Different Responses to Gefitinib in Lung Adenocarcinoma Coexpressing Mutant- and Wild-Type Epidermal Growth Factor Receptor Genes

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Response to gefitinib is strongly associated with the status of the epidermal growth factor receptor gene. Here we report the different treatment responses in a case of lung adenocarcinoma coexpressing mutant-type gene in the primary lung mass and a wild-type gene in the metastatic bone lesions. This case demonstrated that at least two strains of tumor cells were present in a single patient. This may be one of the mechanisms of gefitinib resistance.

Key words: lung cancer (diagnosis & staging, translational oncology – gene profiling, translational oncology – signal transduction, EGFR mutation)

INTRODUCTION

Mutation of the epidermal growth factor receptor (EGFR) tyrosine kinase domain leads to autophosphorylation of tyrosine kinase, which in turn activates the intracellular signal transduction cascades, mainly mitogen-activated protein kinase (MAPK pathway), phosphoinositol 3 kinase (PI3K-AKT pathway) and STAT pathway (1). Gefitinib (AstraZeneCa Inc, UK) selectively binds to ATP-binding site of EGFR, thus inhibiting autophosphorylation of tyrosine kinase. Lynch et al. have shown consistent gene mutations of exons 18–21 in the gefitinib responders, while no mutation existed in the non-responders (2). Paez et al. (3) confirmed the same EGFR-mutation points with Lynch study at the same time.

We previously (4) showed that mutation rate is much higher (55%) in Chinese patients with lung adenocarcinoma than that in Western patients (6–10%) (5). The positive predicting rate of treatment response was 87% and the negative predicting rate was 75%. Subsequent reports from Italy (5) and Korea (6) confirmed the high predictive value of EGFR mutation and clinical response of gefitinib. The most common mutations were exon 19 deletion (LREA) and exon 21 point mutation (L858R).

Why do mutations in the EGFR tyrosine kinase domain predict for the responsiveness to gefitinib? There may be at least two potential explanations. The first is that mutation may result in conformational changes near the ATP-binding site; which is also the site of gefitinib binding (2). A second theory proposed by Settleman et al. suggested that mutation receptors preferentially signal through anti-apoptotic pathways including AKT and STAT pathway. Blocking mutant EGFR leads to rapid apoptosis of tumor cells (7).

Here we report the differential responses to gefitinib in a patient with lung adenocarcinoma coexpressing mutant-type EGFR gene in the primary lung mass and a wild-type gene in the metastatic bone lesions.

CASE REPORT

A 59-year-old non-smoker female presented with a 2 month history of low back pain and weight loss on 22 November 2001. Physical examination disclosed multiple tender points at lumbar spines, thoracic spines, bilateral sacro-iliac joints, right humeral head and rib cage. Hemogram showed mild normocytic anemia. Biochemical tests showed normal transaminase, creatinine and calcium levels; carcinoembryonic antigen (CEA) = 414 ng/ml. Chest X-ray showed a 2 cm left upper lung mass. Chest computed tomography (CT) revealed a 2 cm mass (Fig. 1a). Bone scan disclosed multiple bone...
metastases. CT-guided biopsy on 28 November 2001 of the lung mass disclosed an adenocarcinoma. She received systemic chemotherapy with 1000 mg/m² gemcitabine on days 1, 8 and 15 plus 80 mg/m² cisplatin on day 15 every 28 days following lumbar spine irradiation. Because of the radiation dermatitis (recall phenomenon), gemcitabine was temporarily substituted by vinorelbine 25 mg/m² on days 1, 8 and 15 in the 2nd cycle. After 3 cycles of chemotherapy, her disease progressed in both lungs and bones. Magnetic resonance imaging (MRI) disclosed cervical (C)-2, -3, -6 vertebral body signal enhancement as well as C-2, -6 dural sac and cord compressions by the metastatic lesions (Fig. 2a). She underwent laminectomy and vertebroplasty for the C-spine metastases on 5 February 2002. Pathological examination of the C-spine specimen disclosed metastatic adenocarcinoma. CEA increased progressively to 940 ng/ml on 22 July 2002. She was subsequently treated with gefitinib 250 mg QD on 10 August 2002 according to a protocol approved by the institutional review board of Chang Gung Memorial Hospital in Taiwan. Chest CT scan evaluation of the response 3 months following gefitinib treatment showed a >30% reduction of the diameter of the lung mass (Fig. 1b). CEA decreased to 109 ng/ml in a month. However, her bone pain did not improve. MRI of the C-spine showed new signal-enhanced lesions (Fig. 2b). The lung mass disappeared completely following further gefitinib treatment. Unfortunately, her bone metastases progressed. This patient expired 13 months after gefitinib treatment.

