TP53 Loss of Heterozygosity Induces De Novo SCLC Formation in EGFR-Mutated Lung Adenocarcinoma: A Case Report

Kei Kunimasa, MD, PhD,a,* Yosuke Hirotsu, PhD, b Kenji Amemiya, MSH, b Harumi Nakamura, MD, PhD,c Kazumi Nishino, MD, PhD,a Keiichiro Honma, MD, PhD, d Jiro Okami, MD, PhD, e Masao Omata, MD, PhD, b,f Toru Kumagai, MD, PhDa

aDepartment of Thoracic Oncology, Osaka International Cancer Institute, Osaka, Japan
bGenome Analysis Center, Yamanashi Central Hospital, Yamanashi, Japan
cLaboratory of Genomic Pathology, Osaka International Cancer Institute, Osaka, Japan
dDepartment of Diagnostic Pathology and Cytology, Osaka International Cancer Institute, Osaka, Japan
eDepartment of General Thoracic Surgery, Osaka International Cancer Institute, Osaka, Japan
fThe University of Tokyo, Tokyo, Japan

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ABSTRACT

SCLC transformation in EGFR-mutated lung adenocarcinoma is one of the major phenotypic changes that is observed during the resistance to EGFR tyrosine kinase inhibitors. However, the mechanism of this transformation remains unclear. In this study, we found a small de novo SCLC component in surgically resected specimens of EGFR-mutated lung adenocarcinoma before EGFR tyrosine kinase inhibitor treatment. By using laser microdissection and whole-exome sequencing, TP53 loss of heterozygosity was found to be possibly involved in SCLC transformation.

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Introduction

Transformation to SCLC or the presence of de novo SCLC has been reported as a mechanism of resistance or a contributing factor of EGFR-activating mutations of EGFR tyrosine kinase inhibitor (TKI) in lung adenocarcinoma.1,2 Although de novo SCLC harboring major EGFR-activating mutations are rare, they likely share part of the biological background of transformed SCLC.2

The baseline co-occurrence of TP53 and RB1 mutations is a risk factor for future or de novo SCLC transformation.1 In addition to TP53 and RB1 mutations, large-scale genomic alterations such as whole-genome...
doubling and APOBEC mutation signatures have been implicated in SCLC transformation. It is speculated that clones carrying or acquiring these genetic alterations transform to SCLC clones under the influence of EGFR TKIs or during natural cancer evolution. Here, we present a case of surgically resected EGFR-mutated lung adenocarcinoma in which early formation of de novo SCLC was confirmed. The use of laser microdissection and whole-exome sequencing (WES) suggested that TP53 loss of heterogeneity (LOH) may have triggered transformation to SCLC.

Case Presentation

A 38-year-old woman, who was a former light smoker (1.5 pack-years), presented to our hospital for examination of an abnormal shadow in her left lung field, observed in Figure 1A. Bronchoscopic biopsy was performed and the specimen was pathologically analyzed. She was diagnosed with clinical stage IB (cT2aN0M0) lung adenocarcinoma after a systemic screening investigation (Fig. 1B). EGFR mutation testing with reverse-transcription polymerase chain reaction identified Ex.19 deletion (p.E746_A750del). Left upper lobectomy was performed, which revealed pleural dissemination; the postsurgical pathologic TNM classification was pT2aN0M1a, stage IVa. Histopathologic analysis of the resected specimen revealed that SCLC occupied a relatively small area (approximately 3%) of the adenocarcinoma (50% papillary, 40% acinar, and 7% lepidic) (Fig. 2A-C). In the disseminated pleural nodules, only an adenocarcinoma component was identified. For the SCLC component, she was treated with four cycles of etoposide (100 mg/m²) in addition to cisplatin (80 mg/m²) treatment as adjuvant chemotherapy after once-daily osimertinib 80mg treatment for advanced EGFR-mutated lung adenocarcinoma. She has been taking osimertinib for two years, and no recurrence has been observed.

To clarify the genetic differences between EGFR-mutant lung adenocarcinoma and SCLC, only the SCLC component was excised from the surgically resected specimen using laser microdissection (Fig. 2D), and WES was performed on each of the distant adenocarcinoma components to compare genetic alterations (Supplementary Method). Buffy coat from the patient’s blood sample was used as a normal control reference. WES exhibited 136 nonsynonymous mutations and 97 copy number alterations in the adenocarcinoma component in addition to 198 nonsynonymous mutations and 153 copy number alterations in the SCLC component (Supplementary Table 1). Of these alterations, nonsynonymous mutations annotated in large databases are detailed in Table 1. TP53 gene copy number

Figure 1. (A) Plain chest CT at the first visit revealed a lung nodule in the left upper lobe. (B) FDG-PET scan revealed FDG uptake in the nodule and no uptake in any other organs except for physiological uptakes. CT, computed tomography; FDG, fluorodeoxyglucose; PET, positron emission tomography.
was one and TP53 LOH was observed in the SCLC component.

Discussion

In the present case, we were, fortunately, able to detect a very small de novo SCLC component in EGFR-mutated adenocarcinoma before EGFR TKI treatment. Furthermore, by using laser microdissection and WES, we were able to evaluate the genetic alterations associated with relatively early SCLC transformation. Analysis of the relationship between SCLC transformation and genetic alteration during EGFR TKI treatment for patients with EGFR-mutated lung cancer revealed that RB1 mutation could precede the acquisition of resistance to the TKI.\(^1\) RB1 mutation was not detected in the SCLC component in our case. The TP53 variant allele frequency in the SCLC component was 98.5\%, whereas the variant allele frequency in the adenocarcinoma component was 50\%. These results suggest that TP53 LOH is the first genetic alteration and that RB1 mutation may be a subsequent event in SCLC transformation from EGFR-mutated adenocarcinoma.

It has been reported that loss of heterozygosity, in which the wild-type allele is deleted, increases the tumor malignancy in cancer harboring the TP53 mutant allele.\(^3\) In SCLC transformation in EGFR-mutated lung cancer after EGFR TKI administration, TP53 mutation was detected in 60\% to 80\% of the cases, and RB1 mutation was seen in about 40\% to 58\% of the cases.\(^2,4\) RB1 mutation was not detected in approximately half of the cases with SCLC transformation, suggesting that RB1 mutation is not an essential factor for the morphologic change to SCLC from adenocarcinoma. Concomitant TP53 mutation has been reported to attenuate the effect of EGFR TKI,\(^5\) whereas the high TP53 variant allele frequency of the SCLC component suggests that the effect is stronger than that of the adenocarcinoma component. When EGFR TKI was administered to the present case, the SCLC component remained as a persistent clone, and when progression was recognized radiologically, the SCLC may form a major component. In WES, a total of 351 genetic alternations were identified in the SCLC component and 233 in the adenocarcinoma component. It is possible that more genetic alterations were observed in SCLC because of the genome instability associated with TP53 mutation. