Clinical characteristics of patients with non-small cell lung cancers harboring anaplastic lymphoma kinase rearrangements and primary lung adenocarcinoma harboring epidermal growth factor receptor mutations

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ABSTRACT. Echinoderm microtubule associated protein like 4-anaplastic lymphoma kinase (*EML4-ALK*) gene rearrangements and epidermal growth factor receptor (*EGFR*) mutations in non-small cell lung cancer (NSCLC) have been intensively studied. The objective of this study was to determine the clinicopathological characteristics in genotype-specific subsets of patients with NSCLC to help ensure the optimal identification of patients whose tumors harbor these two driver mutations. The incidence of *ALK* rearrangements was investigated in 763 NSCLC specimens by immunohistochemistry using a D5F3 antibody, and *EGFR* mutations were assessed by amplification refractory mutation system (ARMS) in 222 patients with lung adenocarcinoma. Of these, 73 (9.6%) were detected

as being *ALK*-positive; this designation was associated with young age, female gender, never-smokers, lymph node metastasis, and poor tumor differentiation, but not with histology. *EGFR* mutations were identified in 102 (45.9%) of 222 adenocarcinoma samples, and were more frequent in females and never-smokers. No difference in age was observed. Specifically, we identified several cases of complex *EGFR* mutations, and concomitant *EGFR* mutations and *ALK* rearrangements. These results suggest that young women and never-smokers are at risk for *ALK* rearrangement. We also identified concomitant mutations of *EGFR* and rearrangements of *ALK* in this study.

Key words: Non-small cell lung cancer; Epidermal growth factor receptor; Echinoderm microtubule associated protein like 4-anaplastic lymphoma kinase; Adenocarcinoma; D5F3 antibody

INTRODUCTION

Echinoderm microtubule associated protein like 4-anaplastic lymphoma kinase (*EML4-ALK*) gene rearrangement in lung cancer is a specific non-small cell lung cancer (NSCLC) category that is characterized by *ALK* gene inversion or translocation (Li et al., 2013). However, the incidence of *EML4-ALK* fusion in patients with NSCLC is very low, approximately 3-13% (Shaw et al., 2009; Kwak et al., 2010; Zhang et al., 2010; Wang et al., 2012; Li et al., 2013; Wu et al., 2013).

Recently, crizotinib, a small-molecule dual inhibitor of ALK receptor tyrosine kinase, showed a significant clinical benefit in patients with *ALK* rearrangements in NSCLC (Crinò et al., 2011; Wang et al., 2014a). Thus, the identification of cases with *ALK*-rearrangements is essential for the selection of an appropriate therapy.

Immunohistochemistry (IHC) for ALK protein overexpression is a promising screening modality, with D5F3 antibodies showing excellent sensitivity and specificity for *ALK*-rearranged NSCLC (Takahashi et al., 2010; To et al., 2013; Shan et al., 2014; Zhang et al., 2013; Zhang et al., 2014). Furthermore, the ALK fusion protein IHC diagnostic kit developed by Roche Ventana Medical Systems (Oro Valley, AZ, USA) has been approved by the European Medicines Agency (EMEA) and the China Food and Drug Administration (CFDA) as an aid for identifying patients who are eligible for treatment with crizotinib.

Epidermal growth factor receptor (*EGFR*) mutations have been reported in approximately 50% of patients with lung adenocarcinomas. The *EGFR* mutations are always associated with good responses to EGFR tyrosine kinase inhibitors (EGFR-TKIs) (Pao et al., 2004; Kim et al., 2014).

This article summarizes the clinicopathological characteristics of *ALK* rearrangements in patients with NSCLC and *EGFR* mutations in adenocarcinomas, with the purpose of identifying additional useful information on candidate selection for ALK tyrosine kinase inhibitor (ALK-TKI) or EGFR-TKI therapy. We also identified three patients with complex *EGFR* mutations and three with concomitant *EGFR* mutations and *ALK* rearrangements, and analyzed their characteristics, treatment, and outcomes; the results from this analysis will provide additional clues for the treatment of these special case patients.

