

Epidermal growth factor receptor (EGFR) in lung cancer: an overview and update

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ABSTRACT Epidermal growth factor receptor is a trans-membrane glycoprotein with an extracellular epidermal growth factor binding domain and an intracellular tyrosine kinase domain that regulates signaling pathways to control cellular proliferation. Epidermal growth factor receptor binding to its ligand results in autophosphorylation by intrinsic tyrosine/kinase activity, triggering several signal transduction cascades. Constitutive or sustained activation of these sequences of downstream targets is thought to yield more aggressive tumor phenotypes. Mutations in epidermal growth factor receptor have been discovered in association with some lung cancers. Lung adenocarcinomas with mutated epidermal growth factor receptor have significant responses to tyrosine kinase inhibitors, although for unselected patients it does not appear to have a survival benefit. However, in a subset of patients (non-smoking Asian women with adenocarcinoma, particularly with a bronchioloalveolar carcinoma), there appears to be a significant survival advantage. Both EGFR mutation and gene amplification status may be important in determining which tumors will respond to tyrosine kinase inhibitors.

KeyWords: lung cancer; epidermal growth factor receptor; tyrosine kinase inhibitors

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Lung cancer is one of the leading causes of cancer-related deaths among both men and women, and there continues to be limited treatment options available for advanced-stage disease (1,2,3). Non-small cell lung cancer (NSCLC) which comprises the majority (about 75%) of lung cancer, has proven difficult to treat due to poorly understood pathological mechanisms. Recent advances in our understanding of cell signaling pathways that control cell survival have identified genetic and regulatory aberrations that suppress cell death, promote cell division, and induce tumorigenesis. One such discovery is that of epidermal growth factor receptor (EGFR). EGFR is a transmembrane receptor tyrosine kinase protein that is expressed in some normal epithelial, mesenchymal, and neurogenic tissue. Overexpression of EGFR has been reported and implicated in the pathogenesis of many human malignancies, including NSCLC (4,29). Some studies have shown that EGFR expression in NSCLC is associated with reduced survival (30,31,32), frequent lymph node metastasis and poor chemosensitivity (33,34).

Two oral anti-cancer drugs that inhibit EGFR, gefitinib (Iressa)

and erlotinib (Tarceva), have recently been approved for use in advanced non-small cell lung cancer, and mutations in EGFR have been discovered in association with some lung cancers. Since then, considerable effort has been made to identify clinical, morphologic, and molecular factors that can predict response rates to these drugs. This review provides an introduction to the role of EGFR in lung cancer and presents some of the recent literature on this topic.

EGFR function and its role in lung cancer

EGFR belongs to the erbB family of closely related receptor tyrosine kinases, which include erbB1 (also known as EGFR), erbB2 (HER2), erbB3, and erbB4. Although their basic structures are similar, each one has distinct properties, including variation in tyrosine kinase activity. It has an extracellular ligand binding domain, a transmembrane portion, and intracellular tyrosine kinase and regulatory domains. Upon binding of a specific ligand (eg. epidermal growth factor), the normally functioning EGFR undergoes conformational change and phosphorylation of the intracellular domain occurs, leading to downstream signal transduction by various pathways. These include the Raf1-extracellular signal-regulated kinase, PI3K/Akt, and signal transducer and activator of transcription (STAT) factors. Depending on the pathway, the end result is cell proliferation or cell maintenance by inhibition of apoptosis (4).

DNA mutations in EGFR as detected by polymerase chain reaction (PCR) can occur in regions corresponding to the extracellular or intracellular portions of the protein. In non-small cell lung can-

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cer, overexpression of EGFR or mutations in intracellular EGFR have been observed in 43-89% of cases (5). Others report that one quarter of NSCLC had mutations in the EGFR tyrosine kinase domain and these were associated with increased receptor expression in 75% of cases (35,36). Of the known EGFR tyrosine kinase domain mutations, greater than 90% occur as short in-frame deletions in exon 19 or as point mutations in exon 21, the latter resulting in arginine replacing leucine at codon 858 (L858R) (6). These mutations can result in constitutive activation of signal transduction pathways, leading to cell proliferation or anti-apoptosis, regardless of the presence of extracellular ligand. Two less common mutations occur at exons 18 and 21. Of note, EGFR and KRAS mutations appear to be mutually exclusive (7)

