CASE REPORT

Role of YES1 amplification in EGFR mutation-positive non-small cell lung cancer: Primary resistance to afatinib in a patient

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Keywords
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Abstract
Epidermal growth factor receptor (EGFR) mutation-positive non-small cell lung cancer (NSCLC) patients benefit from EGFR tyrosine kinase inhibitors (TKIs), while some patients demonstrate a resistance to EGFR-TKIs. In the case reported here, the NSCLC patient harboring an EGFR-sensitive mutation and YES1 amplification was treated with afatinib as first-line therapy, but was found to have progressive disease four weeks later. During subsequent chemotherapy, this patient’s disease progressed rapidly. Mechanisms of primary resistance to EGFR-TKIs remain unclear. This case suggested that YES1 amplification might be associated with primary resistance to EGFR-TKIs and that YES1 amplification might be a negative predictor of EGFR-TKI treatment in NSCLC patients harboring EGFR sensitive mutations.

Introduction
Lung cancer is the leading cause of cancer mortality worldwide. Non-small cell lung cancer (NSCLC) comprises up to 90% of all lung cancers. Conventional treatment for advanced NSCLC consists of chemotherapy which only has a small impact on patient survival. Molecular targets, such as epidermal growth factor receptor (EGFR), which is involved in cell signaling have led to the development of new, targeted therapies. Patients with EGFR mutation-positive NSCLC have been reported to benefit from EGFR tyrosine kinase inhibitors (TKIs), although some patients demonstrate a resistance to this treatment. In the case reported here, the patient was diagnosed with NSCLC harboring an EGFR-sensitive mutation and YES1 amplification and was treated with afatinib as first-line therapy. However, he was determined to have progressive disease four weeks later and after subsequent chemotherapy, his disease progressed rapidly. Mechanisms of primary resistance to EGFR-TKIs remain unclear. This case suggested that YES1 amplification might be associated with primary resistance to EGFR-TKIs and that YES1 amplification might be a negative predictor of EGFR-TKI treatment in NSCLC patients harboring EGFR sensitive mutations.

Case report
A 68-year-old never-smoking male who complained of left chest pain without apparent cause visited our hospital on 23 June 2019. He was diagnosed with stage IV lung adenocarcinoma with intrapulmonary, osseous and mediastinal lymph node metastasis and pleural effusion as shown in Fig 1a. Next-generation sequencing (NGS) examination was performed to evaluate the genomic alterations of this patient before treatment and the results of NGS examination are provided in Table 1. It revealed that the patient harbored the missense mutation in exon 21 p.L858R of EGFR, YES1 amplification, CCND1 amplification, FGF19 amplification, FGF3 amplification and FGF4 amplification.

The patient received first-line afatinib therapy on 12 July 2019, but chest computed tomography (CT) on day 14 of treatment showed a slight enlargement of the left primary tumor. However, after four weeks of afatinib treatment, the
The tumor had enlarged significantly and the pleural effusion increased rapidly, resulting in a classification of progressive disease (PD) as shown in Fig 1b, and as evaluated according to Response Evaluation Criteria in Solid Tumors 1.1 (RECIST 1.1). After two cycles of second-line chemotherapy including pemetrexed plus cisplatin, the patient experienced further PD. The patient was administered third-line therapy of the regimen which consisted of albumin-bound paclitaxel, cisplatin and bevacizumab. Unfortunately, the patient’s disease was evaluated as PD after only one cycle treatment of this regimen.

Discussion

Approximately 37.5%–51.4% of Chinese patients with advanced NSCLC have been found to have somatic activating mutations in EGFR.\textsuperscript{1–3} Patients with NSCLC who have somatic mutations of the EGFR gene have been found to benefit from EGFR-TKIs including gefitinib, erlotinib, afatinib, dacomitinib, osimertinib and so on. In previous studies, targeted therapies have led to longer progression-free survival (PFS) compared to treatment with platinum-based chemotherapy.\textsuperscript{4–6} However, about 20% of NSCLC patients have less or no benefit from EGFR-TKIs because of various primary resistance mechanisms. In this case, the EGFR-positive NSCLC patient harboring YES1 amplification demonstrated a primary resistance to afatinib.