MATERIAL AND METHODS

Patients

In this study, we included 763 consecutive patients with NSCLC who presented at our clinic between October 2012 and December 2014. Tumor tissues were collected within half an hour of resection and were fixed in 10% neutral-buffered formalin and then stored as paraffin-embedded archival samples until use. The tumor-lymph node-metastasis (TNM) staging was reviewed according to the 7th edition of the American Joint Committee for Cancer (AJCC) staging system. Patients who had a previous history of other cancers, or pre-surgical chemotherapy or radiotherapy were excluded from this study. Patients who did not smoke or those with a history of smoking <100 cigarettes were categorized as never-smokers. Written informed consent was obtained from each participant before the initiation of any study-related procedure. The study was approved by the Affiliated Cancer Hospital of Xiangya School of Medicine.

ALK immunohistochemistry

Formalin-fixed and paraffin-embedded tissues were sectioned at a thickness of 4 μm and stained with an antibody for ALK (mouse monoclonal, D5F3 antibody, Ventana Medical Systems), using a Ventana automated immunostainer, and the OptiView DAB IHC detection kit and the OptiView Amplification kits were used according to the manufacturer instructions. The results from this method were reported as positive or negative, as depicted in Figure 1. Positive immunostaining for ALK was clearly visible as strong, granular, cytoplasmic staining in tumor cells. A negative result referred to no positive cell staining.

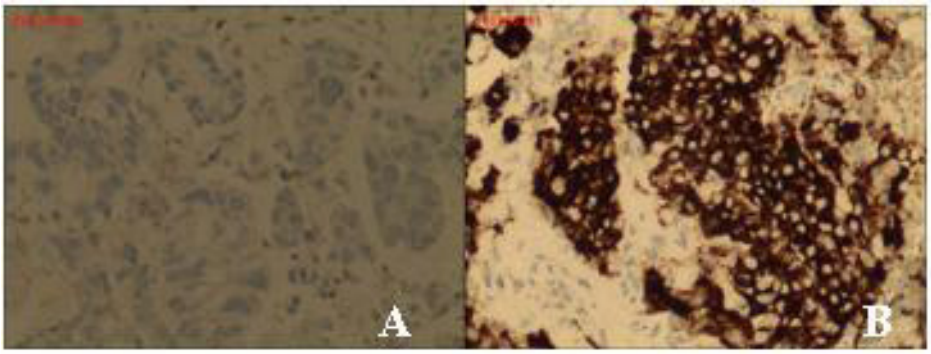

Figure 1. Detection of *ALK* rearrangements using Ventana IHC (200X). **A.** An ALK-negative case without cytoplasmic staining. **B.** An EML4-ALK-positive case with strong granular cytoplasmic staining in NSCLC. ALK = anaplastic lymphoma kinase; IHC = immunohistochemistry; EML4 = Echinoderm microtubule associated protein like 4; NSCLC = non-small cell lung carcinoma.

Detection of *EGFR* mutations in exons 18-21 by amplification refractory mutation system (ARMS)

ARMS, as a real-time PCR-based test, is a highly sensitive method. The AmoyDx *EGFR* Mutation Test Kit (Amoy Diagnostics Co., Ltd., Suzhou, P.R., China) has been widely used in the

clinical laboratory. We chose this kit to detect *EGFR* mutation hotspots in exons 18-21. The assay was carried out according to the manufacturer protocol for the kit with the LightCycler® 480 II real-time PCR system (Hoffman-La Roche Ltd., (Basel, Switzerland). Upon completion, the results were analyzed according to the criteria defined by the manufacturer instructions.

Statistical analysis

Statistical analyses were performed using SPSS, v.19.0 (IBM). A Pearson χ^2 test or Fisher's exact test was used to assess the association between gene mutation status and patient clinical factors. All statistical tests were conducted at a two-sided level of significance of $P < 0.05$.

RESULTS

Clinicopathological characteristics of ALK-positive patients with NSCLC

The clinicopathologic features of the 763 patients recruited for this study are summarized in Table 1. We identified 73 patients (9.6%) with *ALK* rearrangements. The ALK-positive patients were significantly younger (53.5 *vs* 57.9 years old, P = 0.007). Other obvious differences were found between ALK-positive and -negative patients with respect to gender (P = 0.044), smoking status (P = 0.002), tumor differentiation (well *vs* moderate, poor, and not available, P = 0.007), and lymph node metastasis, not only in the local nodal involvement referred to as N from TNM staging classification (N0 *vs* N1-3, P = 0.046), but also in distant lymph node metastasis (P = 0.004). *ALK* rearrangement had no statistical correlation with other clinical characteristics such as disease stage (I-IIIA *vs* IIIB-IV, P = 0.184), histologic subtypes (adenocarcinoma *vs* squamous cell and others, P = 0.174), and M factors including visceral pleural invasion, and brain, bone, lung, and liver metastasis (P = 0.151, P = 0.102, P = 0.857, P = 0.168, P = 0.174, respectively).

