Epidermal Growth Factor Receptor Mutations in Patients with Non–Small Cell Lung Cancer

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Abstract
A year has passed since mutations of the tyrosine kinase domain of the epidermal growth factor receptor (EGFR) were discovered in patients with non–small cell lung cancer (NSCLC) who had dramatic clinical responses to treatment with gefitinib. Additional laboratory and clinical studies have provided further insight into the biological impact of EGFR mutations in cell culture experiments and in patients with NSCLC. In vitro characterization of NSCLC cell lines and host cell lines transfected with these mutant and wild-type EGFR show that most cell lines with mutated EGFR are growth-inhibited by 10- to 100-fold lower concentrations of gefitinib and erlotinib compared with wild-type EGFR. NSCLC lines with mutations of the EGFR treated with concentrations of gefitinib and erlotinib that are achievable in the plasma undergo apoptosis rather than growth arrest. Retrospective studies of patients with NSCLC-treated gefitinib have reported a close association between EGFR mutations, increased chance of clinical response and longer survival. This review will provide information on the impact of EGFR mutations on gefitinib and erlotinib treatment by in vitro experiments, the outcome of NSCLC patients with these mutations when treated with gefitinib and erlotinib, and the subsets of patients with NSCLC in whom these mutations arise. (Cancer Res 2005; 65(17): 7525-9)

Background
The epidermal growth factor receptor (EGFR) is detected by immunohistochemistry on 40% to 80% of non–small cell lung cancers (NSCLC; ref. 1, 2). This prompted multiple pharmaceutical companies to develop small molecule inhibitors of the tyrosine kinase domain of the wild-type EGFR during the 1990s (3, 4). Two therapeutic agents, gefitinib and erlotinib, have been extensively studied in patients with NSCLC and are approved by the Food and Drug Administration for treatment of patients with relapsed NSCLC in the U.S. (4). The initial testing of gefitinib and erlotinib in phase II trials showed that ~10% of patients of European background and 30% of patients from Japan had clinical responses when treated with the EGFR inhibitors, gefitinib and erlotinib (5–7). The clinical characteristics that are associated with responses to gefitinib and erlotinib were defined in these studies (5–7). Clinical responses to these agents were more common in women than men, in patients from Japan than from Europe and the U.S., patients with adenocarcinoma than other histologic subtypes, and patients who had never smoked cigarettes.

Studies of another solid tumor, gastrointestinal stromal tumor, showed that activating mutations of the C-KIT gene were associated with clinical responses to the small molecule inhibitor of the tyrosine kinase domain of the C-KIT gene, imatinib (8). This led multiple groups to identify patients with NSCLC and dramatic clinical responses following treatment with gefitinib and erlotinib for sequencing of the tyrosine kinase domain of EGFR in their tumor.

Therapeutic Impact of EGFR Mutations
The association between somatic mutations of EGFR and dramatic clinical response to gefitinib was reported online in April of 2004, with the two articles being published in May and June of 2004, respectively (1, 9). Thirteen of the 14 patients with a clinical response to gefitinib therapy had somatic mutations of the EGFR identified, whereas all 11 patients whose tumors did not respond to gefitinib treatment had a somatic wild-type EGFR sequence. These mutations were all in the tyrosine kinase domain of the EGFR. Research in the past year has shown that these mutations in NSCLC are associated with in vitro sensitivity to treatment with gefitinib and erlotinib, transfection of the mutant epidermal growth factor gene into host cell lines confers sensitivity to gefitinib and erlotinib, and mutations of the EGFR gene are common in patients with dramatic clinical responses to gefitinib and erlotinib. Research from other groups has identified candidate factors other than EGFR mutations that may be involved in determining response to gefitinib and erlotinib, including increased EGFR copy number and K-ras mutations (10, 11). However, the published information on these two genomic markers is limited at the current time, so we are going to restrict our comments to EGFR mutations in this review.

The in vitro studies of NSCLC cell lines and host cell lines transfected with different mutations of the EGFR have provided important insights into how the mutations activate the tyrosine kinase domain of EGFR and the dramatic impact gefitinib and erlotinib have on EGFR signaling (1, 2, 9, 12, 13). EGFR is constitutively activated as assessed by phosphorylation of the different tyrosine residues on EGFR, the downstream pathways are activated, and gefitinib and erlotinib inhibit both this signaling and the growth of the NSCLC cells (Fig. 1). Investigators have identified six cell lines with deletions and mutations of EGFR that are sensitive to treatment with gefitinib and erlotinib (2, 12–15). These include PC-9, HCC-827, NCI-H1650, NCI-H1975, NCI-H3255, and DFCLIU-011. Four NSCLC cell lines have deletions of exon 19 (PC-9, H1650, HCC827, and DFCLIU-011) and two have point mutations of exon 21 (NCI-H1975 and NCI-H3255). The IC_{50} of these six NSCLC lines range from 20 to 200 nmol/L of gefitinib when assessed in dye conversion assays.

