

EGFR-mutated pulmonary adenocarcinoma with concurrent PIK3CA mutation, and with acquired RET fusion and EGFR T790M mutation after afatinib therapy

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Generally, receptor tyrosine kinase (*RTK*) fusions are mutually exclusive of epidermal growth factor receptor (*EGFR*) mutations in non-small cell lung cancer (NSCLC). However, *RTK* fusions have recently emerged as mechanisms of actionable resistance to *EGFR*-tyrosine kinase inhibitors (TKIs) in *EGFR*-mutated NSCLC [1]. More than half of the acquired *RET* fusions following *EGFR*-TKI therapy occur in response to osimertinib (third-generation) treatment. To the best of our knowledge, only one case of NSCLC with acquired *RET* fusion following afatinib (second-generation) therapy has been reported in the English literature. Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (*PIK3CA*) mutations are found infrequently (approximately 7%) in pulmonary adenocarcinoma [2]. Although other oncogenic driver mutations in NSCLC are mutually exclusive, *PIK3CA* mutations frequently coexist with other mutations [2,3]. Here, we report a rare case of *EGFR*-mutated pulmonary adenocarcinoma with concurrent *PIK3CA* mutation, displaying acquired *RET* fusion and *EGFR* T790M mutation following afatinib therapy.

CASE REPORT

A 64-year-old man without a known underlying disease was

transferred with dyspnea and pleural effusion. He was a smoker with a 30 pack-year history. The clinical course is shown in Fig. 1. The cytological examination of the pleural fluid confirmed the diagnosis as metastatic adenocarcinoma of the lung (Fig. 1A). Chest computed tomography (CT) (Fig. 2A) suggested lung malignancy with pleural and lymph node metastases. Positron emission tomography showed a 3-cm-sized hypermetabolic mass in the right upper lobe of the lung. CT-guided needle biopsy was performed on the mass in the right upper lobe, and adenocarcinoma was diagnosed (Fig. 1B). The *EGFR* mutation status was tested using real-time polymerase chain reaction clamping method on the pleural fluid and our results revealed exon 19 deletion. The patient received afatinib treatment, and as a result, the size of the lung mass and amount of pleural fluid were found to be decreased on chest CT after 3 months (Fig. 2B). However, the disease was stable for 6 months, and next-generation sequencing (NGS) was performed on the previous needle biopsy specimen of the lung on an Ion Torrent S5 sequencer (Thermo Fisher Scientific, Waltham, MA, USA) using a commercially available targeted gene panel (OncoPrint Comprehensive Assay v3, Thermo Fisher Scientific). The NGS results showed exon 19 deletion of *EGFR* and *PIK3CA* G118D mutation. The disease progressed in 7.5 months (Fig. 2C) and afatinib had to be discontinued. Video-assisted thoracoscopic wedge resection was performed on the right upper lobe of the lung, and the status of the *EGFR* mutation was re-evaluated. Microscopic examination of the specimen showed tumor heterogeneity with solid and cribriform components (Fig. 1C), and acinar and papillary components (Fig. 1D). A second *EGFR* mutation test showed T790M

