Diffuse Micronodular Pulmonary Metastasis of Lung Adenocarcinoma Predicts Gefitinib Response in Association with Epidermal Growth Factor Receptor Mutations

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Abstract. Female, non-smoker, Asian ethnicity and adenocarcinoma histology are the major clinical predictors of gefitinib response in non-small cell lung cancer, as shown in previous studies. Recently, response to gefitinib has been associated with epidermal growth factor receptor (EGFR) mutations. Higher rates of mutation were seen in females, patients with adenocarcinomas, the Asian population and never-smokers, which may explain the clinical response predictors. The presence of diffuse micronodular pulmonary metastasis on chest imaging as a novel clinical predictor of its response is proposed here. Two cases of lung adenocarcinomas in men presenting with diffuse micronodular pulmonary metastasis were encountered. Both patients showed a major response to gefitinib. The dramatic reduction of micronodular pulmonary nodules throughout both lungs on computed tomography scans was achieved after treatment for a couple of months with 250 mg of oral gefitinib. In the molecular analysis, one patient had a heterozygous delL746-A750 mutation and the other had a heterozygous L858R EGFR mutation. In conclusion, patients with lung adenocarcinoma, even men, who presented with bilateral diffuse micronodular metastatic spread to the lungs tended to have an activated EGFR mutation. Therefore, they are most likely to receive benefits from molecular target drugs such as gefitinib and possibly erlotinib.

Gefitinib (ZD1839, Iressa; AstraZeneca, Osaka, Japan) is a specific inhibitor of the epidermal growth factor receptor (EGFR) tyrosine kinase. It has shown favorable efficacy, especially in the treatment of non-small cell lung cancer (NSCLC) (1, 2). Recently, activating mutations of EGFR were found to have a significant association with the response to gefitinib, suggesting the promising role of EGFR mutations as a predictive marker of gefitinib response (3-5). These mutations occurred near the adenosine tri-phosphate (ATP) cleft of the tyrosine kinase domain in which 4-anilinoquinazoline compounds, such as gefitinib, compete with ATP for binding (3, 4). Consequently, gefitinib binding with the ATP cleft abrogates the downstream signal transduction by blocking autophosphorylation, leading to the arrested growth and ultimate apoptosis of cancer cells (3-6). Higher rates of mutation were seen in females, patients with adenocarcinomas, the Asian population and never-smokers, which may explain the clinical response predictors (3-9).

During our practice of treating NSCLC with gefitinib, we noticed that a diffuse micronodular pattern of pulmonary metastasis might be another clinical predictor of response in patients with lung cancer in the Japanese population. An investigation of the pertinence of our hypothesis is attempted here by describing case reports and their EGFR mutational analysis.

Case Reports

Patient 1. A 40-year-old salesman and former light smoker developed a cough and general fatigue one month before hospital admission. At the end of June 2004, a chest radiograph and computed tomography (CT) scan taken in a local hospital revealed a nodule with diameter of 2.0 cm in the apical segment of the lower lobe of the right lung and
numerous micronodular opacities on both lungs. On July 1, 2004, he was referred to Kochi Medical School Hospital, Japan, for further examination. Bronchoscopy with brushing to collect samples for cytological analysis demonstrated adenocarcinoma cells in the apical segment of his right lower lobe. His condition was diagnosed as pulmonary adenocarcinoma with multiple pulmonary metastases. A systematic survey showed no metastasis other than in the lungs. Arterial blood gas testing showed a PaO2 of 82.6 mmHg and a PaCO2 of 42.5 mmHg for the inhalation of room air. Regarding treatment, the patient wanted to receive a molecular target drug, gefitinib, instead of conventional platinum-containing chemotherapy. He began to take gefitinib soon after hospital admission. The diffuse micronodular opacities on the chest radiograph began to disappear after one week. Thereafter, skin eruptions involving his face, especially surrounding his mouth and the upper trunk were observed as an adverse effect, but were tolerable during the entire course of his illness. Two months after the start of the therapy, the maximum reduction in number and size of micronodular opacities was attained with associated shrinkage of the primary tumor on CT scans (Figure 1 A, B). Eight months after the start of gefitinib therapy, the patient developed right pleural effusion and multiple liver metastases as recurrence of the disease and was admitted to our hospital for a change in therapy. At that time, a portion of the pleural effusion was kept for later DNA analysis after obtaining informed consent. Gefitinib therapy was stopped and one cycle of chemotherapy using the combination of carboplatin and paclitaxel was instituted. However, the patient refused continuation of chemotherapy and requested a transfer to another hospital, where he died 2 months later of progressive disease.

