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Preexisting interstitial lung disease is inversely correlated to tumor epidermal growth factor receptor mutation in patients with lung adenocarcinoma

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ABSTRACT

Introduction: Interstitial lung disease (ILD), especially idiopathic pulmonary fibrosis, has been shown to be associated with lung carcinogenesis. However, an association between epidermal growth factor receptor (*EGFR*) mutation status and preexisting ILD in patients with lung adenocarcinoma is unknown.
Methods: Between January 2008 and April 2012, we analyzed 602 patients with lung adenocarcinoma. *EGFR* mutation status was analyzed using the peptide nucleic acid-locked nucleic acid polymerase chain reaction clamp method, and preexisting ILD was diagnosed based on clinical features, chest high-resolution computed tomography (HRCT) findings, and histological findings.
Results: There were 555 patients with pulmonary adenocarcinoma with tumor *EGFR* mutation data available for analysis. Of them, 31 patients (6%) had preexisting ILD, and *EGFR* mutations were detected in 246 of the 555 patients (46%). In the comparison between patients with *EGFR* mutations and those with wild-type *EGFR*, there was a significant inverse association between occurrence of tumors with *EGFR* mutations and ILD (1/246 vs. 30/309, $P < 0.001$). Based on the multivariate analysis of age, gender, smoking status, Eastern Cooperative Oncology Group Performance Status, stage, and ILD, *EGFR* mutations were found to be independently associated with females (OR, 1.58; 95% CI, 1.01–2.46; $P = 0.048$), never-smokers (OR, 3.31; 95% CI, 2.12–5.20; $P < 0.001$), and the absence of ILD (OR, 17.41; 95% CI, 3.54–315.34; $P < 0.001$).
Conclusions: This study showed that patients with pulmonary adenocarcinoma and ILD had a lower probability of carrying tumor *EGFR* mutations.

1. Introduction

Epidermal growth factor receptor tyrosine kinase inhibitors (EGFR-TKIs) are well-established therapy for the treatment of non-small cell lung cancer (NSCLC) [1]. Since patients of NSCLC with somatic activating mutations of the *EGFR* gene (*EGFR* mutations) generally respond to *EGFR* tyrosine kinase inhibitors (gefitinib or erlotinib) and can achieve long progression-free survival, the presence of *EGFR* mutations is a very useful marker for facilitating the choice of treatment for NSCLC [2–4]. Several clinicopathologic factors have been identified to be related to the frequency of *EGFR* mutations, including adenocarcinoma histology, female gender, never-smoker status, and East Asian ethnicity [5,6].

Interstitial lung disease (ILD) is characterized by damage to the lung parenchyma by varying patterns of inflammation and fibrosis [7]. ILD, especially idiopathic pulmonary fibrosis (IPF), has been shown to be associated with lung carcinogenesis [8]. This is partly because inflammation and fibrosis may induce genetic damage, which leads to carcinogenesis of the pulmonary parenchymal tissue [9,10]. Cigarette smoking, which is known to be a negative predictive factor for tumors with activating *EGFR* mutations, also induces inflammation and lung cell damage and is associated with ILD, chronic obstructive pulmonary disease (COPD), and lung carcinogenesis [7,8,11,12]. From previous reports, the pathogenic features of ILD itself appear to be similar to carcinogenesis related to cigarette smoking in several aspects, such as oxidative stress, mutagenesis, angiogenesis, and epithelial to mesenchymal transformation [12–14]. In addition, these similarities have been also

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reported in the pathogenesis of COPD and IPF [15]. Therefore, we hypothesized that there was a negative association between occurrence of tumors with *EGFR* mutations and preexisting ILD, which was independent of the other established predictive factors. In this study, we analyzed patients for preexisting ILD and *EGFR* status.

2. Patients and methods

2.1. Patients

Between January 2008 and April 2012, we analyzed 602 patients with lung adenocarcinoma at Kobe City Medical Center General Hospital. Results were analyzed retrospectively using case and radiographic records. Patients who reported never smoking in their lifetime were defined as never smokers, those who had smoked within 1 year of the diagnosis were categorized as current smokers, and the rest were considered to be former smokers. All chart reviews were approved by the Ethics Committee of Kobe City Medical Center General Hospital.

2.2. Analysis of EGFR, KRAS and anaplastic lymphoma kinase (ALK) rearrangements

We isolated tumor DNA from various specimens, and *EGFR* mutation status at exons 18–21 was analyzed using the peptide nucleic acid-locked nucleic acid polymerase chain reaction clamp method, as previously reported [16]. Specific mutations in *KRAS* exon 2 (codons 12/13) were identified as described previously [17,18].

To identify *ALK* rearrangements, immunohistochemistry (IHC) for *ALK* expression was performed. We used the 5A4 antibody with a highly sensitive detection system (Envision FLEX+ system) as described previously [19].

