

[CASE REPORT]

Small Cell Lung Cancer Derived from Adenocarcinoma with Mutant Epidermal Growth Factor Receptor Provides a Signature of Transcriptional Alteration in Tumor Cells

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Abstract:

Small cell lung cancer (SCLC) transformation of epidermal growth factor receptor (EGFR) mutant adenocarcinoma (ADC) during EGFR tyrosine kinase inhibitor (TKI) treatment is an example of a rare subset of acquired drug resistance. We herein report the case of a 75-year-old man treated with afatinib who was then diagnosed with SCLC transformation. After two years of successful treatment with afatinib, the tumor relapsed, and a re-biopsy revealed SCLC harboring EGFR exon 19 deletion. We encountered a case of transcriptional alteration, potentially important for SCLC transformation of EGFR mutant lung ADC, that was recognized via the expression of NOTCH, ASCL1 and RB1 on immunohistochemical staining.

Key words: small cell lung cancer, epidermal growth factor receptor, adenocarcinoma, transcriptional alteration, acquired drug resistance

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Introduction

Lung cancer is a leading cause of cancer-related mortality worldwide. Non-small cell lung cancer (NSCLC) accounts for approximately 85% of all lung cancer cases, while small cell lung cancer (SCLC) represents the remaining 15%. In recent years, clinical studies have focused on molecular-targeted drugs, such as epidermal growth factor (EGFR)-tyrosine kinase inhibitors (TKIs) used for adenocarcinoma (ADC) with mutant EGFR. Although EGFR-TKIs show good therapeutic effects, patients relapse due to the development of resistance to these drugs. Histological transformation of ADC with mutant EGFR to SCLC is one such resistance mechanism (1).

SCLC is considered one of the most aggressive malignant neuroendocrine (NE) tumors. Despite a high rate of response

to first-line treatment, these cases show rapid growth and metastasis and acquire multidrug resistance. Basic studies on molecular mechanisms underlying small cell carcinogenesis are ongoing, and rapid progress in the exploration of novel therapeutic modalities is anticipated.

Case Report

A 75-year-old Japanese man who was a 16-pack-year light former smoker, was diagnosed with primary lung ADC and underwent left upper lobectomy and mediastinal lymphadenectomy in July 2015. He received four cycles of adjuvant chemotherapy with cisplatin plus vinorelbine. In July 2016, the tumor marker serum carcinoembryonic antigen (CEA) was elevated (40.9 ng/mL, reference: ≤ 3.5 ng/mL), and ¹⁸F-fluorodeoxyglucose positron emission tomography (¹⁸F-FDG-PET) showed right rib, thoracic spine and pelvic

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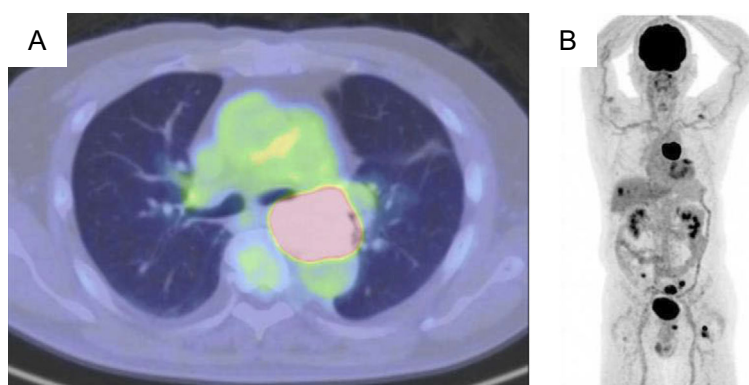


Figure 1. ^{18}F -Fluorodeoxyglucose positron emission tomography images revealing the high radio-tracer accumulation in (A) mediastinal lymphadenopathy and (B) multiple bone metastases.