The EGFR gene may also undergo amplification, as detected by fluorescence in situ hybridization (FISH) or chromogenic in situ hybridization (CISH). In addition to mutated EGFR, there is now evidence that increased EGFR gene copy number, as defined as high polysomy or amplification, is associated with a better response to TKIs (13-16). Although some cases of adenocarcinoma show both EGFR mutations and increased gene copy number, others may show only one or the other. Recently it was reported that approximately 50% of EGFR mutated cases show an increased EGFR copy number, while approximately 75% of cases with increased gene copy number have mutations (6,17).

EGFR receptor tyrosine kinase inhibitors

The discovery of the EGFR receptor tyrosine kinase inhibitors (TKIs) gefitinib and erlotinib resulted in a large phase III trial of nearly 1700 advanced stage lung cancer patients, treated with either gefitinib or placebo. For all lung cancers as there was no survival benefit in the group treated with gefitinib (8). However, in a subset of patients (non-smoking Asian women with adenocarcinoma, particularly with a bronchioloalveolar carcinoma), there was a significant survival advantage.

Subsequently, three landmark trials in 2004 showed that lung adenocarcinomas with mutated EGFR had significant responses to gefitinib and erlotinib (9,10,11). This was supported by other phase III clinical trials with chemotherapy plus erlotinib or gefitinib versus chemotherapy plus placebo in NSCLC patients that showed a survival improvement for EGFR mutated cases irrespective of the treatment they received (38,39). Overall, response rates to TKIs in adenocarcinomas with mutations in EGFR are in the range of 65-90% (6). Despite the increased response rates to these drugs in NSCLC cases with an EGFR mutation, they may not have an overall survival benefit (22). One study showed that presence of EGFR mutations was not associated with survival, although a trend for shorter survival was observed in the subgroup of patients harboring an exon 19 deletion (37). One earlier study reported that treatment of NSCLC patients with gefitinib improved symptoms and caused a radiologic response in only 10% of cases (44), suggesting that EGFR activation may be a minor component of the tumorigenic

process.

Gefitinib and erlotinib are orally-administered small molecule inhibitors of the intracellular tyrosine kinase domain of EGFR and are approved for second or third line treatment of advanced lung cancer. Interestingly, intracellular mutations in EGFR at exon 19 and 21 appear to confer increased affinity for these drugs, which may impart a dependence on these mutations for drug efficacy (10). Acquired resistance mutations may also occur in cancers treated with TKIs, most commonly the threonine to methionine shift at codon 790 (T790M) (9). This has been reported in up to 50% of tumors exhibiting acquired resistance to gefitinib (12).

Assessment of EGFR abnormalities

As mentioned, gefitinib and erlotinib are effective in only a subset of patients with non-small cell lung cancer. Some studies have reported morphologic characteristics that appear to be associated EGFR mutations or with better responses to TKIs. These include adenocarcinoma with a non-mucinous bronchioloalveolar component, hobnail cell type, as well as papillary and micropapillary patterns (18,19). It has also been reported that tumors with amplified EGFR are more likely to have a significant component of solid growth, suggesting an association between EGFR amplification and a more aggressive tumor (20).

The utility of detecting EGFR overexpression by immunohistochemistry (IHC) is controversial. In some studies, IHC has been shown to have value in predicting response to TKIs, while others have not been able to show its predictive value (14,21,22,23). There has been intense interest in developing novel antibodies that are able to identify abnormal EGFR or more reliably detect overexpression, and thus better predict response to targeted therapies (21).

