



Clinical Note

Activating and Resistance Mutations of the Epidermal Growth Factor Receptor (EGFR) Gene and Non-Small Cell Lung Cancer: A Clinical Reality

Álvaro Taus, ^{a,*} Iván Vollmer, ^b Edurne Arriola ^a

^aServicio de Oncología Médica, Hospital del Mar, Barcelona, Spain

^bIDIMAS-CRC-Mar, Servicio de Radiología, Hospital del Mar, Barcelona, Spain

ARTICLE INFO

Article history:

Received February 18, 2010

Accepted June 10, 2010

Keywords:

Epidermal growth factor receptor

Mutation

T790M

Cancer

Lung

Tyrosine-kinase inhibitors

Erlotinib

Gefitinib

ABSTRACT

In non-small cell lung cancer, EGFR gene mutations identify a patient sub-population with different clinical characteristics and treatment responses than those who do not present these mutations. There are mutations that lead to increased sensitivity to EGFR-targeted therapy, as well as mutations that result in resistance. The determination of EGFR mutations involves a change in the therapeutic approach to lung cancer patients in current clinical practice. In this article we present a case of a patient suffering from a metastatic lung adenocarcinoma with an activating mutation on diagnosis, initially responding to treatment with erlotinib, who subsequently developed a secondary resistance due to acquiring the T790M mutation in exon 20 of the EGFR gene.

© 2010 SEPAR. Published by Elsevier España, S.L. All rights reserved.

Isoenzimas de lactatodeshidrogenasa en el músculo esquelético de pacientes con EPOC

RESUMEN

En el cáncer de pulmón de célula no pequeña, las mutaciones del gen de EGFR identifican una subpoblación de pacientes con unas características clínicas y de respuesta al tratamiento diferente de aquellos que no presentan dichas mutaciones. Existen tanto mutaciones que derivan en un aumento de sensibilidad al tratamiento dirigido contra estas alteraciones génicas como mutaciones que confieren resistencia a los mismos tratamientos. La determinación de las mutaciones de EGFR implica cambios en la actitud terapéutica de los pacientes con cáncer de pulmón en la práctica clínica habitual. En este artículo presentamos un caso de una paciente afecta de un adenocarcinoma pulmonar metastásico con una mutación activadora al diagnóstico, inicialmente respondedora a tratamiento con erlotinib, que posteriormente desarrolla una resistencia secundaria mediante la adquisición de la mutación T790M en el exón 20 del gen EGFR.

© 2010 SEPAR. Publicado por Elsevier España, S.L. Todos los derechos reservados.

Palabras clave:

Receptor del factor de crecimiento

epidérmico

Mutación

T790M

Cáncer

Pulmón

Inhibidores tirosin-kinasa

Erlotinib

Gefitinib

Introduction

Lung cancer is a tumor with poor prognosis despite the advances in the understanding of the disease and the approval of new treatments in recent years.¹ One of the greatest revolutions in the

treatment of cancer in general, and in lung cancer in particular, is the identification of molecular alterations (mutations, gene amplifications) that are responsible for tumor survival. The prognosis of patients whose tumors express molecular alterations and are treated with specific therapies is more favorable than that of the general lung cancer population. One of these targets in lung cancer is the epidermal growth factor receptor (EGFR). EGFR is part of a signaling network that is the central component of several critical cell processes, such as cell growth, proliferation and motility.²

* Corresponding author.

E-mail address: ATaus@imas.imim.es (A. Taus).

Gefitinib (Iressa®) and erlotinib (Tarceva®) are two tyrosine-kinase inhibitors (TKI) used in the treatment of non-small cell lung cancer (NSCLC) that reversibly inhibit EGFR, interrupting the transduction of its growth signal and producing an anti-tumor effect. Most of the tumors that respond to the TKI of EGFR present activating mutations in the tyrosine-kinase domain of the EGFR gene.³⁻⁵ Overall, the frequency of the mutation in NSCLC is 5-20% depending on the population studied, being more frequent in women, non-smokers, histology of adenocarcinoma and Asian ethnic origin.^{6,7} In our setting, the mutation of the EGFR gene is observed in approximately 15% of NSCLC.^{8,9} Around 75% of the patients with a mutation in the EGFR gene respond to treatment with erlotinib/gefitinib, while among the non-mutation patients only 10% respond.^{5,6,10} In spite of the good, prolonged initial response, practically all cases acquire resistance to the tyrosine-kinase inhibitors of EGFR (EGFR-TKI).¹¹

In this article, we present the case of a patient affected by metastatic pulmonary adenocarcinoma, initially responding to treatment with erlotinib, that later developed a secondary resistance by acquiring the T790 M mutation in the EGFR gene.