Although ALK-positive status had no statistical correlation with histologic subtypes, we still explored its clinicopathological characteristics in adenocarcinoma. We identified 54 instances (11.0%) of ALK rearrangement in 493 patients with lung adenocarcinoma. Many characteristics of these patients were consistent with those of patients with NSCLC. The ALK-positive patients were significantly younger (51.4 *vs* 57.3 years old, P = 0.001). Obvious differences were found with respect to smoking status (P = 0.005), tumor differentiation (well *vs* moderate, poor, and not available, P = 0.006), lymph node involvement (N0 *vs* N1-3, P = 0.036; distant lymph node metastasis *vs* no metastasis, P = 0.003). No obvious differences were found with respect to sex (P = 0.106) and disease stage (I-IIIA *vs* IIIB-IV, P = 0.088). *ALK* rearrangement had no statistical correlation with other clinical characteristics such as M factors.

EGFR mutations and clinical characteristics of patients

EGFR status was assessed in 222 patients among the 493 patients with lung adenocarcinoma, as shown in Table 1. Mutations were found in 102 patients (45.9%). Mutations primarily consisted of deletions in exon 19 (64 patients, 62.7%) or exon 21 L858R or L861Q mutations (32 patients, 31.4%), with two patients carrying exon 20 T790M point mutations and one carrying exon 20 S768I. One patient harbored overlapping mutations: exon 18 G719X/exon 19 deletion. Mutations in exon 19 and mutations in exon 21 were exclusive, but two special cases

of coexistence were identified in our study, one with exon 19 deletion/exon 21 L858R, and the other with exon 19 deletion/exon 20 T790M/exon 21 L858R. The patient with overlapping *EGFR* mutations did not have an *ALK* rearrangement.

Table 1. Clinicopathological characteristics of *ALK* rearrangements in NSCLC and *EGFR* mutations or *ALK* rearrangements in adenocarcinoma.