Several studies have investigated how best to evaluate EGFR abnormalities. A meta-analysis and systemic review of the literature involving nearly 5000 patients with lung adenocarcinoma from 27 studies was recently undertaken in order to determine the value to EGFR tests in predicting response to targeted therapy (21). IHC, FISH, and PCR were assessed and it was concluded that all three methods significantly correlate with response to TKIs. Positive predictive values of IHC, FISH, and PCR were 6.5-82%, 11-89%, and 7-100%, respectively. The authors of this review note that there was significant variation in study methodology, and highlighted the importance of standardizing these methods. Further studies are required in order to identify the best method for selecting patients who will benefit from TKIs.

Direct sequencing of mutations by PCR has obvious advantages in that it can detect specific mutations, and thus identify the mutations known to be associated with better responses to TKIs. For example, it has been observed that tumors with EGFR exon 19 mutations have better overall survival than those with exon 21 mutations when treated with TKIs (24,25). In addition, PCR can identify mutations that are thought to carry resistance to TKIs, including the exon 20 insertion that confers primary resistance, as well as the ac-

quired resistance mutation T790M. Identification of a KRAS mutation also indicates primary resistance to TKIs, as these mutations are mutually exclusive with EGFR mutations.

On the other hand, not all tumors with the susceptible EGFR mutations respond to TKIs, and some tumors respond to TKIs that do not show mutations by direct sequencing. There are several potential reasons for this. It has been postulated that mutations in the tyrosine kinase domain of EGFR are early events in lung carcinogenesis, since these mutations have been found in about 50% of atypical adenomatous hyperplasia, as well as in normal lung tissue surrounding a tumor (26). Second, it is known that mutations may be missed if the sample submitted for direct sequencing contains less than 25% tumor cells (6). Thus, although PCR is able to identify the exact mutation status in many cases, it is not a perfect method for predicting TKI response, and DNA sequencing is yet to be available for routine clinical use in most laboratories.

EGFR gene copy number, as evaluated by FISH or CISH, has also been associated with response to TKIs (27). However, other studies indicate that no difference in survival is observed regarding EGFR gene status measured by FISH analysis (40,41,42,43). There continues to be ongoing debate regarding the relative importance of mutation status versus gene amplification status. A certain degree of co-existence occurs, in that about 50% of tumors with EGFR mutations show increased gene copy number, while about 75% of tumors with increased copy number contain mutations (6). In accordance with the concept that EGFR mutations occur early in lung carcinogenesis, it has been suggested that EGFR gene amplification is a later event (20,28). One recent study found that among 65 cases of never-smoking Asian women with adenocarcinoma, 80% had mutations in EGFR, of which nine showed gene amplification by FISH (20). All of the amplified cases had the exon 19 deletion mutation. Patients with tumors containing amplified EGFR were significantly more likely to have a pattern of solid growth on histology, show a strong staining pattern by IHC, and have a worse overall survival compared to patients with tumors containing non-amplified EGFR. Interestingly, amplified EGFR was not associated with acinar or BAC patterns, the latter of which has been reported to predict responsiveness to TKIs. These findings support the concept that EGFR amplification may occur after a mutation and result in a higher-grade, more aggressive tumor.

The introduction of EGFR tyrosine kinase inhibitors and the subsequent discovery of EGFR mutations in non-small cell lung cancers have resulted in a considerable amount of research and publication in this area. To date, EGFR mutation analysis by direct sequencing has been the most studied and reliable method of predicting response to TKIs. However, the most recent evidence suggests that increased gene copy number may also be involved, with mutations occurring as early events in the carcinogenesis of some lung cancers, and gene amplification occurring later. Thus, both EGFR mutation and gene amplification status may be important in determining which tumors will respond to TKIs. However, further studies are required in order to standardize methodologies for mu-

tation and gene amplification analysis. In addition, efforts are ongoing to develop IHC markers that reliably can detect abnormal EGFR and thus guide the use of targeted therapies.