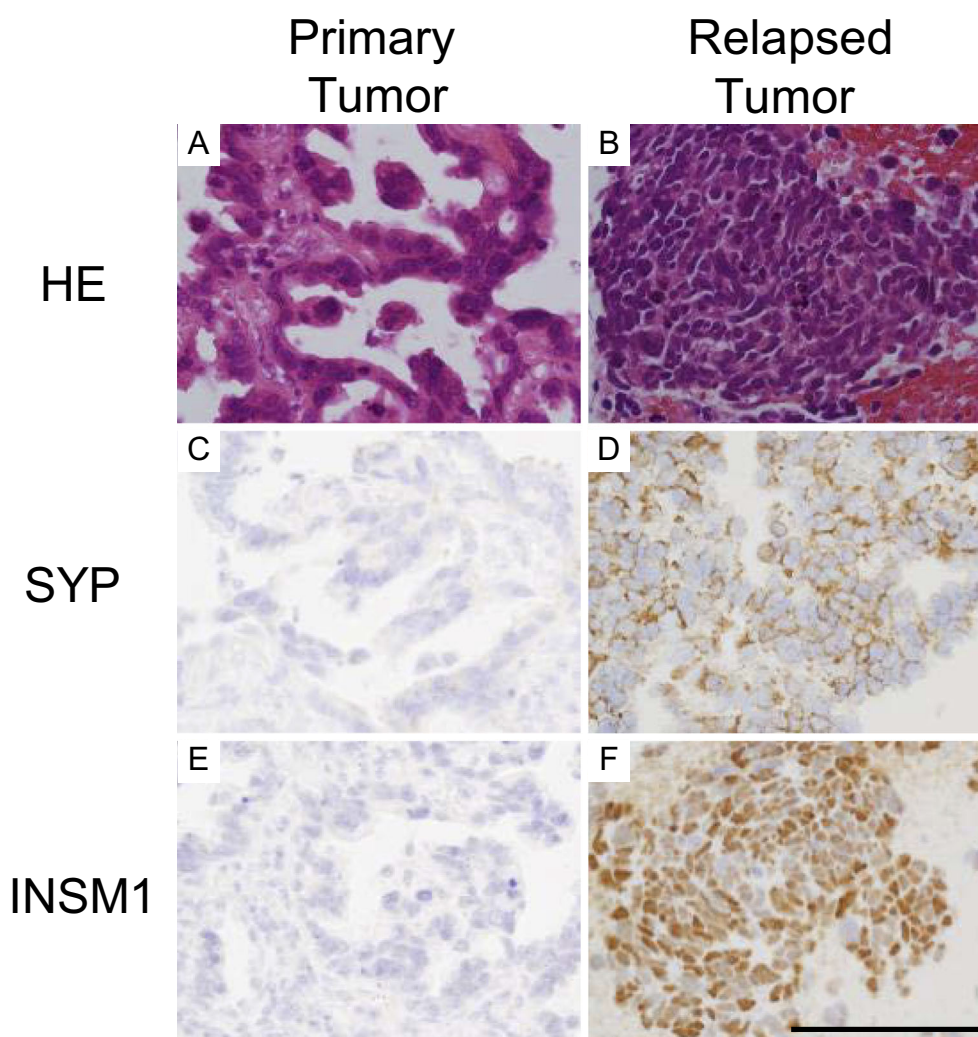


Figure 2. A comparison of the histopathological findings of primary and relapsed tumor samples. (A) Primary tumor sample revealing adenocarcinoma morphology and (B) a relapsed tumor sample showing small cell carcinoma morphology, both under Hematoxylin and Eosin staining. The protein expression of SYP and INSM1 in both primary and relapsed samples was assessed via immunohistochemistry (C-F). Scale bar=100 μm .

bone metastases. *EGFR* exon 19 deletion was detected in resected lung specimens. He was treated with afatinib (40 mg once a day), a second-generation *EGFR*-TKI. Following the initiation of afatinib treatment, the serum CEA level de-

creased gradually to 5.6 ng/mL, suggesting that treatment with afatinib had presented an anti-tumor response.

