SMAD4 Mutation in Small Cell Transformation of Epidermal Growth Factor Receptor Mutated Lung Adenocarcinoma

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Disclosures of potential conflicts of interest may be found at the end of this article.

ABSTRACT

The large screening of exons 18 to 21 of the epidermal growth factor receptor (*EGFR*) gene may lead to the discovery of rare, atypical molecular alterations for which the sensitivity to tyrosine kinase inhibitors (TKIs) remains uncertain. We are reporting a rare exon 18 *EGFR* mutation

(p.E709_710 > D) that confers sensitivity to second-generation *EGFR* TKI (afatinib), lasting for 1 year. Tumor progression biopsy showed small cell lung cancer transformation, associated with a *SMAD4* mutation. *The Oncologist* 2019;24:9–13

KEY POINTS

- A rare exon 18 epidermal growth factor receptor mutation with sensitivity to afatinib is reported.
- Small cell transformation was observed at tumor progression.
- Acquisition of a *SMAD4* mutation was observed at tumor progression.

PATIENT STORY

A 57-year-old female presented at our hospital for hemoptysis. She had no remarkable medical history. She was a nonsmoker and had no history of passive smoking. An upper left lobe lung adenocarcinoma with pathologically documented mediastinal lymph node infiltration (4R and 4 L) was discovered. The clinical stage was cT2N3M0 (seventh TNM classification).

Endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) was performed on the left and right laterotracheal and subcarinal lymph nodes. Cytological examination revealed malignant TTF1 positive glandular cells, consistent with the diagnosis of lung adenocarcinoma (Fig. 1A, 1B).

AKT1, *ALK*, *BRAF*, *CTNNB1*, *DDR2*, epidermal growth factor receptor (*EGFR*), *ERBB2*, *ERBB4*, *FBXW7*, *FGFR1*, *FGFR2*, *FGFR3*, *KRAS*, *MAP2K1*, *MET*, *NOTCH1*, *NRAS*, *PIK3CA*, *PTEN*, *SMAD4*, *STK11* and *TP53*, as already described [1]. An atypical deletion in exon 18 of the *EGFR* gene was detected: p. E709_710 > D with an allelic frequency of 25% (Fig. 1C) without any other molecular alteration.

Functional and Clinical Significance

The delE709_T710insD in exon 18 of the *EGFR* gene is a rare event. In the COSMIC and the cBioportal databases, this mutation has only been reported nine times in non-small cell lung cancer (NSCLC), without any data about the sensitivity to tyrosine kinase inhibitors (TKIs). OncoKB annotations specify that “patients with NSCLC harboring the exon 18 E709_T710delinsD mutation can respond to *EGFR* TKIs.” Some data about TKI sensitivity were reported for first-generation TKI gefitinib or erlotinib with one response among three patients [2]. In an in vitro study, Kobayashi et al. showed that this exon 18 deletion is oncogenic and that Ba/F3 cells transfected with exon 18 mutations are more sensitive to second-generation TKIs than to other TKIs [2]. In addition, the same authors reported one case that responded to afatinib.

MOLECULAR TUMOR BOARD 1

Genotyping Results

DNA was extracted from the formalin-fixed paraffin-embedded cell block and subjected to gene panel analysis using next-generation sequencing (NGS). Gene mutations were analyzed using the Colon and Lung Cancer panel (Ampliseq; Life Technologies, Merelbeke, Belgium) for sequencing 1,825 hotspot mutations in 22 genes including

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Figure 2. Evolution of the computed tomography images. Computed tomography images from primary tumor (1, 2) and lung metastases (3) before starting afatinib (A), at the best afatinib response with almost complete disappearance of lung metastases (B), and at afatinib progression (C).

PATIENT UPDATE 1

Concomitant radio- and chemotherapy with cisplatin-vinorelbine was administered. A partial response was observed. Nine months after the end of the treatment, local recurrence and pulmonary metastases (Fig. 2A) justified the introduction of afatinib (40 mg/day), based on the exon 18 *EGFR* mutation. A partial response lasted for 1 year (Fig. 2B). At this time, the patient was paucisymptomatic, presenting minor hemoptysis. However, a local recurrence (Fig. 2C) and new bone metastases were documented.

A new biopsy of the primary tumor was taken. Biopsy material consisted of necrotic fragments containing small tumor cells, densely packed, with scant cytoplasm and finely granular nuclear chromatin (Fig. 3A, 3B). Tumor cells showed immunoreactivity for synaptophysin, CD56, and TTF1 (Fig. 3C, 3D), but no immunoreactivity for chromogranin.

Ki-67 immunohistochemistry revealed a high proliferation index (Fig. 3E). TP53 immunohistochemistry showed an absence of TP53 expression in tumor cells (Fig. 3F). Small cell lung cancer (SCLC) transformation was diagnosed.

