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CASE REPORT

A case of *EGFR* mutant lung adenocarcinoma that acquired resistance to EGFR-tyrosine kinase inhibitors with *MET* amplification and epithelial-to-mesenchymal transition

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Abstract: *EGFR* mutant lung cancer responds to EGFR tyrosine kinase inhibitors (TKIs), but all patients eventually develop resistance to EGFR-TKIs. Herein we report a case of *EGFR* mutant lung adenocarcinoma that acquired resistance to EGFR-TKI with *MET* amplification and epithelial-to-mesenchymal transition (EMT). A 73-year-old woman was diagnosed with adenocarcinoma harboring an *EGFR* exon 19 deletion. She received gefitinib as second-line therapy. Tumors were reduced 1 month after gefitinib therapy. However, only a few months later, chest computed tomography results indicated cancer progression. Gefitinib therapy was stopped and docetaxel therapy started. However, she died 13 days after admission. Microscopic examination of postmortem specimens revealed a diffuse proliferation of atypical giant cells in primary and metastatic lesions, but no adenocarcinomatous components as in the antemortem specimens. Immunohistochemical analyses showed that antemortem tumor specimens were positive for CDH1 but negative for VIM. In contrast, postmortem tumor specimens were positive for VIM but negative for CDH1. Genetic analyses revealed *MET* amplification. We concluded that resistance to EGFR-TKI might be caused by *MET* amplification and EMT. To our knowledge, no clinical studies have reported that *MET* amplification and EMT together may be associated with acquired resistance to EGFR-TKI. Second biopsy after the development of EGFR-TKI resistance may be recommended to determine the best therapeutic strategy.

Keywords: epidermal growth factor receptor tyrosine kinase inhibitor, *MET* amplification, epithelial-to-mesenchymal transition

Background

Patients with *EGFR* mutant lung cancer derive significant therapeutic benefit from treatment with EGFR tyrosine kinase inhibitors (TKIs). However, acquired resistance is an inevitable consequence of this treatment strategy, with a broad variety of resistance mechanisms.[1,2] Herein we report a case of potential acquired resistance to EGFR-TKI in primary lung adenocarcinoma with both *MET* amplification and epithelial-to-mesenchymal transition (EMT).

Case report

A 73-year-old woman was admitted to our hospital due to progressive dyspnea. She had been diagnosed with T2bN1M1a adenocarcinoma (stage IV with visceral pleural nodules) harboring an *EGFR* exon 19 deletion by computed tomography (CT)-guided lung tumor biopsy (biopsy was performed twice) and by visceral pleural nodule biopsy using video-assisted thoracoscopy (biopsy was performed once) (Figure 1). She had

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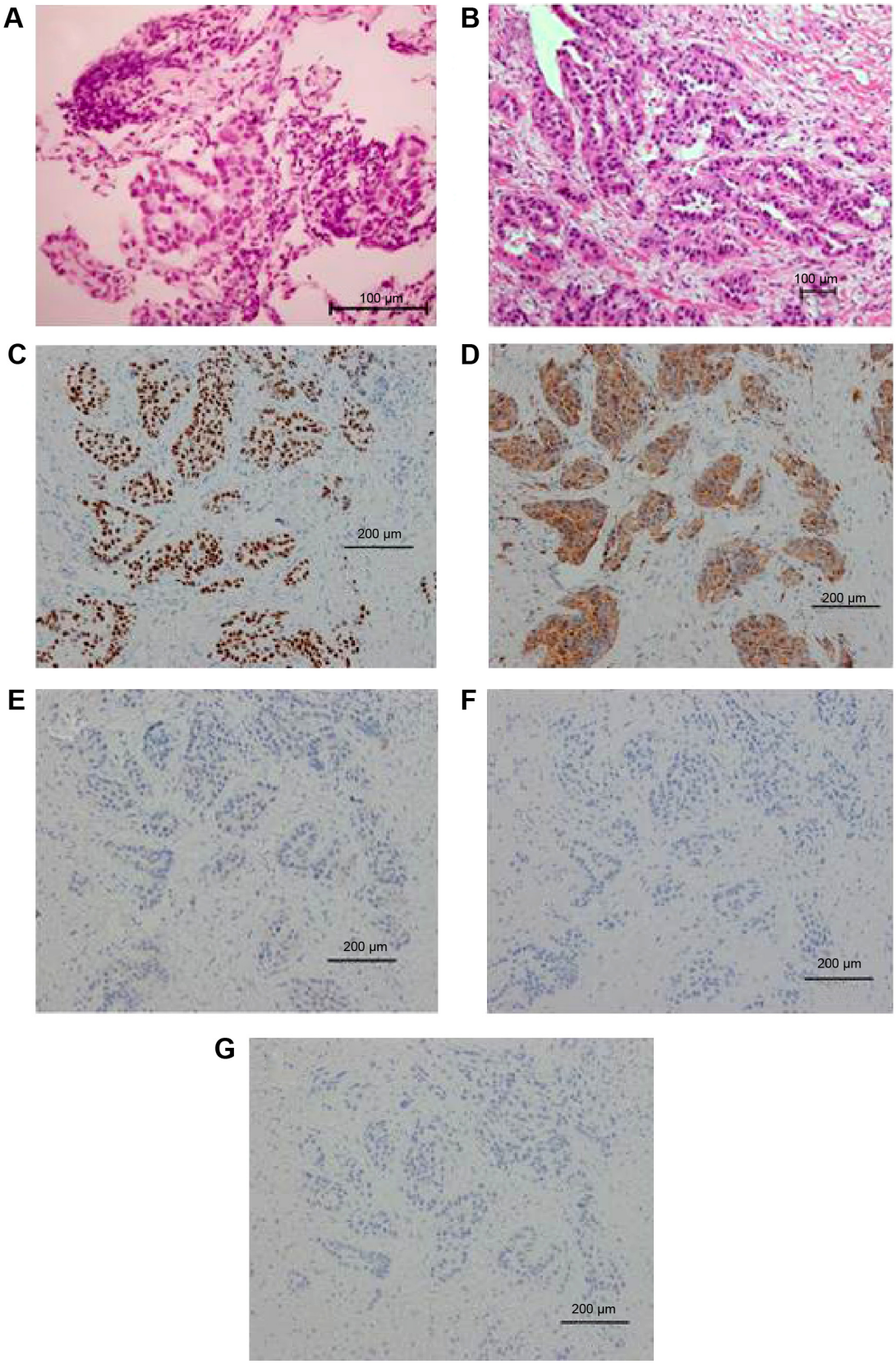