For mutational analysis of the kinase domain of EGFR-coding sequence, we analyzed the lung specimen obtained during diagnostic procedures and bone specimen obtained at the time when she underwent C-spine surgery. Tissue sections were obtained and stained with hematoxylin and eosin first to examine the tumor proportion of the tissue. Only the tumor portion was dissected from the unstained tissue section slides and sent for DNA extraction performed with DEXPAT reagent (Takara Biomedical, Japan) following the instruction of the manufacture. The tyrosine kinase domain of EGFR-coding sequence, exons 18, 19, 20 and 21 were amplified with four pairs of primers specific to the flanking sequences of individual exons and PCR amplificons were subjected to direct sequencing (the PCR primers and amplification procedures). Forward and reverse sequencing reactions were performed using the same primers for PCR amplification and ABI BigDye Terminator kit v3.1 (Applied Biosystems, CA, USA) according to manufacturer’s instructions. Sequencing reactions were electrophoresed on an ABI3700 genetic analyzer. Sequence variations were determined by using Seqcape software (Applied Biosystems) with the EGFR reference sequence (NM_005228.3, NCBI). All sequence variations were confirmed by multiple independent PCR amplifications and repeated sequencing reactions. This patient achieved a complete response to gefitinib treatment in the lung mass expressing A839T mutation (Fig. 3). The C-spine metastasis expressing wild-type EGFR did not respond to gefitinib treatment.

**DISCUSSION**

Gefitinib as second-line therapy for advanced non-small-cell lung cancer (NSCLC) after cisplatin-based regimen failure leads to 8.8–19.0% clinical response, with median survival of 6–8 months (8,9). Erlotinib also achieved 9–12.3% response rate and 6.7–8.4 months median survival in the same setting (10,11). These were more effective and less toxic than Docetaxel monotherapy as shown in historical comparisons (12). Unfortunately, combination of either gefitinib or erlotinib with chemotherapy, compared with chemotherapy alone, did not improve either response rate or overall survival as first line therapy for advanced NSCLC (13–16). Clinical practice with EGFR tyrosine kinase inhibitor (TKI) in NSCLC observes around 10–15% of patients achieve remarkable clinical benefit with dramatic shrinkage of tumor and improvement of quality of life. Several trials had concluded that non-smokers, women, histological type with adenocarcinoma and patients of Asian heritage have a better outcome with EGFR TKI treatment (9,17,18). Overexpression of EGFR by immunohistochemical staining did not predict clinical response (19).

Lynch et al. (2) amplify EGFR 28 exons in nine TKI-sensitive NSCLC specimens. They found consistent gene

![Figure 1](image-url). Chest CT scans before treatment (a) and after 3 months treatment of gefinitib (b). (a) A 2 cm mass was observed in the left upper lobe of the lung. (b) More than 30% reduction of the diameter was obtained.
mutations of exons 18–21 in the TKI responders, while no mutation was observed in non-responders. Approximately 80–90% of EGFR mutations in the tyrosine kinase domain are exon 19 deletion or exon 21 missense mutations. All EGFR mutations occur around the ATP-binding domain, leading to higher affinity TKI binding than the wild-type EGFR. He also reported that EGFR mutations were observed predominantly in adenocarcinoma histological type, non-smoker, female gender and East Asian patients. Paez et al. (3) confirmed the same EGFR-mutation points with Lynch study at the same time. EGFR mutations have the highest incidence in East Asian patients with adenocarcinoma histological type, with around 57% incidence compared with <10% incidence in Caucasians.