Patient 2. A 51-year-old male construction worker was admitted to our hospital with increasing diffuse micronodular opacities on both lungs. He had regularly undergone annual check-ups and had undergone chest radiograph examination, once annually, for 3 years before the first admission. An abnormal chest radiograph result was followed by CT imaging. The patient was suspected of having pneumoconiosis and was advised to stop smoking. Due to the substantial increase in micronodular opacities on both lungs over the previous 2 years, he was referred to our hospital for further examination in mid-December 2003. Bronchoscopy was immediately performed. A transbronchial lung biopsy and bronchoalveolar lavage fluid cytology showed the presence of suspected adenocarcinoma cells. In the meantime, he did not report discomfort from any respiratory symptoms. Arterial blood gas testing showed a PaO2 of 103.3 mmHg and a PaCO2 of 39.9 mmHg for the inhalation of room air. For the purpose of establishing the diagnosis, video-assisted thoracoscopic surgery (VATS) was performed in January 2004. Partial resection of the middle lobe demonstrated well-differentiated papillary adenocarcinoma with multiple pulmonary metastases. No evidence of either pneumonia or of any other kind of interstitial pneumonia was found in the remaining resected lung tissue. A systematic survey showed no metastasis except for in the lungs, as with Patient 1. Combination chemotherapy using carboplatin and paclitaxel was started. The first cycle of chemotherapy was performed on an in-patient basis. The remaining 5 cycles of chemotherapy were continued on an out-patient basis, with minor response of the primary tumor and no change in the multiple micronodular pulmonary metastases. As the serum carcinoembryonic antigen levels gradually became elevated in February 2005, we proposed treatment with gefitinib and obtained informed consent for the investigation of EGFR mutations as well. In mid-April, gefitinib therapy was started, because a heterozygous L858R EGFR mutation was demonstrated on lung tumor tissue obtained through VATS. Improvement was seen on the chest radiograph after 2 weeks. The maximum reduction in the size and numbers of multiple pulmonary metastatic foci and shrinkage of the primary tumor were achieved after 3 months of therapy (Figure 1 C, D). The only adverse effect was eruptions involving the face (especially surrounding the mouth) and the anterior chest, as observed in Patient 1. The serum carcinoembryonic antigen level had reduced from 125 ng/ml to 25 ng/ml. Major response and the continuing decrease in serum carcinoembryonic antigen level are currently evident.

Mutational Analysis of Epidermal Growth Factor Receptor

Genomic DNA was extracted from the tumor specimens according to the conventional method. Pleural effusion from Patient 1 and formalin-fixed paraffin-embedded lung tumor tissue obtained at VATS from Patient 2 were subjected to DNA extraction. EGFR mutations in exons 18 through 21 were examined according to the methods previously described by Lynch et al. (2); primers for EGFR (exons 18-21) analysis were the same as those reported in their paper. Nested polymerase chain reaction (PCR) amplification was conducted on the paraffin-embedded tissue sections. All sequencing reactions were performed in both the forward and reverse directions. Direct DNA sequencing on amplification products was performed using an ABI PRISM 310 genetic analyzer with a Big-Dye terminator cycle sequencing kit (Applied Biosystems, Foster City, CA, USA). All mutations were confirmed at least twice from independent amplification products.
Results

On the CT scans, the dramatic response to gefinitib can be seen in both patients in terms of the remarkable reduction in micronodular pulmonary metastases, as shown in Figures 1 and 2. CT imaging before the start of gefitinib treatment revealed a primary solid tumor with multiple micronodular opacities in both cases. Most of the micronodules seemed to be distributed in the centrilobular region.