Clinical Case

We present the case of a non-smoker Caucasian female, who was diagnosed in May 2007, at the age of 78, with a stage IV pulmonary adenocarcinoma with bilateral pulmonary and pleural metastasis (fig. 1A), positive thyroid transcription factor 1 (TTF1), who presented the L858R mutation in the exon 21 of the EGFR gene. In June 2007, chemotherapy was initiated with one drug (oral vinorelbine 60 mg/m² days 1, 8 & 15, every 28 days) due to deterioration of the functional state (Karnofsky index 60%). In August of 2007, we observed progression of the disease at the pulmonary level, therefore treatment was begun with erlotinib at a dosage of 150 mg/d, later lowered to 100 mg/d due to cutaneous toxicity. The patient presented initial clinical and radiological response (fig. 1B), and the disease remained stable for 20 months, maintaining an optimal quality of life. In May 2009, a palpable right mammary nodule was discovered. Mammary and axillary Doppler ultrasound revealed the presence of a nodule in the lower internal quadrant of the right breast that was highly suggestive of malignancy, adenopathies with a pathological appearance throughout the entire internal mammary chain and a right axillary adenopathy. Biopsy was taken from breast lesion. Cytology of the axillary adenopathy was reported to be high-grade infiltrating carcinoma, and positive TTF1 was compatible with dissemination of the lung neoplasia. Determinations were done for mutations in the EGFR gene in the sample, observing the mentioned

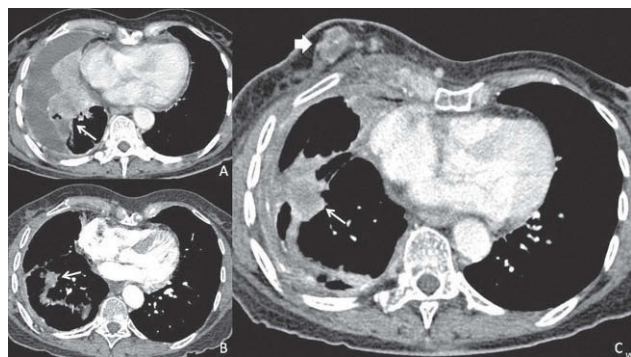


Figure 1. Chest axial tomography: A) baseline at the time of diagnosis, in which multiple pulmonary metastases and pleural effusion are observed; B) after 15 months of treatment with erlotinib, with a marked decrease in pleural effusion as well as lung parenchyma lesions; and C) progression at 20 months after the start of erlotinib, with an increase in lung affection and appearance of nodule in the breast (arrows).

mutation in exon 21 and the presence of a new mutation, T790 M in exon 20. In the extension study, cerebral and lung progression (fig. 1C) were also observed. The patient died in August 2009 due to progression of her oncological disease.

Discussion

The analysis of the clinical samples and the cell lines with sensitivity mutations in the EGFR gene after treatment with gefitinib or erlotinib that develop resistance to treatment demonstrated the presence of a secondary mutation in the exon 20 of the EGFR gene, which consists of the substitution of a methionine residue for one of threonine in the position 790 of the kinase domain (T790 M).^{12,13} This mutation is present in approximately 50% of the tumors with acquired resistance to the treatment with EGFR-TKI.^{4,13,14} There is a second known mechanism of resistance to the TK inhibitors that is the amplification of the MET oncogene.¹⁵