Received: September 1, 2020 Revised: October 21, 2020

Accepted: November 2, 2020

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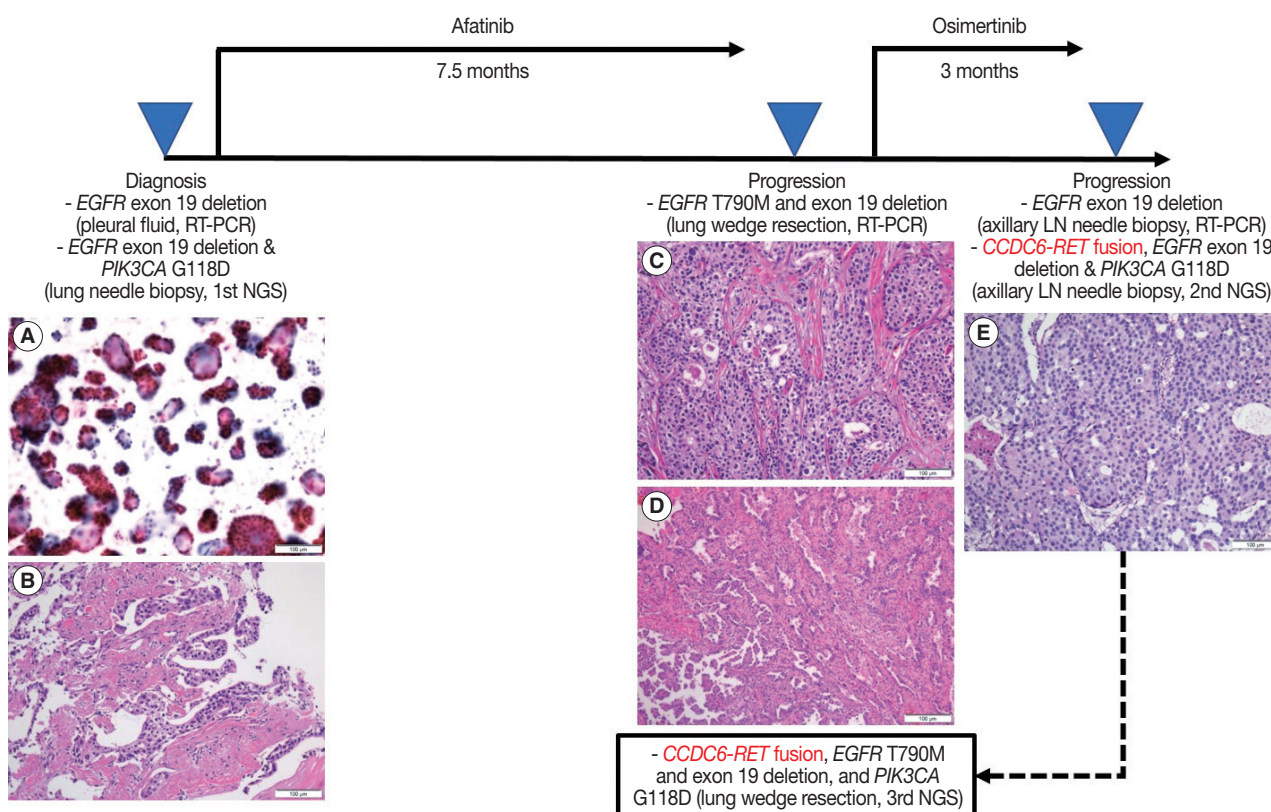


Fig. 1. Clinical course and pathological diagnosis of the patient. Adenocarcinoma was diagnosed using pleural fluid (A), and lung needle biopsy (B). Lung wedge resection specimen showed tumor heterogeneity; solid and cribriform components (C), and acinar and papillary components (D). (E) Metastatic adenocarcinoma was observed in the axillary LN. *EGFR*, epidermal growth factor receptor; *PIK3CA*, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha; NGS, next-generation sequencing; RT-PCR, reverse transcription; LN, lymph node.

mutation and exon 19 deletion. The patient was prescribed with osimertinib, but the disease progressed in 3 months (Fig. 2D). Needle biopsy of the right axillary lymph node was performed for further molecular evaluation of the cancer. Metastatic adenocarcinoma with a solid and cribriform pattern was observed in the lymph node (Fig. 1E). The third *EGFR* mutation test showed exon 19 deletion but not T790M mutation. The second NGS test on axillary lymph node specimens revealed a new *CCDC6-RET* fusion in addition to exon 19 deletion of *EGFR* and *PIK3CA* G118D mutation. Additionally, a third NGS test was performed on the wedge resection specimen (second biopsy), which showed *CCDC6-RET* fusion with T790M mutation and exon 19 deletion of *EGFR* and *PIK3CA* G118D.

DISCUSSION

Viola et al. analyzed 86 cases of *RTK* fusions as acquired resistance in *EGFR*-mutated NSCLC [1]. The acquired *RTK* fusions occurred most frequently (57%) after the third generation *EGFR*-TKI therapy (first, 24%; second, 12%). The most fre-

quently reported acquired *RTK* fusion in *EGFR*-TKI-resistant NSCLC was *RET* fusion, whereas *CCDC6-RET* fusion was the most common variant. Combined *EGFR* and *RET* inhibition with osimertinib and BLU-667 may be an effective therapeutic strategy in *EGFR*-TKI-resistant NSCLC with acquired *RTK* fusion [4]. The concurrent *PIK3CA* mutation is a known poor prognostic and predictive marker for *EGFR*-TKI therapy in pulmonary adenocarcinomas [2]. *CCDC6-RET* fusion and *PIK3CA* G118D mutation are not the most common variants in NSCLC with a single oncogenic driver mutation [1,5,6].

In this case, considering the third NGS result, the *CCDC6-RET* fusion probably occurred with *EGFR* T790M mutation after afatinib treatment. In lung wedge specimens resected after afatinib treatment, we observed tumor heterogeneity (Fig. 1C, D). We could not confirm which tumor population had *CCDC6-RET* fusion or *EGFR* T790M mutation or both. Considering morphological features (Fig. 1C, E), we assumed that tumor population with solid and cribriform patterns (Fig. 1C) had *CCDC6-RET* fusion.