Characteristics	*ALK* in NSCLC (N = 763)		P value	*ALK* in adenocarcinoma (N = 493)		P value	*EGFR* in adenocarcinoma (N = 222)		P value
	Positive	Negative		Positive	Negative		Positive	Negative	
	73 (9.6%)	690 (90.4%)		54 (11.0%)	439 (89.0%)		102 (45.9%)	120 (54.1%)	
Age			0.007			0.001			0.192
≤55 years	38 (52.1%)	249 (36.1%)		34 (63.0%)	172 (39.2%)		48 (47.1%)	67 (55.8%)	
>55 years	35 (47.9%)	441 (63.9%)		20 (37.0%)	267 (60.8%)		54 (52.9%)	53 (44.2%)	
Age (means ± SD)	53.5 ± 10.9	57.9 ± 8.9		51.4 ± 11.6	57.3 ± 9.2		55.6 ± 9.3	53.7 ± 9.9	
Gender			0.044			0.106			0.000
Female	32 (43.8%)	222 (32.2%)		30 (55.6%)	193 (44.0%)		57 (55.9%)	39 (32.5%)	
Male	41 (56.2%)	468 (67.8%)		24 (44.4%)	246 (56.0%)		45 (44.1%)	81 (67.5%)	
Smoking status			0.002			0.005			0.000
Never smoker	43 (58.9%)	277 (40.1%)		39 (72.2%)	229 (52.2%)		74 (72.5%)	46 (38.3%)	
Ever smoker	30 (41.1%)	413 (59.9%)		15 (27.8%)	210 (47.8%)		28 (27.5%)	74 (61.7%)	
Nodal involvement			0.046			0.036			0.867
N0	16 (21.9%)	244 (35.4%)		11 (20.4%)	152 (34.6%)		17 (16.7%)	19 (15.8%)	
N1-3	57 (78.1%)	446 (64.6%)		43 (79.6%)	287 (65.4%)		85 (83.3%)	101 (84.2%)	
Disease stage			0.184			0.088			0.895
I-IIIA	30 (41.1%)	340 (49.3%)		16 (29.6%)	183 (41.7%)		18 (17.6%)	22 (18.3%)	
IIIB-IV	43 (58.9%)	350 (50.7%)		38 (70.4%)	256 (58.3%)		84 (82.4%)	98 (81.7%)	
Histologic type			0.174						
Adenocarcinoma	54 (74.0%)	439 (63.6%)							
Squamous cell carcinoma	16 (21.9%)	193 (28.0%)							
Others*	3 (4.1%)	58 (8.4%)							
Tumor differentiation			0.007			0.006			0.000
Well	3 (4.1%)	110 (15.9%)		3 (5.6%)	96 (21.9%)		22 (21.6%)	11 (9.2%)	
Moderate	35 (47.9%)	341 (49.4%)		24 (44.4%)	201 (45.8%)		56 (54.9%)	47 (39.2%)	
Poor	28 (38.4%)	202 (29.3%)		21 (38.9%)	122 (27.8%)		16 (15.7%)	55 (45.8%)	
Not available	7 (9.6%)	37 (5.4%)		6 (11.1%)	20 (4.6%)		8 (7.8%)	7 (5.8%)	
Metastasis									
Distant lymph node	9 (12.3%)	28 (4.1%)	0.004	8 (14.8%)	18 (4.1%)	0.003	4 (3.9%)	16 (13.3%)	0.015
Visceral pleural invasion	11 (15.1%)	67 (9.7%)	0.151	11 (20.4%)	58 (13.2%)	0.152	23 (22.5%)	27 (22.5%)	0.993
Brain	3 (4.1%)	69 (10.0%)	0.102	3 (5.6%)	54 (12.3%)	0.144	26 (25.5%)	16 (13.3%)	0.021
Bone	16 (21.9%)	145 (21.0%)	0.857	13 (24.1%)	122 (27.8%)	0.563	52 (51.0%)	39 (32.5%)	0.005
Lung	12 (16.4%)	76 (11.0%)	0.168	11 (20.4%)	59 (13.4%)	0.169	23 (22.5%)	23 (19.2%)	0.535
Liver	1 (1.4%)	41 (5.9%)	0.174	0 (0%)	32 (7.3%)	0.079	16 (15.7%)	7 (5.8%)	0.016

*Other cell types include adenosquamous cell carcinoma, large cell carcinoma, neuroendocrine tumor from which small cell lung cancer was excluded, sarcomatoid cancer, and mixed lung cancer. ALK = anaplastic lymphoma kinase; NSCLC = non-small cell lung carcinoma; EGFR = epidermal growth factor receptor.

Certain characteristics were found to be associated with *EGFR* mutations, such as smoking status (P = 0.000), sex (P = 0.000), tumor differentiation (well, or moderate *vs* poor, P = 0.013), and M factors including distant lymph node, brain, bone, and liver metastasis (P = 0.015, P = 0.021, P = 0.005, and P = 0.016, respectively). *EGFR* mutation had no statistical correlation with other clinical characteristics such as age (55.6 *vs* 53.7 years old, P = 0.192), local nodal involvement (N0 *vs* N1-3, P = 0.867), disease stage (I-IIIA *vs* IIIB-IV, P = 0.895).

We stratified *EGFR* mutations in exons 19 and 21 in Table 2, and found that the mutation in exons 19 or 21 did not correlate with female gender or never/light-smoking status.

Table 2. Comparison of clinicopathological characteristics according to *EGFR* mutation status in exons 19 or 21.

Characteristics	*EGFR* exon 19	*EGFR* exon 21	P value
N	64 (62.7%)	32 (31.4%)	
Age			0.885
≤55 years	29	15	
>55 years	35	17	
Gender			0.663
Female	35	19	
Male	29	13	
Smoking status			1.000
Never smoker	46	23	
Ever smoker	18	9	

EGFR = epidermal growth factor receptor.