The patterns of EGFR mutation show a striking correlation with anti-tumor response to gefitinib. Lung adenocarcinoma with substitution mutation of L858R and deletion within codons 746–753 of EGFR are particularly sensitive to gefitinib treatment (2,3). These sequence alterations cluster around the active site of the EGFR kinase (activation loop and glycine-rich P loop), which is important for autoregulation in protein kinase (20). Patients with EGFR mutations other than L858R and 746–753 deletion had also been reported responsive to gefitinib (4).

The position of A839 is in the activation loop of EGFR tyrosine kinase, which is located between L836 and T845 (21). Although there were no biological studies of A839T mutation cell lines with regard to gefitinib treatment, we demonstrated the anti-tumor responsiveness to gefitinib in the lung tumor specimen bearing A839T in this case report. To the best of our knowledge, this is the first report showing such new mutation in the gefitinib responder. The conformational change in the EGFR-TK domain of A839T mutation may explain gefitinib responsiveness in this case.

Marchett et al. (5) examined 375 lung adenocarcinoma specimens for EGFR and k-ras mutation. He observed all of the 39 specimens with EGFR mutation did not have k-ras mutation, while two-third of 339 patients without EGFR mutation expressed k-ras mutation. He concluded EGFR mutation and k-ras mutations are mutually exclusive. Baselga et al. (22) presented a hypothesis that some carcinogens such as those that result from smoking lead to carcinogenesis by activating AKT.

![Figure 2](image-url)  
**Figure 2.** MRI before treatment (a) and after 3 months treatment of gefitinib (b). (a) C-2, -3, -6 vertebral body signal enhancement was observed as well as C-2, -6 dural sac and cord compressions by the metastatic lesions. (b) C-spine showed new signal-enhanced lesions.

![Figure 3](image-url)  
**Figure 3.** This patient achieved a partial response to gefitinib treatment in the lung mass expressing A839T mutation (a) and the C-spine metastasis expressing wild-type EGFR (b) did not respond to gefitinib treatment.
pathway via k-ras mutation, while other carcinogens stimulate carcinogenesis via MAPK and STAT pathway by inducing EGFR mutation.

Pao et al. (23) showed that in two out of five patients with acquired resistance to gefitinib or erlotinib, progressing tumors contain, in addition to a primary drug-sensitive mutation in EGFR, a secondary mutation in exon 20, which leads to substitution of methionine for threonine at position 790 (T790M) in the kinase domain.

Kobayashi et al. (24) reported the case of a patient possessing EGFR mutation with gefitinib-responsive, advanced NSCLC who had a relapse after 2 years of complete remission during treatment with gefitinib. The DNA sequence of the EGFR gene in his tumor biopsy specimen at relapse revealed the presence of a second point mutation, resulting in the same mutation of EGFR as Pao’s report. Structural modeling and biochemical studies showed that this second mutation led to gefitinib resistance.

We demonstrated that both mutant- and wild-type EGFRs were present in a patient with adenocarcinoma. It disclosed heterogeneity of the tumor cells. Because of the ability of self-activating the intracellular signal transduction cascades, the EGFR-mutation cells may be the predominant cells before TKI therapy. When the tumor cells bearing mutant EGFR underwent apoptosis soon after TKI therapy, the wild-type tumor cells became the predominant strain. This may be one of the mechanisms of TKI resistance.

We conclude that the TKI did work for the mutant EGFR tumor as was demonstrated in the primary lung tumor of this case and the resistance of TKI was caused by the heterogeneity of the tumor cells. In patients with NSCLC expressing EGFR mutations, it is reasonable to give TKI as the main therapy. Further trial of combination therapies of TKI with other therapeutically modalities to overcome the heterogeneity of tumor cells is warranted.

References