Somatic mutations in epidermal growth factor receptor underlying complete responsiveness to gefitinib in a Taiwanese female patient with metastatic adenocarcinoma of lung

Kun-Huei Yeh[a–d], Shiou-Hwei Yeh[e], Joeu-Pei Wan[b,e], Ying-Chun Shen[a,b] and Ann-Lii Cheng[c,d]

A 61-year-old never-smoker female suffered from adenocarcinoma of the lung with chest wall invasion and peri-adrenal lymph node metastases. After palliative resection of all clinically detectable primary and metastatic adenocarcinoma, she received cisplatin and gemcitabine combination chemotherapy for a total of 3 cycles. New metastatic lesions were found in spleen and para-aortic lymph nodes. Her tumor tissue was subjected to mutation analysis for epidermal growth factor receptor (EGFR) and had been shown to have a T→G missense mutation in nucleotide 2819 of EGFR full-length cDNA (accession no. NM_005228.3), within exon 21 of the EGFR gene, resulting in amino acid substitutions from leucine to arginine at codon 858 (L858R) as expected. She received oral gefitinib 250 mg/day after mutation analysis. She had a very good tumor response with more than 90% tumor reduction shown by abdominal computed tomographic scan, normalization of previously elevated carcinoembryonic antigen level, and complete resolution of previous uptake signals in spleen and para-aortic lymph nodes shown by positron emission tomographic scan. She has been kept in clinical complete remission for 11+ months after the initiation of gefitinib treatment. Our patient supports the proposition that somatic mutation L858R in exon 21 of the EGFR gene accounts for complete responsiveness to gefitinib in a Taiwanese female patient with metastatic adenocarcinoma of lung. *Anti-Cancer Drugs* 16:739–742 © 2005 Lippincott Williams & Wilkins.

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[a]Division of Oncology and Hematology, Department of Internal Medicine, [b]Deparment of Medical Research, Far Eastern Memorial Hospital, Taipei, Taiwan, [c]Department of Oncology, National Taiwan University Hospital, Taipei, Taiwan, [d]Cancer Research Center and [e]Department of Microbiology, National Taiwan University College of Medicine, Taipei, Taiwan.

Correspondence to K.-H. Yeh, Division of Oncology and Hematology, Department of Internal Medicine, Far Eastern Memorial Hospital, No. 21, Section 2, Nan-Ya S. Road, Ban-Ciao, Taipei 220, Taiwan.
Tel.: +886 2 89667000; fax: +886 2 89668505;
e-mail: khyeh@mail.femh.org.tw

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Introduction

Non-small-cell lung cancer (NSCLC) is the leading cause of death from cancer worldwide, and the second leading cause of death from cancer in Taiwan. At the cost of clinically significant adverse effects, chemotherapy slightly prolongs survival among patients with advanced disease [1].

Overexpression of epidermal growth factor receptor (EGFR) and its ligands in many human epithelial cancers and their association with tumor progression and poor prognosis provided a rationale for targeting this signaling pathway as a treatment strategy [2]. Clinical studies using monoclonal antibody blockade [such as cetuximab (Erbitux), etc.] and EGFR tyrosine kinase inhibitors (TKIs) [such as gefitinib (Iressa), erlotinib (Tarceva), etc.] suggest that EGFR signaling blockade is a well-tolerated and effective treatment strategy [3]. Preclinical and early clinical studies suggest that both approaches cause little toxicity to the tumor-bearing host [2,3].

EGFR is overexpressed in 40–80% of NSCLC and many other epithelial cancers [4]. EGFR requires activation by binding of ligands (such as epidermal growth factor [EGF]) to its extracellular domain, whereas its cellular effects depend on activation of its cytoplasmic tyrosine kinase (TK) by homodimerization of EGFR molecules or heterodimerization with other closely related receptors (such as HER2/*neu*). Autophosphorylation and transphosphorylation of the receptors through their TK domains leads to the recruitment of downstream effectors, and the activation of proliferative and survival signals [5]. Gefitinib or erlotinib target the ATP cleft within the TK of EGFR [6].

Initially, in a randomized, double-blind, parallel-group, multicenter phase II trial conducted for pre-treated advanced NSCLC patients, efficacy for gefitinib was similar, with objective tumor response rates of 18.4% [95% confidence interval (CI) 11.5–27.3) and 19.0% (95% CI 12.1–27.9), and with median overall survival times of

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7.6 and 8.0 months for the groups treated with 250 and 500 mg/day gefitinib, respectively. At 250 mg/day, gefitinib had a favorable profile of adverse effects [7]. In the US, most patients with NSCLC have no response to the TKI gefitinib; however, only about 10% of patients have a rapid and often dramatic clinical response. The molecular mechanisms underlying responsiveness to gefitinib were unknown until 2004 [8–10].

Lynch *et al.* reported somatic mutations with either small, in-frame deletions or amino acid substitutions clustered around the ATP-binding pocket of the TK domain in the EGFR gene [8]. Somatic mutations were identified in the TK domain of the EGFR gene in eight of nine patients with gefitinib-responsive lung cancer, as compared with none of the seven patients with no response ($p < 0.001$) [8]. *In vitro*, EGFR mutants demonstrated enhanced TK activity in response to EGF and increased sensitivity to inhibition by gefitinib [8]. At the same time, similar somatic mutations of the EGFR gene were found in 15 of 58 unselected tumors from Japan and one of 61 from the US [9]. Treatment with gefitinib causes tumor regression in some patients with NSCLC, more frequently in Japan [9]. These results suggest that EGFR somatic mutations may predict sensitivity to gefitinib and screening for such mutations in lung cancers may identify patients who will have a response to gefitinib.

The somatic mutations of EGFR genes are much more frequently seen in Japan—up to 25.8% [9]. Among the East Asian countries, we report here one Taiwanese female patient with metastatic adenocarcinoma of lung who has the same activating somatic mutations (L858R) in exon 21 of the EGFR gene underlying complete responsiveness to gefitinib.

Case presentation

A 61-year-old female suffered from chronic cough for 3 months. She was a never-smoker, led an uneventful life in the past and denied having major systemic diseases. In February 2004, she was diagnosed as having a nodule 2.5×2.0 cm in size at the left upper lung field shown by chest roentgenogram and computed tomographic (CT) scan. Staging work-ups for her lung tumor revealed chest wall invasion and left adrenal metastasis. She received the palliative, minimally invasive video-assisted thoracoscopic (VATS) wedge resection of a left upper lung nodule, excision of the chest wall mass and laparoscopic resection of adrenal metastasis at the Far Eastern Memorial Hospital with informed consent. The surgical specimen from the wedge resection of the left upper lung consisted of a tissue fragment measuring $8.0 \times 3.0 \times 2.0$ cm in size. On the cut surface, a whitish, firm nodule measuring $2.5 \times 2.0 \times 1.8$ cm in size was noted. Microscopically, the resected tissue from the left upper lobe of lung showed adenocarcinoma mixed with a bronchoalveolar, tubular and focal solid growth pattern. Lymphovascular permeation was discernible. Section margins were close to tumors. Microscopically, the resected tissue from the laparoscopic left adrenalectomy showed adenocarcinoma to the peri-adrenal lymph nodes and the resected tissue from the chest wall also showed metastatic adenocarcinoma. Pathological diagnosis was adenocarcinoma of the lung with chest wall and peri-adrenal lymph node metastases.

After resection of all clinically detectable primary and metastatic adenocarcinoma, she received cisplatin and gemcitabine combination chemotherapy (cisplatin 60 mg/m^2, 3-h i.v. infusion, day 1, and gemcitabine 1000 mg/m^2, 30-min i.v. infusion, days 1, 8, 15, repeated every 4 weeks) for a total of 3 cycles from March to May 2004. New metastatic lesions were found in the spleen and para-aortic lymph nodes by CT scan and whole-body positron emission tomographic (PET) scan using i.v. injection of [^{18}F]2-fluoro-2-deoxy-D-glucose (FDG).

Under the patient's informed consent, her tumor tissue was subjected to mutation analysis for EGFR using the methods reported by Lynch *et al.* [8] and briefly described in the next section.

Methods for polymerase chain reaction (PCR) and DNA sequencing

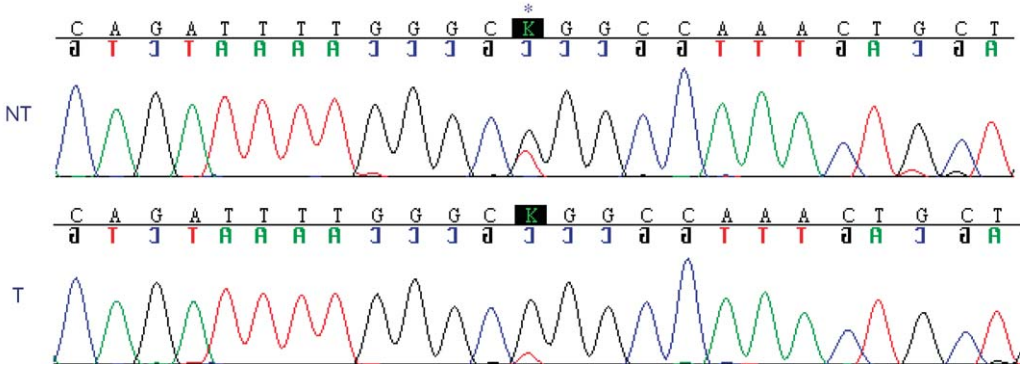
PCR was used to amplify the exons 19 and 21 comprising the EGFR gene using DNA isolated from microdissected tumor tissue, surrounding non-tumor tissue and mononuclear cells from peripheral blood.