We identified three patients (2.9%) with complex *EGFR* mutations; the details are listed in Table 3. All of these three patients exhibited moderate or poor differentiation, were of IIIB-IV stage, and two were never-smokers. The three patients received the first-line treatment of EGFR-TKIs (two of erlotinib, and one of gefitinib), and all achieved partial response.

Table 3. Details of three patients with complex *EGFR* mutations in lung adenocarcinoma.

Patient	Gender	Age (years)	Smoking status	Differentiation	IHC of ALK	*EGFR* mutation type	Diagnostic date	Stage	First-line treatment	Response
159	Female	65	Never	Moderate	Negative	Exon 19 deletion/exon 21 L858R	2013.8.26	IV	Erlotinib	PR
328	Male	47	Never	Moderate	Negative	Exon 18 G719X/exon 19 deletion	2014.4.22	IIIB	Erlotinib	PR
720	Male	54	Ever	Poor	Negative	Exon 19 deletion/exon 20 T790M/exon 21 L858R	2014.10.22	IV	Gefitinib	PR

EGFR = epidermal growth factor receptor; IHC = immunohistochemistry; ALK = anaplastic lymphoma kinase; PR = partial response.

Three patients harbored concomitant *EGFR* mutations and were ALK-positive; the details are listed in Table 4. The overall frequency of concomitant *EGFR* mutations and *ALK* rearrangements was 1.3% (3/222). All three patients were never smokers, exhibited moderate differentiation or not-available, and were of stage IV. *EGFR/ALK* co-alterations were found in 2.9% (3/102) patients with *EGFR* mutations and in 12.5% (3/24) patients with *ALK* rearrangements. Two patients received the first-line treatment of EGFR-TKI (erlotinib), and while one achieved partial response, the other achieved stable disease.

Table 4. Details of three patients with concomitant EGFR mutations/ALK rearrangements.

Patient	Gender	Age (years)	Smoking status	Differentiation	IHC of ALK	Mutation type of EGFR	Diagnostic date	Stage	First-line treatment	Response
582	Female	40	Never	Moderate	Positive	Exon 19 deletion	2014.8.14	IV	Erlotinib	PR
647	Female	51	Never	Moderate	Positive	Exon 19 deletion	2014.9.16	IV	Erlotinib	SD
704	Female	59	Never	Not-available	Positive	Exon 21 L858R	2014.10.17	IV	Not-available	-

EGFR = epidermal growth factor receptor; IHC = immunohistochemistry; ALK = anaplastic lymphoma kinase; PR = partial response; SD = stable disease.

DISCUSSION

Recently many studies have reported the efficacy of Ventana IHC for detecting *ALK* rearrangements with D5F3 antibodies and have supported that Ventana IHC is a method with

excellent sensitivity and specificity to identify ALK rearrangements in paraffin-embedded lung cancer specimens (Takahashi et al., 2010; Zhang et al., 2013; Shan et al., 2014; Wang et al., 2014a; Zhang et al., 2014). The sensitivity and specificity of Ventana IHC were found to be 100 and 98.2%, respectively, and the concordance rate between the fluorescence *in situ* hybridization (FISH) and the Ventana IHC was 98.4% (Wang et al., 2014a). The 100% sensitivity measure suggested that IHC analysis is an effective way to screen patients in the clinical diagnosis process (Shan et al., 2014). Although break-apart FISH is the standard method for diagnosis of *ALK* rearrangements, it is expensive and not readily available. IHC might provide a reliable and cost-effective diagnostic approach in routine pathologic laboratories for the identification of suitable candidates for ALK-targeted therapy.

The *EML4-ALK* fusion genes were discovered in NSCLC in 2007. The incidence of *ALK* rearrangements was found to be 9.6% (73/763) in NSCLC and 11.0% (54/493) in adenocarcinoma in our study, which was similar to the results previously published in Chinese (Shan et al., 2014; Wang et al., 2014a; Yang et al., 2014) and Asian cohorts (Kim et al., 2014). As previously reported, patients who were ALK-positive were predominantly young women and never/light smokers (Lynch et al., 2004; Rodig et al., 2009; Shaw et al., 2009; Wong et al., 2009; Jokoji et al., 2010; Takahashi et al., 2010; Zhang et al., 2013; Zhang et al., 2014). In our study, young age, female gender, and never-smokers were significantly associated with *ALK* rearrangement. Further analysis revealed that *EML4-ALK* was present at a frequency of 13.2% (38/287) in young patients, 13.4% (43/320) in never-smokers, 12.60% (32/254) in females, and 19.27% (21/109) in young never-smoker women in selected NSCLCs in our study. However, significant differences were not observed between histology subtypes.