In July 2018, follow-up PET-computed tomography (CT) revealed an enlarged mediastinal lymph node, 45×30 mm in

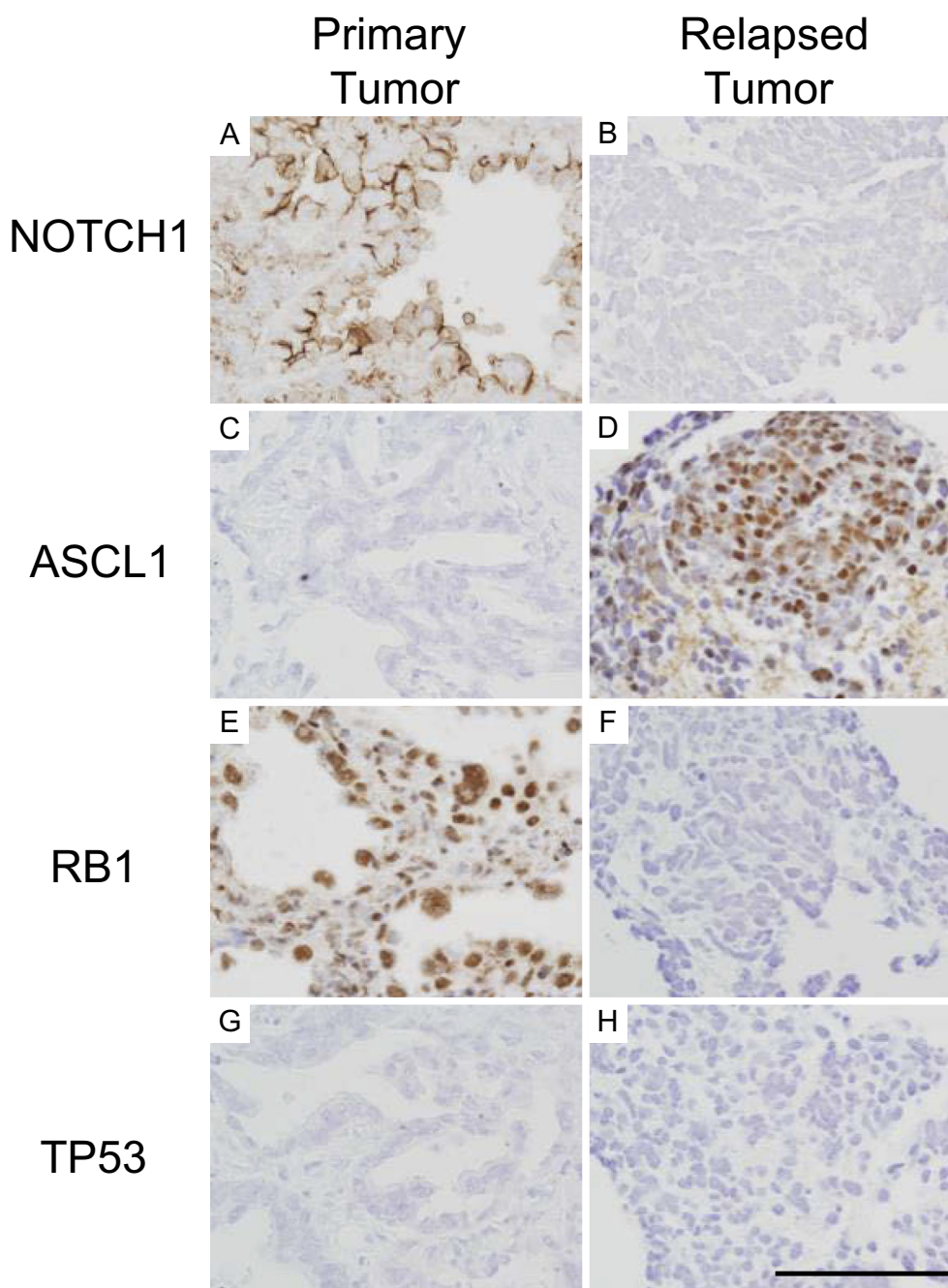


Figure 3. A signature of transcriptional alteration in tumor cells. The expression of NOTCH1, ASCL1, RB1, and TP53 in both primary and relapsed samples was assessed via immunohistochemistry (A-H). Scale bar=100 μ m.