Primer pairs used were: exon 19, TGGTAACATCCACCCAGATCAC (sense) and GAGGCCAGTGCTGTCTCTAAGG (antisense); exon 21, GATGATCTGTCCCTCACAGCAG (sense) and CAGCTCTGGCTCACACTACCAG (antisense). Annealing temperatures were 60°C (exons 19 and 21).

PCR amplicons were purified using exonuclease I (US Biochemical, Cleveland, Ohio, USA) and shrimp alkaline phosphatase (US Biochemical) prior to sequencing. Purified DNA was diluted and cycle-sequenced using the ABI BigDye Terminator kit version 1.1 (ABI, Foster City, California, USA) according to the manufacturer's instructions. Sequencing reactions were electrophoresed on an ABI-3700 genetic analyzer. Electropherograms were analyzed in both sense and antisense direction for the presence of mutations, using Sequencher software version 4.1.4. All sequence variants were confirmed in multiple independent PCR amplifications and sequencing reactions.

Her tumor tissue had a T→G missense mutation in nucleotide 2819 of EGFR full-length cDNA (accession

Fig. 1

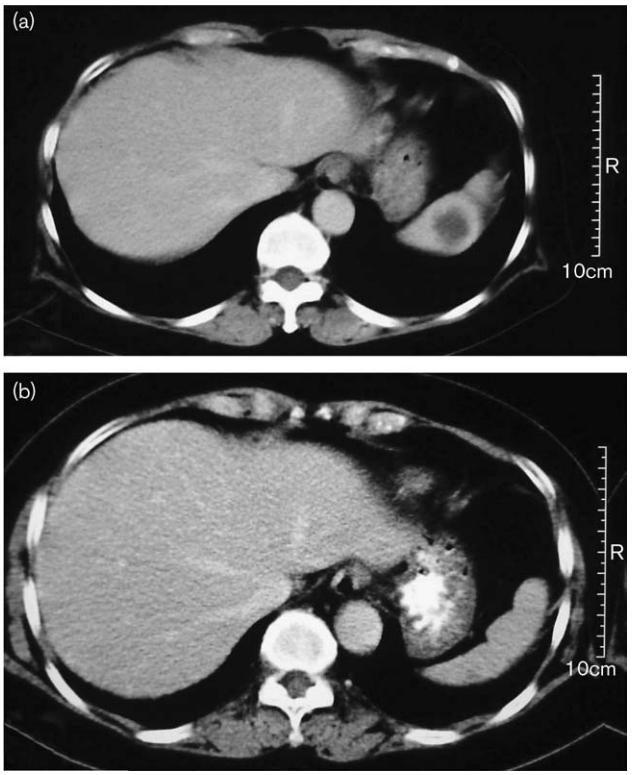


The patient's tumor tissue (T) has a T→G missense mutation in nucleotide 2819 of EGFR cDNA (accession no. NM_005228.3), within exon 21 of the EGFR gene, resulting in amino acid substitutions from leucine to arginine at codon 858 (L858R) as expected. The microdissected surrounding non-tumor tissue (NT) also has a T/G heterozygous pattern which may imply pre-malignant changes with somatic mutation.

no. NM_005228.3) (shown in Fig. 1), within exon 21 of the EGFR gene, resulting in amino acid substitutions from leucine to arginine at codon 858 (L858R) as expected. The microdissected surrounding non-tumor tissue also had a T/G heterozygous pattern which may imply pre-malignant changes with somatic mutation. DNA sequencing from the PCR products of EGFR exon 21 of peripheral blood mononuclear cells did not show the T→G somatic mutation (data not shown), which implies the T→G mutation is not a germline mutation or common single-nucleotide polymorphism. PCR was also used to amplify exon 19 comprising the EGFR gene using DNA isolated from microdissected tumor tissue, surrounding non-tumor tissue and peripheral blood mononuclear cells. The results did not show any somatic mutation in exon 19 (data not shown).