Figure 1 Microscopic findings in the antemortem specimens.
Notes: Hematoxylin and eosin staining for computed tomography-guided lung tumor biopsy specimens (**A**) and visceral pleura specimens using video-assisted thoracoscopic biopsy (**B**) revealed adenocarcinoma. Immunohistochemical analyses showed that tumor cells were positive for TTF-1 (**C**) and NAPSA (**D**), and negative for CK 5/6 (**E**), CgA (**F**) and SYP (**G**).
Abbreviations: CgA, chromogranin; CK 5/6, cytokeratin 5/6; NAPSA, Napsin A; TTF-1, thyroid transcription factor-1.

a performance status of 1 and was a never smoker. As first-line chemotherapy, she received carboplatin and pemetrexed, because there have been no reports that using EGFR-TKI, compared with cytotoxic agents, as first-line chemotherapy significantly prolongs the overall survival of patients with *EGFR* mutant lung cancer. In addition, our patient had a good performance status that withstood cytotoxic chemotherapy at the time of disease diagnosis. After a five-course regimen, progressive disease was observed, and gefitinib was administered as second-line therapy. Chest CT showed that the tumor and right hilar lymphadenopathy were reduced 1 month after gefitinib therapy was initiated (the longest dimension of the tumor decreased by 62.1% after gefitinib therapy) (Figure 2A–D). However, only a few months after gefitinib therapy (on the present admission), CT showed atelectasis in the right middle and lower lobes (Figure 2E and F). Upon suspicion of recurrence, gefitinib therapy was stopped and docetaxel therapy started as third-line chemotherapy according to the Japanese Clinical Practice Guideline for Lung Cancer.[3] However, she died 13 days after admission.

We performed an autopsy on the patient with her son's consent. All organs within the chest were resected. Postmortem macroscopic examination showed a tumor in the right lower lobe and right hilar and mediastinal lymphadenopathy. Tumor invasion was also observed in the esophagus and trachea. Surprisingly, microscopic examination revealed a diffuse proliferation of atypical giant cells in the primary and metastatic lesions, but no adenocarcinomatous components such as those seen in the antemortem specimens were observed (Figure 3). We assessed CDH1 and VIM expression using immunohistochemistry in both antemortem and postmortem specimens. The antemortem pretreatment specimens showed staining for CDH1, but not for VIM. In contrast, postmortem specimens stained for VIM, but not for CDH1. These results suggested that the resistant tumor had undergone an EMT, which caused a phenotypic transformation to giant cell carcinoma.