size, with the accumulation of ^{18}F -FDG (maximum standardized uptake value: 49.0) and multiple bone metastases (Fig. 1). A re-biopsy by transesophageal endoscopic ultrasound-guided fine-needle aspiration (EUS-FNA) for the enlarged mediastinal lymph node was performed to investigate acquired mutation resistance to afatinib. Although exon 19 deletion was detected in the tumor tissue, a secondary *T790M* mutation was not.

Hematoxylin and Eosin stained sections showed small atypical cells with scant cytoplasm, which was completely different from the previously detected ADC tumor cells (Fig. 2A and B). While the relapsed tumor cells were posi-

tive for the protein expression of synaptophysin (SYP) and insulinoma-associated protein 1 (INSM1), the ADC tumor cells were not (Fig. 2C-F). These results indicated histological SCLC transformation. Thereafter, standard chemotherapy for SCLC was initiated with carboplatin (AUC 5) plus etoposide (80 mg/m^2). Following three treatment cycles, the mediastinal lymphadenopathy shrank remarkably.

We also confirmed the difference in the transcriptional signature between these tumors by immunohistochemical staining. Achaete-scute complex homologue 1 (ASCL1) expression was detected in the SCLC sample, whereas neither ASCL1 nor any other NE markers were expressed in the re-

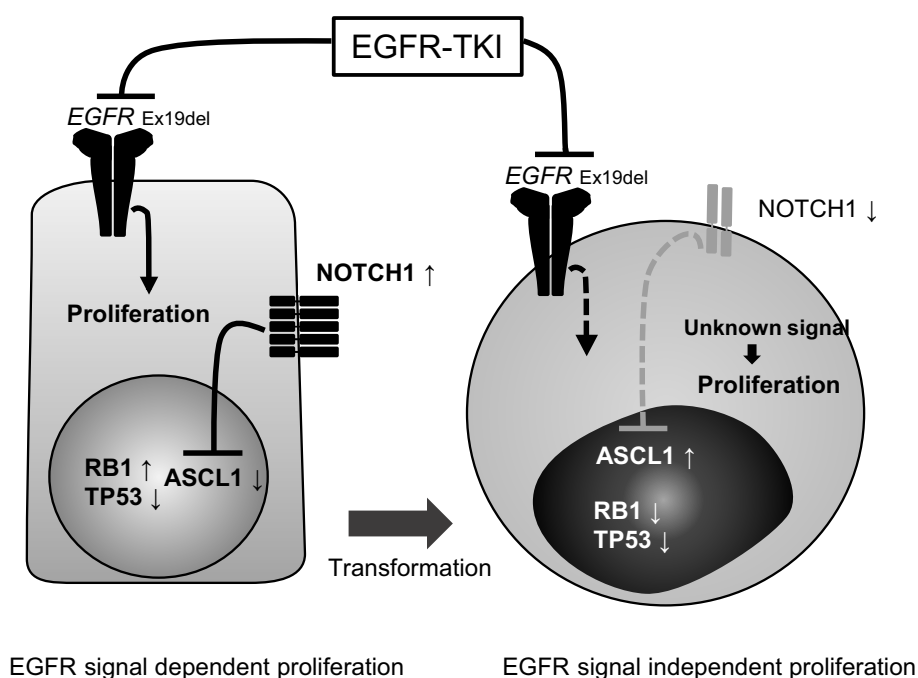


Figure 4. The protein expression observed in the case is summarized. The survival and proliferation of the primary tumor depended on the sustained signal from mutant EGFR. In contrast, the survival and proliferation of the transformed tumor depended on another signal. Transformed SCLC may originate from EGFR mutant ADC that acquires an NE phenotype by inactivating Notch signaling and ASCL1 expression. Additional RB1 loss and inactivating TP53 may be beneficial to small cell carcinogenesis.

sected ADC sample. Instead, both retinoblastoma 1 (RB1) and NOTCH1 were highly expressed in the ADC sample, while loss of RB1 and NOTCH1 was observed in the SCLC. TP53 was not detected in either of the tumor samples (Fig. 3).