Given that the T790 M mutation confers resistance against erlotinib/gefitinib, increasing the affinity of EGFR for ATP,¹⁶ it is possible to overcome this resistance by developing new EGFR-TKI with greater affinity for kinase T790 M, compared with the affinity of ATP for mutated kinase. Various types of the so-called second generation EGFR-TKI are in the developmental phase. BIBW2992,¹⁷ PF00299804¹⁸ and HKI-272¹³ are examples of the new molecules that irreversibly inhibit the binding of ATP with the tyrosine-kinase domain. The clinical efficacy of these new inhibitors is limited, as they are not able to inhibit EGFR T790 M in vitro with the clinically-tolerated concentrations, and diarrhea and skin rash are the dosage-limiting toxicities. Therefore, new inhibitor drugs with greater clinical potential are being developed.¹⁹ Another strategy for overcoming resistance to erlotinib/gefitinib consists in attacking other parallel or convergent signaling pathways. The mTOR signaling pathway regulates cell proliferation, survival and mechanisms of angiogenesis, and has been implicated in the resistance to the inhibitors of EGFR. In sensitive as well as resistant cell lines, the mTOR inhibitor everolimus reduces the expression of EGFR and collaborates with gefitinib to overcome resistances.²⁰

The knowledge of the molecular alterations that define tumor subtypes in the general population with lung cancer has brought about benefits in the survival of patients with said alterations. This shows the importance of being able to determine these alterations in order to offer the patients the best treatment available for each case. Along these lines, the role of other biomarkers is being researched as a predictive factor for the response to different cytostatic agents or directed therapies in lung cancer, such as the BRCA1 gene (breast cancer susceptibility gene 1) and RAP80 (receptor associated protein 80)²¹ with the aim of individualizing the treatment for each patient.

The appearance of new therapeutic strategies for resistant disease is promising, but we have to be able to identify in clinical practice which molecular alterations cause this resistance and therefore be able to offer patients to participate in clinical assays with drugs that may be effective, such as irreversible TKI or MET inhibitor, for example. One difficulty that we have found is that in very few cases are new biopsies taken during tumor progression, impeding the collection of tumor tissue for analysis. One option would be to consider non-invasive alternatives to biopsy, like the development of techniques for detecting T790 M in plasma samples using mutant-enriched PCR assay, which has been shown to be a sensitive method that is applicable in clinical practice for the detection of EGFR gene mutations.^{22,23} It must be kept in mind that the EGFR-T790 M mutation can coexist with other resistance mutations, such as the amplification of the MET proto-oncogene,¹⁵ in the same patients in different metastasized locations as well as even within the same tumor.¹⁴ These patients could require combined treatment to cover the two resistance mechanisms.²²

Conclusion

Individualized lung cancer treatment in the subgroup of patients with sensitizing EGFR mutation has brought about a benefit in survival and quality of life in this subgroup of patients, which has not been obtained until now with modern chemotherapy. The present case reflects this reality: an older patient with metastatic lung cancer presenting a survival of more than 2 years with excellent quality of life, receiving treatment specifically directed at her tumor. Therefore, it is important to include the genetic evaluation of the mutational state of the EGFR gene of each patient with NSCLC in order to effectively identify the patients that can benefit from treatment with EGFR-TKI as well as those who present resistance mutations and need more specific therapies, such as irreversible TKI or combined treatment. The molecular study of the tumor tissue, at diagnosis as well as during progression, is essential for optimizing the management and improving the prognosis of patients diagnosed with lung cancer.

Conflict of Interest

The authors declare having no conflict of interest.