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Three cell lines with wild-type EGFR have intermediate sensitivities (NCI-1819, NCI-H1666, and Calu-3) with IC_{50}’s between 1 and 5 μmol/L (2, 13, 15). Most other NSCLC cell lines with wild-type EGFR require concentrations of gefitinib >10 μmol/L to inhibit their growth, >10-fold the achievable plasma concentrations in patients (2, 4, 13).

The cell lines with mutant EGFR are more likely to have activated EGFR as assessed by phosphospecific antibodies of the intracellular phosphorylated kinase domains. The phosphorylation of EGFR in these cell lines with mutant EGFR is inhibited by low concentrations of gefitinib and erlotinib (10-100 nmol/L). The signaling pathways downstream from EGFR, pERK and pAKT, are inhibited by these same low concentrations of 10 to 100 nmol/L of gefitinib as well (2, 9, 12, 13). Treatment of cell lines with an EGFR point mutation in exon 21 (NCI-H3255) and a deletion mutant in exon 19 (HCC-827) with 100 nmol/L to 1 μmol/L gefitinib or erlotinib leads to apoptosis as assessed by the sub-G1 fractions on flow cytometry studies and by activation of the caspase pathway (2, 13).

The ability of the mutant EGFR to mediate changes in growth and signaling have been verified by transfection experiments into multiple cell systems. The mutant and wild-type EGFR have been transfected into different host cells including NMuMg, COS-7, Chinese hamster ovary cells, and HEK293 cells to determine differences in biochemical signaling and growth (1, 2, 12, 14, 15). The growth and downstream signaling inhibition of the host cell lines transfected with mutant EGFR are consistently more sensitive to treatment with gefitinib and erlotinib than cells with wild-type receptors. The wild-type EGFR receptor typically sends a downstream signal that ultimately stimulates the growth of the NSCLC cells that are dependent on the receptor and gefitinib or erlotinib can modestly inhibit this relatively weak signal (Fig. 2). The mutated EGFR receptor is constitutively activated with a prominent downstream signal that can be dramatically inhibited by gefitinib and erlotinib.

The in vitro sensitivity of EGFR mutant NSCLC cell lines also has a clinical correlate because the association between EGFR mutations and clinical response to gefitinib and erlotinib has subsequently been documented around the world. Reports from other institutions in the U.S., Japan, Taiwan, and Korea have confirmed that 70 of the 91 of patients (77%) with clinical responses to gefitinib and erlotinib have detectable mutations of the EGFR (1, 9, 11, 16–19). In contrast, only 10 of 133 patients (8%) with stable or progressive disease after treatment with gefitinib or erlotinib have a somatic EGFR mutation (1, 9, 11, 16–19). In addition to the differences observed in the response rates, the patients who have somatic mutations of EGFR are likely to live longer than patients with wild-type EGFR when treated with...
gefitinib. Three different studies of 170 patients from Japan and Korea have provided Kaplan-Meier survival curves of patients treated with gefitinib whose tumors have undergone EGFR sequencing (16, 18, 19). The 59 patients with NSCLC with somatic mutations of EGFR treated with gefitinib had median survivals in excess of 2 years compared with median survivals of 7 to 14 months for the 111 patients with wild-type EGFR.

The types of EGFR mutations in patients treated with gefitinib and erlotinib have been similar around the world (1, 9, 11, 16–19). The mutations involve multiple overlapping deletion mutations of exon 19 in 45% of patients, missense mutations in exon 21 in 40% of patients (predominately L858R), and missense or insertion mutations in exons 18 to 21 in the other 15% of patients. There is no clear difference in the mutational pattern in the tumors from patients of European background versus those from East Asia. There is not yet enough information to make firm conclusions about the efficacy of gefitinib and erlotinib treatment in patients with NSCLC as a function of their different types of mutations of EGFR.

The EGFR sequence has been characterized in large numbers of patients with early stage NSCLC that have not been treated with gefitinib or erlotinib. There have been three large studies sequencing >1,600 resected NSCLCs from patients in the U.S., Europe, and East Asia that provide potential explanations about differences between the response rates of 5% to 10% in patients from the U.S. and Europe versus the response rates of 20% to 30% in patients from East Asia (20–22). The mutations involve deletion mutations of exon 19 in ~50% of patients, missense mutations in exon 21 in 40%, and mutations in exons 18 and 20 in the other 10%. The clinical characteristics of patients with mutations of EGFR closely parallel the subsets of patients who are more likely to respond to treatment with gefitinib and erlotinib. The patients with NSCLC from the U.S., Europe, and Australia have an EGFR somatic mutation frequency of ~10% compared with a mutation rate of 30% in patients from Japan and Taiwan. There is also a close association between adenocarcinoma and mutations in the EGFR. The three studies showed that 263 of 888 patients with adenocarcinomas (30%) had mutations detected, whereas 7 of 764 patients with other types of NSCLC (1%) had a somatic mutation of EGFR (20–22). The somatic mutations of EGFR were also two to three times more likely in women than men and three to five times more likely in nonsmokers than those who were current or former smokers. The findings clearly mirror the increased response rates in these same patient populations when treated with gefitinib and erlotinib.