MOLECULAR TUMOR BOARD 2

Genotyping Results

Molecular studies were performed on the new biopsy. Gene mutations were analyzed using the Colon and Lung Cancer panel (Ampliseq) and the Cancer Hotspot Panel (Ampliseq) for sequencing hotspot mutations in 50 genes including *ABL1*, *AKT1*, *ALK*, *APC*, *ATM*, *BRAF*, *CDH1*, *CDKN2A*, *CSF1R*, *CTNNB1*, *EGFR*, *ERBB2*, *ERBB4*, *EZH2*, *FBXW7*, *FGFR1*, *FGFR2*, *FGFR3*, *FLT3*, *GNA11*, *GNAQ*, *GNAS*, *HNF1A*, *HRAS*, *IDH1*, *IDH2*, *JAK2*, *JAK3*, *KDR*, *KIT*,

Figure 1. Pathological and molecular data from the first EBUS. (A): Diff-Quik stained smear of EBUS-TBNA performed on laterotracheal lymph nodes showing malignant glandular cells. (B): Cell block of the EBUS-TBNA performed on laterotracheal lymph nodes showing malignant glandular cells (H&E). Integrative genomics viewer (IGV) view of *epidermal growth factor receptor* exon 18 (C) and *SMAD 4* exon 9 (D) for the first EBUS-TBNA sample.

Figure 3. Pathological and molecular data from the tumor progression biopsy. Biopsy material from the tumor progression consisted of necrotic fragments of a tumor containing small tumor cells, densely packed, with scant cytoplasm and finely granular nuclear chromatin (A, B, H&E). Tumor cells showed immunoreactivity for CD56 (C) and TTF1 (D). Proliferation index evaluated by Ki-67 immunohistochemistry was high (E). TP53 immunohistochemistry revealed an absence of TP53 expression (F). IGV view of *epidermal growth factor receptor* exon 18 (G) and *SMAD4* exon 9 (H) for the tumor progression biopsy sample.

KRAS, *MET*, *MLH1*, *MPL*, *NOTCH1*, *NPM1*, *NRAS*, *PDGFRA*, *PIK3CA*, *PTEN*, *PTPN11*, *RB1*, *RET*, *SMAD4*, *SMARCB1*, *SMO*, *SRC*, *STK11*, *TP53*, *VHL*. The same *EGFR* alteration (p.E709_710 > D with an allelic frequency of 28%) was observed (Fig. 3G). In addition, the mutation p.R361C in the *SMAD4* gene was detected with an allelic frequency of 41% (Fig. 3H). This mutation was absent in the first EBUS-TBNA sample (Fig. 1D). Although TP53 immunohistochemistry was negative, no mutation was detected in exons 2, 4–8, and 10 of *TP53*. No mutation was detected in exons 4, 6, 10, 11, 14, 17, 18, and 20–22 of *RB1*.

Functional and Clinical Significance

According to the available treatment options, it is now recommended, when feasible, to obtain a new tumor biopsy at the time of progression. European Society for Medical Oncology and National Comprehensive Cancer Network guidelines consider that prior to changing therapy, a biopsy is reasonable for determining the mechanism of resistance [3]. The reported mechanisms of acquired resistance to first-generation EGFR TKI are *EGFR* T790M (the

most common mechanism of acquired resistance; 63%), *MET* amplification (5%), *human epidermal growth receptor 2* amplification (13%), and SCLC transformation (3%) [4].

The mechanisms of acquired resistance to afatinib have not been well studied. In a series of 42 patients with resistance to afatinib, Wu et al. reported that the T790M *EGFR* mutation was observed for 47.6% of patients. No SCLC transformation was recorded [5].

As already described [6], the original *EGFR* mutations are maintained in the case of SCLC transformation, as we observed in the present case. The molecular changes associated with SCLC transformation remain poorly understood. SCLC transformation was recently described as associated with a complete inactivation of both TP53 and RB1 [7]. In the present case, TP53 immunohistochemistry was negative in tumor cells, suggesting a *TP53* alteration. However, no mutation was detected in exons 2, 4–8, and 10 of *TP53* by NGS. No mutation was detected in *RB1* (the NGS panel contains 10 amplicons covering exons 4, 6, 10, 11, 14, 17, 18, and 20–22 of *RB1*). For these two genes, we can't exclude a mutation in another exon or a deletion. In the present case, we observed acquisition of *SMAD4* mutation

after SCLC transformation. *SMAD4*, a tumor suppressor gene, belongs to the transforming growth factor beta signaling pathway, which negatively regulates the growth of epithelial cells. *SMAD4* is frequently mutated in pancreatic and colorectal cancers. Evolutionary conservation programs predict that R to C substitution would negatively affect protein function [8] and prevent both hetero- and homo-oligomerization of *SMAD4* [9]. A preclinical study demonstrated that *SMAD4* inactivation correlates with cetuximab resistance and epithelial-to-mesenchymal transformation in head and neck squamous cell carcinomas [10]. Further studies are needed to elucidate if *SMAD4* mutation could be a pathophysiological step in this rare event.

PATIENT UPDATE 2

A rapid progressive disease was observed after two cycles of carboplatin-etoposide. A combination of doxorubicin, cyclophosphamide, and etoposide was given as well, but her general condition declined and no further treatment

was proposed. The patient died from disease progression around 3 years after the initial diagnosis.

GLOSSARY OF GENOMIC TERMS AND NOMENCLATURE

Allelic frequency: proportion or percentage of reads at a site that contain a mutant allele

Deletion: deletion of bases into the DNA sequence

EGFR: epidermal growth factor receptor

Sequencing: determining the order of the bases in a strand of DNA

AUTHOR CONTRIBUTIONS

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DISCLOSURES

The authors indicated no financial relationships.

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