In conclusion, we report a rare case of *EGFR*-mutated pul-

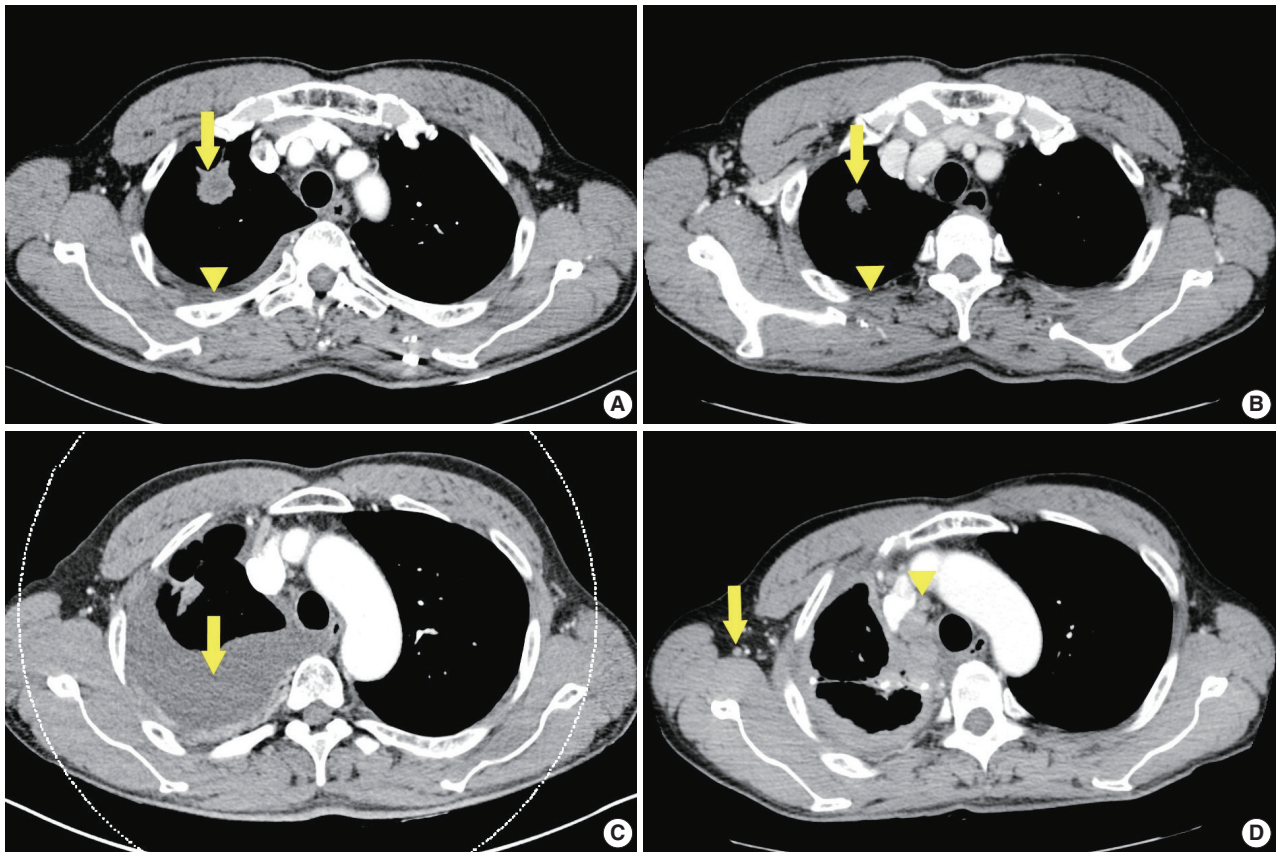


Fig. 2. Chest computed tomography. (A) Lung mass (arrow) in right upper lobe with pleural fluid (arrowhead) at diagnosis. (B) Decreased size of lung mass (arrow) and amount of pleural fluid (arrowhead) after 3 months of afatinib therapy. (C) Increased amount of pleural fluid (arrow) after 7.5 months with afatinib therapy. (D) Increased size of axillary (arrow) and mediastinal (arrowhead) lymph nodes after 3 months with osimertinib therapy.

monary adenocarcinoma with concurrent *PIK3CA* mutation, and acquired *RET* fusion and *EGFR* T790M mutation after *EGFR*-TKI therapy. Since NSCLC has a number of well-known oncogenic driver mutations, we believe that NGS is currently one of the best methods to determine the treatment of NSCLC, especially adenocarcinoma.

Ethics Statement

This study was approved by the Institutional Review Board of Gyeongsang National University Hospital, and informed consent was waived (IRB No. GNUH 2020-08-005).

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Conceptualization: JWY. Data Curation: MK, JWY. Investigation: MK, JMN, JWY. Resources: GWL, SJL, JDK. Supervision: JWY. Visualization: MK, JWY. Writing—original draft preparation: MK, JWY. Writing—review and editing: MK, JWY. Approval of final manuscript: all authors.

Conflicts of Interest

The authors declare that they have no potential conflicts of interest.

Funding Statement

No funding to declare.

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