Our study demonstrated that the *ALK* translocation was found in pure squamous carcinoma of the lung. Among the 209 patients with squamous cell carcinomas, 16 incidences (7.7%) of *ALK* rearrangement were detected, which is less than the incidence (11.0%) in adenocarcinoma, but not significantly so. Caliò et al. (2014) also demonstrated *ALK* rearrangements in squamous lung cancers, as those might represent adenosquamous neoplasms misdiagnosed because of false squamous cell carcinomas, limited biopsy samples not representative of the entire lesion, and lack of use of an immunohistochemical confirmatory panel. However, the 16 patients with squamous carcinoma enrolled in our study comprised nine with whole-tissue from surgery, demonstrating that the *ALK* translocation might be found in pure squamous carcinoma of the lung. Our results indicate that ALK protein expression is not a rare molecular event in squamous lung cancer. However, the question of whether to carry out routine detection of *ALK* rearrangement in squamous carcinoma is worth exploring. The response of lung squamous carcinoma patients with *ALK* rearrangement to targeted crizotinib therapy as a first-line treatment should be explored.

In this study, the ALK-positive patients were more likely to have lymph node metastasis compared to ALK-negative patients. This observation was consistent with another study, which showed that ALK-positive adenocarcinomas might metastasize to lymph nodes early, despite the small size of the primary tumor (Jokoji et al., 2010; Paik et al., 2012; Takamochi et al., 2013). To date, several small sample studies (Takahashi et al., 2010; Zhang et al., 2014; Toll and Maleki, 2015) have reported that *ALK* rearrangements were associated with moderate or poor differentiation status; our study further affirmed this finding in a relatively larger sample size.

We identified 102 patients with *EGFR* mutations, which accounted for 45.9% (102/222) of patients with lung adenocarcinoma. The incidence rate of *EGFR* mutations in our study was similar to those of other studies evaluating cohorts of Asian ethnicity (Pao et al., 2004; Kim et al.,

2014; Wang et al., 2014b). Previous results have indicated that the frequency of *EGFR* mutation was increased in patients with adenocarcinoma, young women, and never smokers (Lynch et al., 2004; Shaw et al., 2009; Li et al., 2013; Wang et al., 2014b). In concordance with previous reports, *EGFR* mutations were identified at high frequencies in never-smokers (P = 0.000) and females (P = 0.000). The frequency of *EGFR* mutation in tumors from Asian females in the PIONEER study was 61% and from never-smokers was 60.7% (Shi et al., 2014), and in our study, the percentages of *EGFR* mutation were 55.9% (57/102) and 72.5% (74/102) in women and nonsmoker patients, respectively. Notably, *EGFR* mutation was frequently identified in well-moderately differentiated adenocarcinoma, in contrast to the findings for *ALK* rearrangements, which were more common in moderate-poorly differentiated adenocarcinoma.

We found correlations between metastasis factors (distant lymph node, brain, bone, and liver metastasis) and *EGFR* mutation, probably due to the relatively greater invasiveness of adenocarcinomas with *EGFR* mutations. However, we were unable to identify an association between *EGFR* mutation status and disease stage or local nodal involvement. Another factor that did not significantly correlate with *EGFR* mutation frequency was age. Some previous studies had shown that patients with *EGFR* mutations were older than those without *EGFR* mutations (Ueno et al., 2012), while others found no significant association (Shi et al., 2014; Tokumo et al., 2015); our study is consistent with the latter.

After stratifying the *EGFR* mutations in exons 19 and 21, we found that the mutations in exons 19 or 21 did not correlate with female gender and never/light-smoking status. Our result is not consistent with other studies of the characteristics of adenocarcinoma and *EGFR* mutation type (Tiseo et al., 2011; Santelmo et al., 2013), which suggested that the association between *EGFR* and female never-smokers might be limited to *EGFR* mutations in exon 21.