Using the antemortem and postmortem specimens, we also evaluated *EGFR* mutations, *PIK3CA* mutations, and *MET* gene amplification (Table 1 and Figure 3). Genetic analyses of the *EGFR* gene and *PIK3CA* gene were performed using direct sequencing. *MET* amplification was analyzed by fluorescent in situ hybridization. Signals were enumerated in at least 60 tumor nuclei per core. We considered *MET*-positive cases to have a mean ≥5 copies per cell according to a previous report.[4] *EGFR* exon 19 deletion was observed in both specimens. *MET* amplification was also observed in both specimens, but *MET* gene copy numbers of postmortem tumor cells were higher than those of antemortem tumor cells. In addition, the expression of HGF, which is a ligand of MET, increased in postmortem, compared with antemortem, tumor cells. We concluded that resistance to EGFR-TKI might be caused by *MET* amplification and EMT.

Discussion

The reported mechanisms underlying acquired EGFR-TKI resistance include *EGFR* T790M mutation, *PIK3CA* mutation, *MET* amplification, small cell lung cancer (SCLC) transformation, and EMT.[1,2] In the present case, we considered that *MET* amplification and EMT together may cause resistance to EGFR-TKI. To our knowledge, no clinical studies have

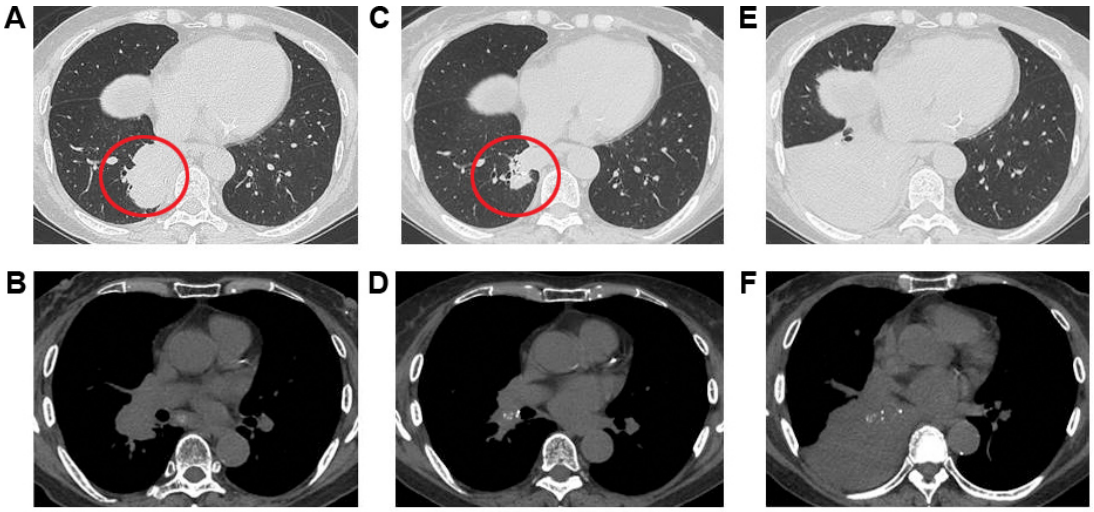

Figure 2 Chest computed tomography (CT) images.
Notes: CT performed before gefitinib treatment showed a mass shadow in the right S^{10} (red circle) and right hilar lymphadenopathy (**A** and **B**). One month after gefitinib therapy, these shadows were reduced (**C** and **D**). However, only a few months after gefitinib therapy (on the present admission), chest CT showed atelectasis in the right middle and lower lobes (**E** and **F**).

