). A study investigated that six NSCLC cell lines showed a lower TS gene expression in cell line H1650 with EGFR mutation, than in the five NSCLC cell lines with wild-type EGFR (Giovannetti et al., 2008), indicating that EGFR mutations may be associated with lower TS gene expression levels, which in turn may cause NSCLC cells with EGFR mutation to become more sensitive to pemetrexed. However, an another study reported finding no significant difference in response rate to pemetrexed monotherapy and no significant difference in PFS between an EGFR mutation group and wild-type EGFR group (Jiang et al., 2015). Thus, even though lung adenocarcinoma patients have EGFR mutations more frequently than non-adenocarcinoma NSCLC patients (Mitsudomi et al., 2006), there has been controversy as to whether the presence of EGFR mutation can predict the efficacy of pemetrexed monotherapy and be a valuable aid to selecting pemetrexed for individual NSCLC patients. On the other hand, there have been no reports of studies that have compared the effects of pemetrexed according to type of activating EGFR mutations. The focus of the present study was lung adenocarcinoma, and to the best of our knowledge this is the first study to show that the type of EGFR mutation might be a useful predictor of pemetrexed monotherapy in patients harboring an EGFR mutation.

One study has reported a more rapid in vivo growth rate of cells with an exon 19 deletion than of cells with a L858R point mutation (Carey KD., 2006). Another study reported finding that the tumor volume doubling time of exon 19 deletion NSCLCs (median: 272 days; range, 95% CI: 28-3061 days) tended to be shorter than in L858R point mutant NSCLCs (median: 817 days; range, 95% CI: 85-5159 days), suggesting that the tumor cells with an exon 19 deletion were more aggressive than the tumor cells with a L858R point mutation (Nakamura R et al., 2014). In a randomized phase III trial comparing afatinib and CDDP plus pemetrexed in patients with advanced NSCLC harboring EGFR mutation, the MST of the CDDP plus pemetrexed group with L858R point mutation and the CDDP plus pemetrexed group with exon 19 deletion were 40.3 months and 21.1 months, respectively, suggesting that MST of L858R group may be expected to be almost twice as long as MST of exon 19 deletion group in response to the pemetrexed based-chemotherapy (Yang JC et al., 2015). Based on the results of our study it seems reasonable to conclude that the type of EGFR mutation may be a useful predictor of pemetrexed efficacy in non-squamous NSCLC patients with an active EGFR mutation.

We recognize that thymidylate synthase (TS) expression in NSCLC has attracted considerable attention because of its potential role as a promising predictor of response to pemetrexed-based chemotherapy. Recent meta-analyses have reported TS expression as a predictor of sensitivity to pemetrexed-based chemotherapy in advanced NSCLC patients (Rossi et al., 2009; Lei Wang et al., 2014; Liu et al., 2015; Ting Wang et al., 2013; Liu et al., 2013). However, the detection of TS expression as a predictor of the treatment efficacy requires a wide availability of TS immunohistochemistry at many institutions, the necessary technical resources and human resources, suggesting the difficulty of widespread.

This study had several limitations. Since it was a retrospective study and the sample size may not have been sufficient, the results cannot be regarded as definitive. Second, because it was a retrospective study, it was impossible to perform a pharmacokinetic validation of the differences in the efficacy of pemetrexed according to type of EGFR mutation. Third, there was no data indicating the relationship between type of EGFR mutation and TS expression level of the tumors in the present study.

In conclusion, the clinical data obtained in this study provided a valuable rationale for considering the type of EGFR mutation as well as non-squamous histology to be a predictor of the efficacy of pemetrexed monotherapy. The type of EGFR mutation should be considered an essential factor in studies of pemetrexed therapy in non-squamous NSCLC patients with an EGFR mutation.

Acknowledgements

We thank the staff of the Department of Respiratory Medicine of Kitasato University School of Medicine for their advice and assistance.

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