Several mechanisms have been reported to be associated with decreased sensitivity to EGFR-TKIs in EGFR mutation patients, including T790M mutation,\textsuperscript{7} exon 20 insertion,\textsuperscript{8} MET amplification,\textsuperscript{9} overexpression of hepatocyte growth factor,\textsuperscript{10} overexpression of NOTCH3,\textsuperscript{11} anaplastic lymphoma kinase (ALK) positive,\textsuperscript{12} loss of PTEN,\textsuperscript{13} activation of IGFR signaling,\textsuperscript{14} NF-κB pathways,\textsuperscript{15} and STAT3...
Table 1  Next-generation sequencing (NGS) results

| Gene name | Alteration                    | Abundance | Targeted drug (Sensibility. evidence level) |
|-----------|-------------------------------|-----------|-------------------------------------------|
| EGFR      | exon 21 p.L858R missense mutation | 25.23%    | Gefitinib (Sensibility. A)                  |
|           |                               |           | Erlotinib (Sensibility. A)                  |
|           |                               |           | Afatinib (Sensibility. A)                   |
|           |                               |           | Dacomitinib (Sensibility. A)                |
|           |                               |           | Crizotinib (Sensibility. A)                 |
| YES1      | Amplification                 | CN: 3.0   | Pembrolizumab (resistance. D)              |
|           |                               |           | Nivolumab (resistance. D)                  |
| CCND1     | Amplification                 | CN: 3.6   | Pembrolizumab (resistance. D)              |
|           |                               |           | Nivolumab (resistance. D)                  |
| FGF19     | Amplification                 | CN: 4.0   | Pembrolizumab (resistance. D)              |
|           |                               |           | Nivolumab (resistance. D)                  |
| FGF3      | Amplification                 | CN: 3.9   | Pembrolizumab (resistance. D)              |
|           |                               |           | Nivolumab (resistance. D)                  |
| FGF4      | Amplification                 | CN: 3.8   | Pembrolizumab (resistance. D)              |
|           |                               |           | Nivolumab (resistance. D)                  |

NGS assay prior to EGFR-TKI therapy. The genes without certain clinical significance were omitted. In this case, NGS assay was performed by Burning Rock Biotech based on Illumina sequencing platform.

signaling. In this case, the genetic test report showed the patient harbored the missense mutation in exon 21 p. L858R of EGFR (25.23%), YES1 amplification (CN:3.0), CCND1 amplification (CN:3.6), FGF19 amplification (CN:4.0), FGF3 amplification (CN:3.9) and FGF4 amplification (CN:3.8). FGF3, FGF4, FGF19 and CCND1 were co-localized on the same chromosomal region (11q13). Previous studies have shown no significant difference in overall survival or –median PFS for first-line therapy in patients with FGF/FGFR-aberrant or wild-type tumors, and that EGFR inhibitors could reduce CCND1 expression via eIf2α phosphorylation. In the case reported here, we therefore conclude that YES1 amplification, rather than FGF/CCND1 might be the critical cause of primary resistance to afatinib.

YES1 is a member of the SRC family kinases (SFKs) which regulate the proliferation, survival, angiogenesis, invasion and migration of cancer cells. Research has shown that YES1 plays a role in nuclear translocation of EGFR and acquired resistance to EGFR inhibitors in EGFR mutation positive NSCLC. However, the relationship between YES1 amplification and primary resistance of EGFR-TKIs remains unclear.

