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High frequency of mutation of epidermal growth factor receptor in lung adenocarcinoma in Thailand

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Abstract

Recent reports have suggested influences of racial difference on the frequency of mutation of *EGFR* in lung cancer. We therefore sought to characterize the frequency and pattern of mutation of *EGFR* in lung adenocarcinoma in Thai patients. Overall, *EGFR* catalytic domain mutations were detected in 35/61 (57.4%). We found 29/60 (48.3%) of exon 19 deletions, 5/54 (9.3%) of exon 21 point mutations, and 1/54 (1.9%) of double-mutation of both exons. The presence of these mutations was significantly associated with non-smoking habit. In summary, we report a strikingly high prevalence of mutation of *EGFR* in Thai lung adenocarcinoma, which may explain the high response rate to the treatment with TKI among Asian populations.

Keywords: EGFR; Adenocarcinoma; Lung cancer; Mutation; Tyrosine kinase inhibitor; Gefitinib

1. Introduction

EGFR is an important membrane receptor protein, which plays crucial roles in multiple cellular functions. Binding of one of its ligands to the extracellular domain leads to phosphorylation and activation of EGFR. Activated EGFR then conveys signals to the downstream targets, which participate in multiple cellular processes involving cell proliferation, differentiation, migration, and survival [1]. Deregulation of EGFR signaling either by overexpression or amplification of the EGFR has been described in many cancers. This supports the idea that the functions of EGFR are critical to abnormal tumor growth and metastasis [2,3]. Also in line with these

Abbreviations used EGFR, *epidermal growth factor receptor*; *NSCLC*, *non small cell lung cancer*; *TKI*, *tyrosine kinase inhibitor*; *bp*, *base pair*.

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notions, recent developments of molecularly targeted therapy for cancer have been focusing on blocking critical molecules that determine malignant phenotypes in EGFR pathways in many types of malignancy that are difficult to be treated, including non-small cell lung cancer (NSCLC) [4]. Several reports indicate that NSCLC utilizes EGFR pathways, which could be demonstrated either by over-expression or amplification, detected in more than 80% of the tumors [5]. Two recent multi-institutional phase II trials showed certain clinical benefits but only in a small number of advanced and chemotherapy refractory NSCLC patients who were treated with a tyrosine kinase chemical inhibitor (TKI), gefitinib [6,7]. The objective response rates in these studies were differentially demonstrated in NSCLC according to their ethnicity, namely, 9–12% in Caucasian [6] versus 18.4–19.0% in Japanese patients [7]. Additional experiences with currently available TKIs, gefitinib and erlotinib, consistently show an association between the responses and certain clinical parameters like the female gender, adenocarcinoma, and non-smoking habit [8–11]. However, molecular predictors of the response are needed for better clinical guidance and insight in the biology of this highly malignant neoplasm related to the response to TKIs.

Several attempts to identify other potential predictor of the response like the expression pattern of EGFR did not clearly demonstrate a significant correlation between EGFR expression and the response to treatment with TKIs [12,13]. Recently, a few studies have identified a convincing molecular marker that predicts the response to TKIs of NSCLC, the mutation of the *EGFR* catalytic domain [14–16]. With systematic mutation analyses, the majority of mutations were detected exclusively within the tyrosine kinase domain of EGFR encoded in exon 18 to 21 and only in lung adenocarcinoma. Characteristically, 88% of the reported mutations are in-frame deletions of exon 19 and point mutations of exon 21, which lead to functioning mutated EGFR proteins [16]. Almost all patients who showed the responses to TKI harbored EGFR mutations in the tumor cells whereas mutations were rarely detected in known TKI resistant patients. The prevalence of mutations coincided well to the described clinical predictors of the response to TKIs including racial background, the female gender, adenocarcinoma, and non-smoking habit [14,15]. The higher number of Japanese patients who governed mutations of *EGFR* in these studies raises an important question: whether or not NSCLC patients with Asian ethnicity follow a similar pattern and prevalence of *EGFR* mutation.

Our experience with a compassionate expanded access program of gefitinib treatment in Thailand showed an overall objective response rate of 20% reminiscent to those of Japanese patients (N.V. and P.L. unpublished observations). The data prompted us to investigate the prevalence and pattern of *EGFR* mutation in Thai NSCLC patients. Hence, we chose to screen for exon 19 and exon 21 mutation of *EGFR* in lung adenocarcinoma patients according to the previous findings that indicated clustering of mutations in these specific regions of the *EGFR* gene and the tumor histology. In principle, the association between the EGFR mutations and the clinical response were demonstrated in a limited set of patients previously treated with gefitinib with known responses. We observed a strikingly high rate of mutations in our patients. In addition, a new pattern of exon 19 mutation, 9-base deletion, and double mutations of both exons were detected in our study.

2. Materials and methods

2.1. NSCLC tissue sample

The samples were paraffin-embedded tissue samples of lung adenocarcinomas taken from the archieve of the Department of Pathology, Faculty of Medicine, Chulalongkorn University, Thailand. The study was approved by the Ethics Committee of the Faculty of Medicine, Chulalongkorn University. All samples were reviewed and marked by a pathologist (C.C.) for an area, approximately 3–8 mm at the greatest diameter which contained more than 70% of the tumor cells. The first panel comprised of five samples with prior treatment with gefitinib and known response. Additional 56 samples were screened for the mutation of *EGFR* gene.

2.2. Mutation analysis

Tumor samples were mapped with hematoxylin-eosin sections, manually microdissected with needle, and subjected to DNA isolation as previously