References

- Jemal A, Thomas A, Murray T, Thun M. Cancer statistics, 2002. *CA Cancer J Clin* 2002;52:23-47.
- Sasaki H, Endo K, Okuda K, Kawano O, Kitahara N, Tanaka H, et al. Epidermal growth factor receptor gene amplification and gefitinib sensitivity in patients with recurrent lung cancer. *J Cancer Res Clin Oncol* 2008;134:469-77.
- Schiller JH, Harrington D, Belani CP, Langer C, Sandler A, Krook J, et al. Comparison of four chemotherapy regimens for advanced non-small-cell lung cancer. *N Engl J Med* 2002;346:92-8.
- Inamura K, Ninomiya H, Ishikawa Y, Matsubara O. Is the epidermal growth factor receptor status in lung cancers reflected in clinicopathologic features? *Arch Pathol Lab Med* 2010;134:66-72.
- Gupta R, Dastane AM, Forozaan F, Riley-Portuguez A, Chung F, Lopategui J, et al. Evaluation of EGFR abnormalities in patients with pulmonary adenocarcinoma: the need to test neoplasms with more than one method. *Mod Pathol* 2009;22:128-33.
- Ladanyi M, Pao W. Lung adenocarcinoma: guiding EGFR-targeted therapy and beyond. *Mod Pathol* 2008;21:S16-22.
- Massarelli E, Varella-Garcia M, Tang X, Xavier AC, Ozburn NC, Liu DD, et al. KRAS mutation is an important predictor of resistance to therapy with epidermal growth factor receptor tyrosine kinase inhibitors in non-small cell lung cancer. *Clin Cancer Res* 2007;13:2890-6.
- Miller VA, Kris MG, Shah N, Patel J, Azoli C, Gomez J, et al. Bronchioloalveolar pathologic subtype and smoking history predict sensitivity to gefitinib in advanced non-small-cell lung cancer. *J Clin Oncol* 2004;22:1103-9.
- Lynch TJ, Bell DW, Sordella R, Gurubhagavata S, Okimoto RA, Brannigan BW, et al. Activating mutation in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. *N Engl J Med* 2004;350:2129-39.
- Paez JG, Janne PA, Lee JC, Tracy S, Greulich H, Gabriel S, et al. EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. *Science* 2004;304:1497-500.
- Pao W, Miller V, Zakowski MF, Doherty J, Politi K, Sarkaria I, et al. EGF receptor gene mutations are common in lung cancers from "never smokers" and correlate with sensitivity of tumors to gefitinib (Iressa) and erlotinib (Tarceva). *Proc Natl Acad Sci USA* 2004;101:13306-11.
- Balak MN, Gong Y, Riely GJ, Somwar R, Li AR, Zakowski MF, et al. Novel D761Y and common secondary T790M mutations in epidermal growth factor receptor-mutant lung adenocarcinomas with acquired resistance to kinase inhibitors. *Clin Cancer Res* 2006;12:6494-501.
- Cappuzzo F, Hirsch FR, Rossi E, Bartolini S, Ceresoli GL, Bemis L, et al. Epidermal growth factor receptor gene and protein and gefitinib sensitivity in non-small-cell lung cancer. *J Natl Cancer Inst* 2005;97:643-55.
- Hirsch FR, Varella-Garcia M, McCoy J, West H, Xavier AC, Gumerlock P, et al. Increased epidermal growth factor receptor gene copy number detected by fluorescence in situ hybridization associates with increased sensitivity to gefitinib in patients with bronchioloalveolar carcinoma subtypes: a Southwest Oncology Group Study. *J Clin Oncol* 2005;23:6838-45.
- Cappuzzo F, Ligoria C, Janne PA, Toschi L, Rossi E, Trisolini R, et al. Prospective study of gefitinib in epidermal growth factor receptor fluorescence in situ hybridization-positive/phospho-Akt-positive or never smoker patients with advanced non-small-cell lung cancer: the ONCOBELL trial. *J Clin Oncol* 2007;25:2248-55.
- Mukohara T, Engelman JA, Hanna NH, Yeap BY, Kobayashi S, Lindeman N, et