YES1 amplification has been found in 15% of lung adenocarcinoma and 25% of lung squamous cell carcinoma patients, and YES1 expression was reported to be related to a shorter OS in NSCLC patients. Garmendia et al. demonstrated that YES1 amplification induces tumor growth as an oncogenic driver alteration in NSCLC. High YES1 protein expression was an independent predictor for poor prognosis in patients with NSCLC, and they indicated that SFKs may serve as potential therapeutic targets by the examination of YES1 genetic alteration in NSCLC.

On 12 January 2018, the Food and Drug Administration (FDA) granted approval to afatinib for a broadened indication in the first-line treatment of patients with metastatic NSCLC whose tumors had nonresistant EGFR mutations as detected by an FDA-approved test. Therefore, in the future, there will be a greater choice of EGFR-TKIs for clinicians to treat patients with EGFR mutation-positive tumors. However, our case suggested that afatinib, including other first line EGFR-TKIs, should be given carefully to patients harboring EGFR mutations combined with YES1 amplification.

Few cases of primary resistance to afatinib for patients harboring EGFR exon 21 L858R missense mutation have been reported and here we present the first case with concurrent alterations of EGFR exon 21 L858R missense mutation and YES1 amplification. Garmendia et al. demonstrated that YES1 amplification presented a high sensitivity to dasatinib, an SFK inhibitor. Another study revealed that the disruption of the SFK pathway may remain a viable method of overcoming TKI resistance. Dasatinib combined with EGFR-TKIs treatment may therefore benefit patients with concurrent alteration of YES1 amplification and EGFR sensitive mutation but this should be verified in future studies.

In conclusion, we suggest that YES1 mutation status should be assessed before EGFR-TKIs treatment in patients with NSCLC harboring EGFR sensitive mutation in clinical
practice, and we believe that clinical research and trials of dasatinib combined with EGFR-TKIs in the use of patients with concurrent alteration of YES1 amplification and EGFR mutation-positive are warranted in the future.

**Disclosure**

The authors report no conflicts of interest.