2.3. Interstitial lung disease (ILD)

Preexisting ILD was diagnosed based on clinical features, pretreatment chest high-resolution computed tomography (HRCT) results, and histological findings. All patients had received HRCT as a clinical practice and the presence of ILD was evaluated by at least 2 pulmonologists who did not know the *EGFR* mutation status. For patients who had undergone surgical treatment for lung cancer, the surgical lung biopsy was used to verify the clinicopathologic diagnosis of ILD. On the basis of the clinical features and laboratory testing, we classified ILD into two groups, idiopathic interstitial pneumonia (IIP) and non-IIP. The latter included collagen vascular disease-associated ILD, occupational lung diseases, and others. For the diagnosis of IIP, we used the American Thoracic Society/European Respiratory Society consensus classification [7]. From the histological and/or HRCT findings, ILD was classified into usual interstitial pneumonia (UIP) pattern or non-UIP pattern (Fig. 1). For all the patients with ILD and lung adenocarcinoma, the chest CT was re-examined, and the patients were divided into two groups according to the location of the cancerous and the ILD lesions: the tumor was located in the ILD area or developed in the non-ILD area (Fig. 1).

2.4. Statistical analysis

Continuous variables were analyzed using Student's *t*-test, and the results are expressed as mean ± standard deviation (SD). Dichotomous variables were analyzed using chi-square test or Fisher's exact test, as appropriate. The relationship between numerical and categorical variables was compared using Wilcoxon signed-rank test. Univariate and multivariate logistic regression models were applied to estimate odds ratios (OR) and 95% confidence interval (CI). All tests were two-tailed, and *P*-values of <0.05 were considered statistically significant. All statistical analyses were performed using the JMP 9 software (SAS Institute, Cary, NC, USA).

3. Results

Of the 602 patients analyzed, 47 were excluded from the study because their *EGFR* mutation status was not investigated: 31 of them did not consent, 14 patients declined cancer treatment, and 2 were excluded due to insufficient amount of tissue for analysis. Of these 47 excluded patients, three had lung adenocarcinoma and ILD.

The clinical characteristics of the 555 patients are summarized in Table 1. All patients were Japanese, including 308 males (56%) and 247 females (44%), with a median age of 69 years (range, 35–90 years). Two hundred and sixty-seven patients (48%) were never-smokers, and 288 patients (52%) were current- or former-smokers. Most patients (84%) had an Eastern Cooperative Oncology Group Performance Status (ECOG PS) of 0 or 1. According to the 7th edition TNM classification, all patients were classified on the basis of clinical stage [20]; almost half of the patients (44%) had stage IV. *EGFR* mutations were detected in 245 of the 555 patients (44%). Tumors from 10 patients harbored double *EGFR* mutations.

Of the 555 patients, 31 (6%) had preexisting ILD. Patient characteristics and comparison between cases with and without ILD are summarized in Table 2. In ILD patients, 11 patients (35%) were diagnosed on the basis of pretreatment HRCT and surgical lung biopsy, and 20 (65%) on the basis of pretreatment HRCT alone. Seventeen patients (55%) were identified with UIP pattern, and 14 (45%) had non-UIP pattern. Most patients (24/31, 77%) had a tumor in the ILD area. Of the 31 ILD cases, only one patient (3%) had a

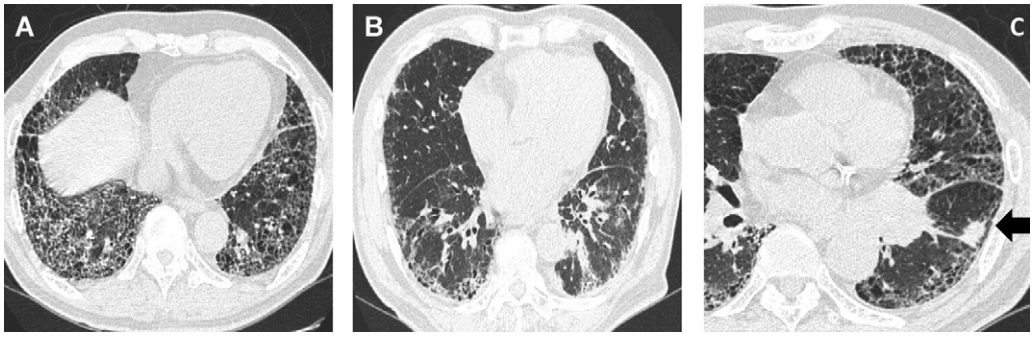

Fig. 1. Chest high-resolution computed tomography (HRCT) image of the chest. (A) HRCT scan showing subpleural distribution, honeycomb cysts, and bronchiectasis (UIP pattern). (B) HRCT scan showing patchy ground glass opacity with reticulation, traction bronchiectasis, and bronchovascular bundle thickening (non-UIP pattern). (C) HRCT scan showing a tumor (arrow) in the ILD area (UIP pattern).