- al. Differential effects of gefitinib and cetuximab on non-small-cell lung cancer bearing epidermal growth factor receptor mutations. *J Natl Cancer Inst* 2005;97:1185-94.
17. Li AR, Chitale DA, Riely GJ, Pao W, Miller VA, Zakowski MF, et al. EGFR mutations in lung adenocarcinomas: clinical testing experience and relationship to EGFR gene copy number and immunohistochemical expression. *J Mol Diagn* 2008;10:242-8.
18. Sartori G, Cavazza A, Sgambato A, Marchioni A, Barbieri F, Lucia Longo, et al. EGFR and K-ras mutations along the spectrum of pulmonary epithelial tumors of the lung and elaboration of a combined clinicopathologic and molecular scoring system to predict clinical responsiveness to EGFR inhibitors. *Am J Clin Pathol* 2009;131:478-89.
19. Ninomiya H, Hiramatsu M, Inamura K, Nomura K, Okui M, Miyoshi T, et al. Correlation between morphology and EGFR mutations in lung adenocarcinomas: significance of the micropapillary pattern and hobnail cell type. *Lung Cancer* 2009;63:235-40.
20. Sholl LM, Yeap BY, Iafrate AJ, Holmes-Tisch AJ, Chou YP, Wu MT, et al. Lung adenocarcinoma with EGFR amplification has distinct clinicopathologic and molecular features in never-smokers. *Cancer Res* 2009;69:8341-48.
21. Gupta R, Dastane AM, McKenna R Jr, Marchevsky AM. The predictive value of epidermal growth factor receptor tests in patients with pulmonary adenocarcinoma: review of current "best evidence" with meta-analysis. *Hum Pathol* 2009;40:356-65.
22. Tsao MS, Sakurada A, Cutz JC, Zhu CQ, Kamel-Reid S, Squire J, et al. Erlotinib in lung cancer - molecular and clinical predictors of outcome. *N Engl J Med* 2005;353:133-44.
23. Parra HS, Cavina R, Latteri F, Zucali PA, Campagnoli E, Morengi E, et al. Analysis of epidermal growth factor receptor expression as a predictive factor for response to gefitinib (Iressa, ZD1839) in non-small-cell lung cancer. *Br J Cancer* 2004;91:208-212.
24. Riely GJ, Pao W, Pham D, Li AR, Rizvi N, Venkatraman ES, et al. Clinical course of patients with non-small cell lung cancer and epidermal growth factor receptor exon 19 and exon 21 mutations treated with gefitinib or erlotinib. *Clin Cancer Res* 2006;12:839-44.
25. Jackman DM, Yeap BY, Sequist LV, Lindeman N, Holmes AJ, Joshi VA, et al. Exon 19 deletion mutations of epidermal growth factor receptor are associated with prolonged survival in non-small cell lung cancer patients treated with gefitinib or erlotinib. *Clin Cancer Res* 2006;12:3908-14.
26. Sakuma Y, Matsukuma S, Yoshihara M, Nakamura Y, Nakayama H, Kameda Y, et al. Epidermal growth factor receptor gene mutations in atypical adenomatous hyperplasia of the lung. *Mod Pathol* 2007;20:967-73.
27. Hirsch FR, Varella-Garcia M, Cappuzzo F, McCoy J, Bemis L, Xavier AC, et al. Combination of EGFR gene copy number and protein expression predicts outcome for advanced non-small-cell lung cancer patients treated with gefitinib. *Ann Oncol* 2007;18:752-60.
28. Yatabe Y, Takahashi T, Mitsudomi T. Epidermal growth factor receptor gene amplification is acquired in association with tumor progression of EGFR-mutated lung cancer. *Cancer Res* 2008;68:2106-11.
29. Ohsaki Y, Tanno S, Fujita Y, Toyoshima E, Fujiuchi S, Nishigaki Y, et al. Epidermal growth factor receptor expression correlates with poor prognosis in non-small cell lung cancer patients with p53 overexpression. *Oncol Rep* 2000;7:603-7.
30. Scagliotti GV, Selvaggi G, Novello S, Hirsch FR. The biology of epidermal growth factor receptor in lung cancer. *Clin Cancer Res* 2004;10:4227-32.