After the EGFR mutation analysis, she received oral gefitinib 250 mg/day for her metastatic diseases of the adenocarcinoma. Good tumor response with more than 90% tumor reduction was achieved under treatment with gefitinib, as documented by abdominal CT scans (shown in Fig. 2a and b), and the follow-up [^{18}F]FDG-PET scan in January 2005 showed complete resolution of all previous uptake signals in spleen and para-aortic lymph nodes. Only dryness of the skin was noted after long-term gefitinib treatment, while no other clinically relevant adverse effects were noted (such as skin rash, paronychia, etc.). The level of carcinoembryonic antigen decreased from 11.4 to 0.8 ng/ml. Currently, she has a clinical complete remission for 11+ months after the start of gefitinib treatment.

Fig. 2



Good tumor response with more than 90% tumor reduction was achieved, as documented by abdominal CT scans before (a) and after (b) gefitinib treatment.

Discussion

EGFR is overexpressed in 40–80% of NSCLCs and many other epithelial cancers [4]. Gefitinib or erlotinib targets the ATP cleft within the TK of EGFR, which has been used for the treatment of NSCLC [6,7]. The molecular mechanisms underlying responsiveness to gefitinib in NSCLC were unknown until 2004 [8–10].

Lynch *et al.* and Paez *et al.* reported that the somatic mutations of EGFR gene underlying responsiveness to gefitinib were either small, in-frame deletions or amino acid substitutions clustered around the ATP-binding pocket of the TK domain. Functionally, EGFR mutants demonstrated enhanced TK activity in response to EGF and increased sensitivity to inhibition by gefitinib *in vitro* [8,9].

Pao *et al.* also reported that seven of 10 gefitinib-sensitive tumors had similar types of somatic mutations with in-frame deletions in exon 19 or point mutations frequently in codon 858 (exon 21) from sequencing the EGFR TK domain; no mutations were found in eight gefitinib-refractory tumors ($p = 0.004$) [10]. Five of seven tumors sensitive to erlotinib (Tarceva), a related EGFR TKI, had analogous somatic mutations, as opposed to none of 10 erlotinib-refractory tumors ($p = 0.003$) [10].

These results suggest that EGFR somatic mutations may predict sensitivity to gefitinib or erlotinib and screening for such mutations in lung cancers may identify patients who will have a response to gefitinib or erlotinib [8–10].

The somatic mutations of EGFR genes are seen much less in the US (8% or less) [8,9], while they are much more frequently seen in Japan, up to between 25.8 (15 of 58 patients) [9] and 32% (38 of 120 patients) [11], and in China, about 24.4% (10 of 41). Among the East Asian countries, Huang *et al.* reported that mutation(s) in the kinase domain (exons 18–21) of the EGFR gene were identified in 38.6% (39 of 101) of Taiwanese patients [12]. All of the mutations occurred in adenocarcinoma, except one that was in an adenosquamous carcinoma. The mutation rate in adenocarcinoma was 55% (38 of 69) [12].

Herein, we report another Taiwanese female patient with metastatic adenocarcinoma of the lung who has the same activating somatic mutations (L858R) in exon 21 of the EGFR gene underlying complete responsiveness to gefitinib.

Intriguingly, Tokumo *et al.* reported that EGFR somatic mutations were frequently associated with adenocarcinoma ($p < 0.0001$), never-smokers ($p < 0.0001$) and female gender ($p = 0.0001$) [11]. Pao *et al.* also reported that most mutation-positive tumors were adenocarcinomas from patients who smoked less than 100 cigarettes in a lifetime (i.e. 'never-smokers'). They screened EGFR exons 2–28 in 15 adenocarcinomas resected from untreated never-smokers. Seven tumors (of 15) had TK domain mutations, in contrast to four of 81 NSCLC resected from untreated former or current smokers ($p = 0.0001$) [10]. Our patient is a never-smoker, female Taiwanese with metastatic adenocarcinoma of the lung. Whether these surrogate characteristics can predict EGFR somatic mutations warrants further prospective clinical studies.

The histopathology of adenocarcinoma accounts for more than 50% (range 48–68%) of lung cancers in Taiwan [12,13]; moreover, the rate of EGFR somatic mutations is very high (55%) in adenocarcinoma of Taiwanese patients [12]. This implies that gefitinib or erlotinib could be equally effective, minimally toxic and a reasonable alternative to combination chemotherapy for lung cancer patients in Taiwan. Up-front mutation analysis for detection of somatic mutation(s) in the kinase domain (exons 18–21) of the EGFR gene can be suggested for Taiwanese lung cancer patients before the start of gefitinib or erlotinib treatment.

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