Most studies on the relationship between *EGFR* mutations and response to EGFR-TKIs show a single site mutation. The deletion in exon 19 and the L858R substitution in exon 21 constitute the most frequent mutations. Tumors expressing one of these mutations are known to show good response to TKIs (Pao et al., 2004; Kim et al., 2014). Two or more concomitant sites of *EGFR* mutations have also been detected despite the low patient number. Chen et al. (2008) showed that the frequency of double *EGFR* mutations is around 6% among all *EGFR* mutations of lung cancer, and concomitant *EGFR* mutations have been termed “complex mutations.” Patients with complex *EGFR* mutations (excluding those with T790M) with the classical mutation pattern had a better response, longer survival time, and longer progression-free survival time after EGFR-TKI therapy than did those without the classical mutation pattern (Wu et al., 2008). In our study, we identified three patients (2.9%) with complex *EGFR* mutations, and all received the first-line treatment of EGFR-TKIs (two of erlotinib, and one of gefitinib) and achieved partial response; the longest follow-up time was one and a half years. Our study supports the conclusion that EGFR-TKIs might still be the best choice of treatment for patients with complex mutations (Wu et al., 2008).

EML4-ALK and other oncogenic drivers such as mutant *EGFR* and oncogenic *KRAS* are generally mutually exclusive. The coexistence of *EGFR* and *ALK-EML4* gene mutations represents a rare event (about 1%) in patients with NSCLC (Yang et al., 2011; Yang et al., 2014). In our study, the overall frequency of concomitant *EGFR* mutations and *ALK* rearrangements was 1.3% (3/222) and 2.9% (3/102) in patients with *EGFR* mutations, and 12.5% (3/24) in patients with *ALK* rearrangements. These data indicate that driver alterations of *EGFR* and *ALK* could coexist in a small group of patients with NSCLC, and more frequently in ALK-positive tumors, which is consistent with previous studies (Yang et al., 2011; Yang et al., 2014).

In limited clinical series of patients reported to have both alterations, isolated responses to EGFR-TKIs (erlotinib or gefitinib) and ALK-TKI (crizotinib) have been described. Several case reports indicated that the presence of concomitant *EGFR* mutations and *ALK* rearrangements might be associated with mutual resistance to single-agent crizotinib or EGFR-TKIs (Shaw et al., 2009; Sasaki et al., 2011; Tiseo et al., 2011; Tanaka et al., 2012). Other studies reported good response to EGFR-TKIs (Wang et al., 2012; Santelmo et al., 2013; Yang et al., 2014) or crizotinib (Chen et al., 2013; Yang et al., 2014). According to a previous study, the objective response rate (80%) for first-line EGFR-TKIs in *EGFR/ALK* co-altered tumors was similar to that in patients with pure *EGFR* mutations (Yang et al., 2014). In our study, two of the three patients with concomitant *EGFR* mutations and *ALK* rearrangements received the first-line treatment of EGFR-TKI (erlotinib), and one achieved partial response, while the other achieved stable disease; to date, the third has shown no progress. However, the longest follow-up time between the two patients is nearly 5 months. Our data also provided a clue for the treatment of patients with the coexistence of *EGFR* mutations and *ALK* rearrangements; however, further large investigations into the efficacy of first-line, sequential, or combination treatment requires more evidence in this specific subgroup.

In conclusion, *ALK* rearrangements were studied in a large unselected sample collection of patients with NSCLC. An occurrence of 9.6% for *ALK* rearrangements was identified, which was associated with a younger age, female gender, and non-smoker status, but no obvious relationship with histology was identified, which is in contrast with the results reported by other studies. In the era of ALK or EGFR-targeted inhibitors, the selective enrichment of patients with NSCLC according to their clinicopathologic features could efficiently screen mutation candidates for molecular targeted therapy. However, our study has several limitations. First, this is a single-institution study and we enrolled patients with NSCLC in our hospital only. We believe these unselected patients are fairly representative of patients with NSCLC across different pathological types. We did not clarify the outcome of therapy with crizotinib or chemotherapy or other therapeutic strategies for patients with NSCLC harboring *ALK* rearrangements, including patients with double genetic aberrations of *ALK* and *EGFR*. Larger studies are required to address these issues.

Conflicts of interest

The authors declare no conflict of interest.

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