Discussion

Transformation from ADC to SCLC may have occurred via one of two proposed mechanisms in this case. First, an SCLC trait may have been acquired during treatment with EGFR-TKI; alternatively, the tumor cells may have contained an SCLC trait as an aspect of tumor heterogeneity from the outset. We did not detect any SCLC components or NE properties in the initial ADC samples. In addition, the SCLC sample did not represent any other histological neoplasms. Furthermore, the common *EGFR* mutant status marker of exon 19 deletion was detected in each tumor sample as well as in other histological types of the tumor. These results enabled us to conclude that our case was derived from a genetically identical cell and to hypothesize that the SCLC trait had been acquired via transformation during treatment.

A characteristic feature of SCLC is the expression of NE phenotypes. Reportedly, various transcription factors determine NE differentiation in normal and neoplastic lung epithelial cells. Inactivation of Notch signaling and expression of ASCL1 may be significant for SCLC carcinogenesis.

Notch1-hairy and enhancer of split-1 pathway is a repressor of NE differentiation through the decreased expression of NE-promoting transcription factors, such as ASCL1 (2-4). ASCL1, a proneural basic helix-loop-helix factor, is regarded as an NE inducer and lineage marker (5). In human SCLC cells, knockdown of ASCL1 induced growth inhibition and apoptosis (6). In our IHC studies, NOTCH1 was highly expressed in the first ADC sample, but ASCL1 was not expressed. While NOTCH1 expression was suppressed following SCLC transformation, ASCL1 was highly expressed. These findings indicate that a change in transcriptional control had occurred.

Another alternative pathway for histological transformation from ADC to SCLC during EGFR-TKI treatment is the loss or inactivation of Rb and p53 (7, 8). Mouse models carrying conditional alleles for both *p53* and *Rb1* developed small cell carcinoma in the lung (9). In accordance with these observations, RB1 expression was absent in the SCLC in this case. As a limitation of our analysis, it might not be reasonable to discuss the TP53 expression. As TP53 is known to be a short-life protein (10), it is usually difficult to assess the normal TP53 expression via immunohistochemistry. Though the TP53 expression was absent via immunohistochemistry in this case, we speculated two possibilities concerning the genomic status of the *TP53* gene. One is a wild-type *TP53* gene status, wherein the gene expresses the functional TP53 protein. On the other hand, the *TP53* gene is reportedly commonly mutated in 46% of all lung ADC

cases (11). In human SCLC samples, inactivating mutations in the *TP53* gene were shown to affect up to 90% of SCLCs (12). Based on these previous findings, the other possible genomic status in this case is a missense or non-sense mutation incapable of expressing any functional protein of TP53.

In summary, we detected the occurrence of signature transcriptional alterations in tumor cells during EGFR-TKI treatment. Tumor cells suggested to depend on mutant EGFR signaling for their proliferation and survival may have escaped from such dependence and acquired the capacity to proliferate and survive via a different mechanism. Although the fundamental factors associated with the acquisition of this trait remain unclear, once acquired, the advantage conferred on the tumor cell survival under EGFR-TKI treatment is clear (Fig. 4). Tumor cell relapse occurs as a result of this new trait.

Conclusion

We demonstrated a signature expression suggestive of transcriptional alterations in tumor cells associated with SCLC transformation in the present case. Although, replicated trials with larger samples and more detailed analyses are warranted, our findings may be important for elucidating the details of SCLC transformation, which is an EGFR-TKI resistance mechanism.

The authors state that they have no Conflict of Interest (COI).

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