Despite the observation that patients with NSCLC and somatic mutations of EGFR treated with gefitinib live longer than patients whose NSCLC have wild-type EGFR with similar

Figure 2. The wild-type EGFR is activated by an agonist, undergoes dimerization, and sends intracellular signals to increase the growth rate of the NSCLC cells. The modest growth signal is inhibited by the addition of gefitinib and erlotinib represented by the band emerging from the receptor. The diminution in the growth signal caused by gefitinib and erlotinib is represented by the modest decrease in the width of the band before and after addition of these drugs.
Acquired Resistance to EGFR Inhibitors

Despite the therapeutic success of EGFR-TKIs in NSCLC patients with EGFR mutations, the majority of such patients will ultimately develop disease progression. The mechanism of acquired resistance to gefitinib is beginning to be understood. Three recent studies reported nine patients who had a clinical and radiographic response to gefitinib or erlotinib and had EGFR mutations documented in their initial tumor. These nine patients then developed progressive NSCLC on gefitinib or erlotinib therapy underwent repeat biopsy of their tumor, and then sequencing of EGFR (23–25). Six of the nine patients had a secondary mutation in addition to the initial EGFR mutation, a substitution of a methionine for a threonine at position 790 (T790M). Structural modeling suggests that the bulkier methionine residue creates a steric hindrance preventing gefitinib or erlotinib from binding to the ATP binding pocket of the EGFR. Furthermore, biochemical studies show that in the presence of T790M EGFR mutation, gefitinib is no longer able to inhibit the phosphorylation of either wild-type or mutated EGFR. However, other irreversible EGFR inhibitors still retain inhibitory activity, suggesting that a further understanding of the structure/function relationship of mutated EGFR and EGFR inhibitors (23, 24). This may ultimately identify second-generation EGFR TKIs that could be used in patients who develop an acquired T790M mutation and lead to sequencing tumors from patients who relapse after successful treatment with an EGFR-TKI.

Meaning and Implications of Mutations of the EGFR

The therapeutic efficacy of conventional combination chemotherapy for previously untreated patients with advanced NSCLC offers a modest 20% response rate, a 2- to 4-month prolongation of survival, and a median survival of 8 to 10 months (26, 27). The identification of a subset of patients with adenocarcinoma with mutations in the EGFR has provided a therapeutic alternative to the consistently modest results with chemotherapy. Previous therapeutic studies of patients with advanced NSCLC have not divided patients by histologic subtypes (adenocarcinoma, squamous cell carcinoma, and others) but have lumped 85% of the lung cancers into NSCLC. The patients with adenocarcinoma of the lung and nonsmokers are more likely to have a response to gefitinib or erlotinib treatment and these responses are associated with the presence of a somatic mutation of EGFR in ~80% of the patients. This may provide a rationale for selecting patients with specific histologies (adenocarcinoma) and smoking status as candidates for clinical trials with EGFR inhibitors, a new concept in the treatment of NSCLC. The retrospective collection of information shows that patients with NSCLC and EGFR mutations have a clinical response rate of ~80%, can be treated for at least a year, and have at least a 2-year median survival after the start of treatment. Prospective clinical trials for patients with NSCLC are ongoing to prospectively assess the impact of giving gefitinib or erlotinib to previously untreated patients with NSCLC and mutations in the EGFR. If these retrospective observations are confirmed in prospective trials, patients with NSCLC who have a mutation identified in their EGFR will likely be offered an EGFR inhibitor as their initial therapy for NSCLC. The prospect of being able to treat a subset of patients (10-30% of the population of NSCLC) with gefitinib or erlotinib for a median of a year or more with the expectation of a median survival of ≥2 years has the potential to dramatically improve the outcome for this subgroup of patients. Clinical trials will also be needed to determine if patients with NSCLC and mutations of the EGFR need to be treated with erlotinib or gefitinib alone or if there is an advantage to adding chemotherapy to their regimen.

The discovery of the association between EGFR mutations and the response to gefitinib and erlotinib has transformed NSCLC from one disease treated with conventional combination chemotherapy to subsets of NSCLC identified by genomic studies. Patients with NSCLC and EGFR mutations can be effectively treated with gefitinib and erlotinib. Although many aspects of EGFR mutations have been characterized, several unknown questions remain (Table 1). The information on the association between EGFR mutations and response to gefitinib and erlotinib has now been available for more than a year. The answers to the remaining important questions will come from additional well-conducted ongoing and planned preclinical and clinical studies of patients and NSCLC cell lines with wild-type and mutated EGFR treated with EGFR inhibitors.

Acknowledgments


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Table 1. The current state of knowledge of EGFR mutations in patients with NSCLC

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<th>Known</th>
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<td>The etiology of the EGFR mutations</td>
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<td>in adenocarcinomas, and East Asians</td>
<td>who do not have the identified T790M secondary EGFR mutation</td>
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<td>to gefitinib and erlotinib in patients and in vitro</td>
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References


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