References

- Jemal A, Siegel R, Ward E, Hao Y, Xu J, Thun MJ, et al. Cancer statistics, 2009. *CA Cancer J Clin.* 2009;59:225-49.
- Shepherd FA, Rodrigues Pereira J, Ciuleanu T, Tan EH, Hirsh V, Thongprasert S, et al. Erlotinib in previously treated non-small-cell lung cancer. *N Engl J Med.* 2005;353:123-32.
- Kosaka T, Yatabe Y, Endoh H, Yoshida K, Hida T, Tsuboi M, et al. Analysis of epidermal growth factor receptor gene mutation in patients with nonsmall cell lung cancer and acquired resistance to gefitinib. *Clin Cancer Res.* 2006;12:5764-9.
- Lynch TJ, Bell DW, Sordella R, Gurubhagavatula S, Okimoto RA, Brannigan BW, et al. Activating mutations 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.
- 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.
- 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.
- Cortes-Funes H, Gómez C, Rosell R, Valero P, Garcia-Giron C, Velasco A, et al. Epidermal growth factor receptor activating mutations in Spanish gefitinib-treated non-small-cell lung cancer patients. *Ann Oncol.* 2005;16:1081-6.
- Rosell R, Moran T, Queralt C, Porta R, Cardenal F, Camps C, et al. Screening for epidermal growth factor receptor mutations in lung cancer. *N Engl J Med.* 2009;361:958-67.
- Mitsudomi T, Kosaka T, Endoh H, Horio Y, Hida T, Mori S, et al. Mutations of the epidermal growth factor receptor gene predict prolonged survival after gefitinib treatment in patients with non-small-cell lung cancer with postoperative recurrence. *J Clin Oncol.* 2005;23:2513-20.
- Morita S, Okamoto I, Kobayashi K, Yamazaki K, Asahina H, Inoue A, et al. Combined survival analysis of prospective clinical trials of gefitinib for non-small cell lung cancer with EGFR mutations. *Clin Cancer Res.* 2009;15:4493-8.
- 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.
- Li D, Shimamura T, Ji H, Chen L, Haringsma HJ, McNamara K, et al. Bronchial and peripheral murine lung carcinomas induced by T790M-L858R mutant EGFR respond to HKI-272 and rapamycin combination therapy. *Cancer Cell.* 2007;12:81-93.
- Engelman JA, Mukohara T, Zejnullahu K, Lifshits E, Borrás AM, Gale CM, et al. Allelic dilution obscures detection of a biologically significant resistance mutation in EGFR-amplified lung cancer. *J Clin Invest.* 2006;116:2695-706.
- Engelman JA, Zejnullahu K, Mitsudomi T, Song Y, Hyland C, Park JO, et al. MET amplification leads to gefitinib resistance in lung cancer by activating ERBB3 signaling. *Science.* 2007;316:1039-43.
- Yun CH, Mengwasser KE, Toms AV, Woo MS, Greulich H, Wong KK, et al. The T790M mutation in EGFR kinase causes drug resistance by increasing the affinity for ATP. *Proc Natl Acad Sci USA.* 2008;105:2070-5.
- Li D, Ambrogio L, Shimamura T, Kubo S, Takahashi M, Chirieac LR, et al. BIBW2992, an irreversible EGFR/HER2 inhibitor highly effective in preclinical lung cancer models. *Oncogene.* 2008;27:4702-11.
- Engelman JA, Zejnullahu K, Gale CM, Lifshits E, Gonzales AJ, Shimamura T, et al. PF00299804, an irreversible pan-ERBB inhibitor, is effective in lung cancer models with EGFR and ERBB2 mutations that are resistant to gefitinib. *Cancer Res.* 2007;67:11924-32.
- Zhou W, Ercan D, Chen L, Yun CH, Li D, Capelletti M, et al. Novel mutant-selective EGFR kinase inhibitors against EGFR T790 M. *Nature.* 2009;462:1070-4.
- Bianco R, Garofalo S, Rosa R, Damiano V, Gelardi T, Daniele G, et al. Inhibition of mTOR pathway by everolimus cooperates with EGFR inhibitors in human tumours sensitive and resistant to anti-EGFR drugs. *Br J Cancer.* 2008;98:923-30.
- Rosell R, Pérez-Roca L, Sánchez JJ, Cobo M, Moran T, Chaib I, et al. Customized treatment in non-small-cell lung cancer based on EGFR mutations and BRCA1 mRNA expression. *PLoS One.* 2009;4:e5133.
- Janne PA. Challenges of detecting EGFR T790M in gefitinib/erlotinib-resistant tumours. *Lung Cancer.* 2008;60 Suppl 2:S3-9.
- He C, Liu M, Zhou C, Zhang J, Ouyang M, Zhong N, et al. Detection of epidermal growth factor receptor mutations in plasma by mutant-enriched PCR assay for prediction of the response to gefitinib in patients with non-small-cell lung cancer. *Int J Cancer.* 2009;125:2393-9.