Pathological/CT correlation could only be analyzed in Patient 2. The pathological diagnosis demonstrated well-differentiated papillary adenocarcinoma with invasion to the parietal pleura of the right middle lobe (Figure 2A).

Numerous multiple foci of bronchioloalveolar cell carcinoma-like tumor growth were seen and these were interpreted as pulmonary metastasis (Figure 2B).

In the molecular studies, a heterozygous exon 19 deletion (delE746-A750) was detected in the pleural effusion from Patient 1 (Figure 3A) and a heterozygous L858R mutation was detected in the formalin-fixed paraffin-embedded lung tumor tissue from Patient 2 (Figure 3B). Although pleural effusion from Patient 1 was aspirated in the recurrence of the disease despite the continued taking of gefitinib, an acquired T790M mutation in exon 20 was not detected; this mutation was quite recently reported to have a close relation with the resistance to gefitinib (10, 11).
Discussion

Somatic EGFR mutations are found more frequently among patients with adenocarcinoma, non-smokers, patients of Asian ethnicity and females (4-9). The most common EGFR mutations include the E746-A750 exon 19 deletion and the missense mutation L858R exon 21. Mutations in the EGFR gene are present only in the tumor; no germline mutations have been reported (3, 4). The response to gefitinib has been reported to be closely associated with these EGFR mutations (3-5).

In both of the patients studied here, multiple micronodular foci were commonly observed throughout the lungs on CT imaging. The marked reduction of these foci was seen after oral gefitinib therapy. The interpretation of diffuse multiple micronodular patterns is realistically difficult on the basis of radiological appearance without histological information. Radiologically, centrilobular distribution of micronodules on a CT scan may suggest bronchioalveolar cell carcinoma features with the cancer cells infiltrating the cells lining the alveoli (12, 13). In fact, differentiating multiple hematogenous metastases of lung adenocarcinomas from multiple micronodular foci of bronchioalveolar cell carcinoma cannot be done easily on chest radiographs or CT scans. In addition, a detailed pathological study of these tumors is more problematic because these tumors are unresectable and they are often diagnosed only from limited biopsy and cytology specimens (14).

In our study, the histological evaluation of the lung tumor tissue of Patient 2, obtained through VATS, demonstrated well-differentiated papillary adenocarcinoma with bronchioalveolar cell carcinoma features. Based upon the World Health Organization criteria, pure bronchioalveolar cell carcinoma has a relatively lower incidence of EGFR mutation, while nearly all adenocarcinomas that include any areas of bronchioalveolar cell carcinoma growth, which are sensitive to gefitinib or erlotinib and harbor EGFR mutations, have bronchioalveolar cell carcinoma features (15). This is consistent with the clinical observation reported by Kris et al. that the response rate to erlotinib, a tyrosine kinase inhibitor similar to gefitinib, in pure bronchioalveolar cell carcinoma was reported to be somewhat lower than that of mixed tumors or bronchioalveolar cell carcinoma variants (16). Histological evaluation is not always feasible. Therefore, recognition of typical radiological patterns, from which to predict the response to gefitinib, is extremely useful from the clinical standpoint.

We believe that the diffuse micronodular opacities observed in our cases represent the findings suggestive of invasive lung adenocarcinoma with bronchioalveolar cell carcinoma features. Furthermore, pulmonary toxicity due to gefitinib, often seen among Japanese patients, did not occur in these responding patients; both of them were non-smokers at the time (17).

Because the most common EGFR mutations were present in both of our cases, we are convinced that the diffuse micronodular pattern of pulmonary metastasis by lung adenocarcinomas might be a novel clinical predictor of gefitinib response. However, further studies are clearly warranted.
Figure 3. (A) The EGFR mutational analysis of exon 19 in pleural effusion from Patient 1 showed a heterozygous delE746-A750 mutation. (B) The EGFR mutational analysis of exon 21 in the formalin-fixed paraffin-embedded tissue specimen from Patient 2, obtained through VATS, showed a heterozygous L858R mutation.
References