**References**

1. Li F, du X, Zhang H et al. Next-generation sequencing of Chinese stage IV lung cancer patients reveals an association between EGFR mutation status and survival outcome. *Clin Genet* 2017; 91 (3): 488–93.
2. Pi C, Xu CR, Zhang MF et al. EGFR mutations in early-stage and advanced-stage lung adenocarcinoma: Analysis based on large-scale data from China. *Thorac Cancer* 2018; 9 (7): 814–9.
3. Wang S, Wang Z. EGFR mutations in patients with non-small cell lung cancer from mainland China and their relationships with clinicopathological features: A meta-analysis. *Int J Clin Exp Med* 2014; 7 (8): 1967–78.
4. Rosell R, Carcereny E, Gervais R et al. Erlotinib versus standard chemotherapy as first-line treatment for European patients with advanced EGFR mutation-positive non-small-cell lung cancer (EURTAC): A multicentre, open-label, randomised phase 3 trial. *Lancet Oncol* 2012; 13 (3): 239–46.
5. Sequist LV, J-CH Y, Yamamoto N et al. Phase III study of afatinib or cisplatin plus pemetrexed in patients with advanced EGFR mutation-positive non-small-cell lung cancer: Results from WJTOG3405. *Am J Respir Crit Care Med* 2010; 182 (4): 335–42.
6. Mitsudomi T, Morita S, Yatabe Y et al. Gefitinib versus cisplatin plus docetaxel in patients with non-small-cell lung cancer harbouring mutations of the epidermal growth factor receptor (WJTOG3405): An open label, randomised phase 3 trial. *Lancet Oncol* 2010; 11 (2): 121–8.
7. Watanabe H, Arcila ME, Hellmann MD, Kris MG, Ladanyi M, Riely GJ. Poor response to erlotinib in patients with tumors containing baseline EGFR T790M mutations found by routine clinical molecular testing. *Ann Oncol* 2014; 25 (2): 423–8.
8. Yasuda H, Kobayashi S, Costa DB. EGFR exon 20 insertion mutations in non-small-cell lung cancer: Preclinical data and clinical implications. *Lancet Oncol* 2012; 13 (1): e23–31.
9. Engelman JA, Zeijlmolhu K, Mitsudomi T et al. MET amplification leads to gefitinib resistance in lung cancer by activating ERBB3 signaling. *Science* 2007; 316 (5827): 1039–43.
10. Gusenbauer S, Vlaicu P, Ullrich A. HGF induces novel EGFR functions involved in resistance formation to tyrosine kinase inhibitors. *Oncogene* 2013; 32 (33): 3846–56.
11. Zhang Y, Chen B, Wang Y et al. NOTCH3 overexpression and posttranscriptional regulation by miR-150 were associated with EGFR-TKI resistance in lung adenocarcinoma. *Oncol Res* 2019; 27 (7): 751–61.
12. Won JK, Keam B, Koh J et al. Concomitant ALK translocation and EGFR mutation in lung cancer: A comparison of direct sequencing and sensitive assays and the impact on responsiveness to tyrosine kinase inhibitor. *Ann Oncol* 2015; 26 (2): 348–54.
13. Sos ML, Koker M, Weir BA et al. PTEN loss contributes to erlotinib resistance in EGFR-mutant lung cancer by activation of Akt and EGFR. *Cancer Res* 2009; 69 (8): 3256–61.
14. Choi YJ, Rho JK, Jeon BS et al. Combined inhibition of IGFR enhances the effects of gefitinib in H1650: A lung cancer cell line with EGFR mutation and primary resistance to EGFR-TK inhibitors. *Cancer Chemother Pharmacol* 2010; 66 (2): 381–8.
15. Bivona TG, Hieronymus H, Parket J et al. FAS and NF-kappaB signalling modulate dependence of lung cancers on mutant EGFR. *Nature* 2011; 471 (7393): 523–6.
16. Chiu HC, Chou DL, Huang CT et al. Suppression of Stat3 activity sensitizes gefitinib-resistant non small cell lung cancer cells. *Biochem Pharmacol* 2011; 81 (11): 1263–70.
17. Koyama S, Omura T, Yonezawa A et al. Gefitinib and Erlotinib lead to phosphorylation of eukaryotic initiation factor 2 alpha independent of epidermal growth factor receptor in A549 cells. *PLOS One* 2015; 10 (8): e0136176.
18. Parish A, Schwaederle M, Daniels G et al. Fibroblast growth factor family aberrations in cancers: Clinical and molecular characteristics. *Cell Cycle* 2015; 14 (13): 2121–8.
19. Summy J, Gallick GE. Src family kinases in tumor progression and metastasis. *Cancer Metastasis Rev* 2003; 22 (4): 337–58.
20. Iida M, Brand T, Campbell DA, Li C, Wheeler DL. Yes and Lyn play a role in nuclear translocation of the epidermal growth factor receptor. *Oncogene* 2013; 32 (6): 759–67.
21. Fan PD, Narzisi G, Jayaprakash AD et al. YES1 amplification is a mechanism of acquired resistance to EGFR inhibitors identified by transposon mutagenesis and clinical genomics. *Proc Natl Acad Sci U S A* 2018; 115 (26): E6030–8.
22. Li HY, Carr LL. Predictive biomarkers of response to Src inhibitors in lung cancer. Getting to YES1. *Am J Respir Crit Care Med* 2019; 200 (7): 802–4.
23. Garmentia I, Pajares MJ, Hermida-Prado F et al. YES1 drives lung cancer growth and progression and predicts sensitivity to Dasatinib. *Am J Respir Crit Care Med* 2019; 200 (7): 888–99.
24. Cereen BC, Gray JE, Tanvetyanon T et al. Phase I trial of dasatinib combined with afatinib for epidermal growth factor receptor- (EGFR-) mutated lung cancer with acquired tyrosine kinase inhibitor (TKI) resistance. *Br J Cancer* 2019; 120 (8): 791–6.