31. Veale D, Kerr N, Gibson GJ, Kelly PJ, Harris AL. The relationship of quantitative epidermal growth factor receptor expression in non-small cell lung cancer to long term survival. *Br J Cancer* 1993;68:162-5.
32. Volm M, Rittgen W, Drings P. Prognostic value of ERBB-1, VEGF, cyclin A, FOS, JUN and MYC in patients with squamous cell lung carcinomas. *Br J Cancer* 1998;77:663-9.
33. Fontanini G, De Laurentiis M, Vignati S, Chinè S, Lucchi M, Silvestri V, et al. Evaluation of epidermal growth factor-related growth factors and receptors and of neoangiogenesis in completely resected stage I-IIIa non-small-cell lung cancer: amphiregulin and microvessel count are independent prognostic indicators of survival. *Clin Cancer Res* 1998;4:241-9.
34. Ogawa J, Iwazaki M, Inoue H, Koide S, Shohtsu A. Immunohistochemical study of glutathione-related enzymes and proliferative antigens in lung cancer. Relation to cisplatin sensitivity. *Cancer* 1993;71:2204-9.
35. Shigematsu H, Gazdar AF. Somatic mutations of epidermal growth factor receptor signaling pathway in lung cancers. *Int J Cancer* 2006;118:257-62.
36. Suzuki M, Shigematsu H, Hiroshima K, Iizasa T, Nakatani Y, Minna JD, et al. Epidermal growth factor receptor expression status in lung cancer correlates with its mutation. *Hum Pathol* 2005;36:1127-34.
37. Shigematsu H, Lin L, Takahashi T, Nomura M, Suzuki M, Wistuba II, et al. Clinical and biological features associated with epidermal growth factor receptor gene mutations in lung cancers. *J Natl Cancer Inst* 2005;97:339-46.
38. Bell DW, Lynch TJ, Haserlat SM, Harris PL, Okimoto RA, Brannigan BW, et al. Epidermal growth factor receptor mutations and gene amplification in non-small-cell lung cancer: molecular analysis of the IDEAL/INTACT gefitinib trials. *J Clin Oncol* 2005;23:8081-92.
39. Eberhard DA, Johnson BE, Amler LC, Goddard AD, Heldens SL, Herbst RS, et al. Mutations in the epidermal growth factor receptor and in KRAS are predictive and prognostic indicators in patients with non-small-cell lung cancer treated with chemotherapy alone and in combination with erlotinib. *J Clin Oncol* 2005;23:5900-9.
40. Hirsch FR, Varella-Garcia M, Dziadziuszko R, Xiao Y, Gajapathy S, Skokan M, et al. Fluorescence in situ hybridization subgroup analysis of TRIBUTE, a phase III trial of erlotinib plus carboplatin and paclitaxel in non-small cell lung cancer. *Clin Cancer Res* 2008;14:6317-23.
41. Hirsch FR, Varella-Garcia M, Bunn Jr PA, Di Maria MV, Veve R, Bremmes RM, et al. Epidermal growth factor receptor in non-small-cell lung carcinomas: correlation between gene copy number and protein expression and impact on prognosis. *J Clin Oncol* 2003;21:3798-807.
42. Jeon YK, Sung SW, Chung JH, Park WS, Seo JW, Kim CW, et al. Clinicopathologic features and prognostic implications of epidermal growth factor receptor (EGFR) gene copy number and protein expression in non-small cell lung cancer. *Lung Cancer* 2006;54:387-98.
43. uzuki S, Dobashi Y, Sakurai H, Nishikawa K, Hanawa M, Ooi A. Protein overexpression and gene amplification of epidermal growth factor receptor in nonsmall cell lung carcinomas. An immunohistochemical and fluorescence in situ hybridization study. *Cancer* 2005;103:1265-73.
44. Kris MG, Natale R, Herbst RS, Lynch TJ Jr, Prager D, Belani CP, et al. Efficacy of gefitinib, an inhibitor of the epidermal growth factor receptor tyrosine kinase, in symptomatic patients with non-small cell lung cancer: a randomized trial. *Jama* 2003;290:2149-58.