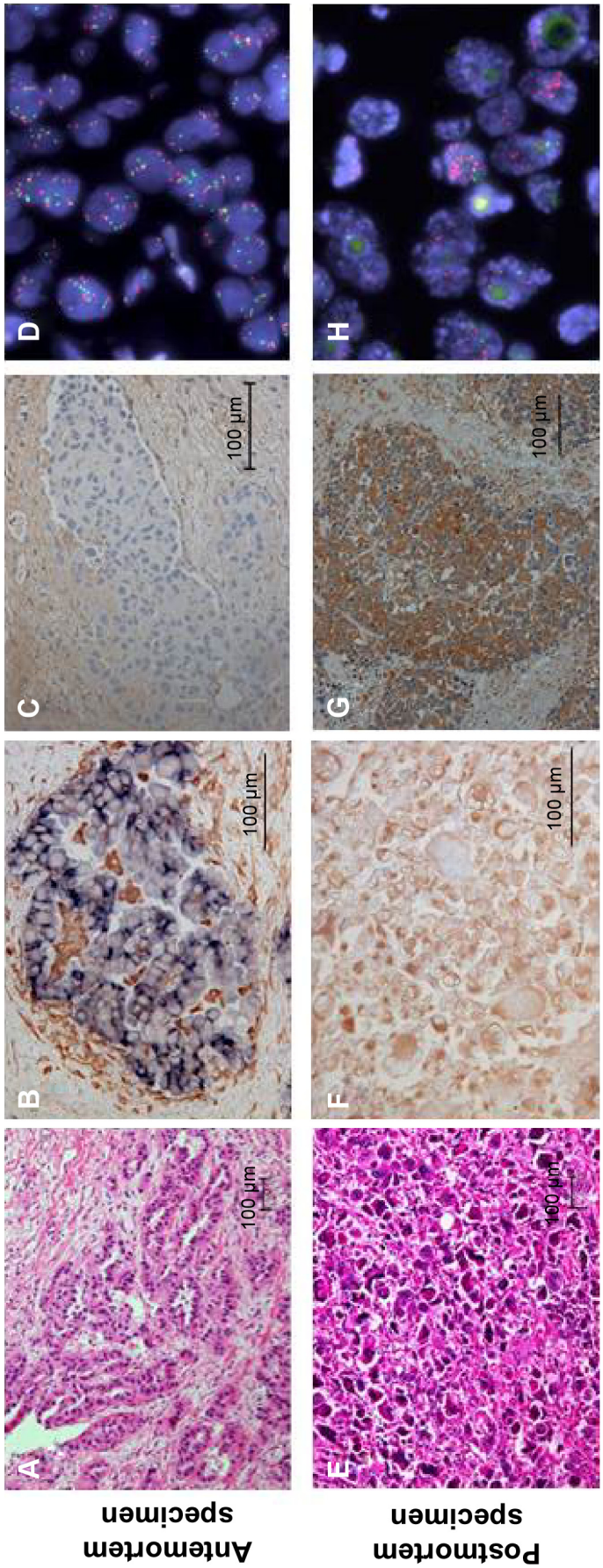

Figure 3 Antemortem and postmortem specimens analysis.
Notes: Antemortem (**A**–**D**) and postmortem specimens (**E**–**H**) analysis. Hematoxylin and eosin staining (**A** and **B**). Double immunohistochemical staining of CDH1 (in blue) and VIM (in brown) (**B** and **F**). Immunohistochemical staining of HGF (**C** and **G**). *MET* gene translocation (fluorescence in situ hybridization, red signal: *MET* gene probe, green signal: Centromere enumeration probe 7) (**D** and **H**).
Abbreviations: CDH1, E-cadherin; HGF, hepatocyte growth factor; VIM, vimentin.

Table 1 Analyses of genetic mutations or amplification in antemortem and postmortem specimens

Histological type	Antemortem specimen	Postmortem specimen
	Adenocarcinoma	Giant cell carcinoma
EGFR exon18 G719X	(–)	(–)
EGFR exon19 Deletion	(+)	(+)
EGFR exon20 T790M	(–)	(–)
EGFR exon21 L858R	(–)	(–)
EGFR exon21 L861Q	(–)	(–)
MET amplification	(+) (5.2/cell)	(+) (15.4/cell)
PIK3CA exon9 E542K	(–)	(–)
PIK3CA exon9 E545K/D	(–)	(–)
PIK3CA exon20 H1047R	(–)	(–)

Notes: +, positive for genetic mutation or amplification; –, negative for genetic mutation or amplification. *EGFR* exon 19 deletion was observed in both specimens. *MET* amplification was also observed in both specimens, but *MET* gene copy numbers of postmortem tumor cells were higher than those of antemortem tumor cells. The other genetic mutations were negative in both specimens.

Abbreviations: EGFR, epidermal growth factor receptor; PIK3CA, phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha.

reported both *MET* amplification and EMT to be associated with acquired resistance to EGFR-TKI.

The association between *MET* amplification and EMT is unclear. However, one in vitro study showed that EGFR-TKI resistance in a non-SCLC cell line harboring *MET* amplification induced EMT following gefitinib removal.[5] The present case also showed that *EGFR* mutant adenocarcinoma harboring *MET* amplification had undergone an EMT, suggesting these mechanisms may lead to rapid progression of tumors.

Second biopsy after the development of EGFR-TKI resistance may be recommended to determine the best therapeutic strategy. The characterization of the mechanisms underlying acquired resistance to EGFR-TKIs has led to the design of several clinical trials.[2] In addition, Sequist et al reported sensitivity to standard SCLC treatments in SCLC tumors transformed from non-SCLC.[1]

Conclusion

We describe EGFR-TKI resistance acquired in non-SCLC with *MET* amplification and EMT. When *EGFR* mutant tumors acquire resistance to EGFR-TKI, second biopsy may be recommended to select the best therapeutic strategy.

Disclosure

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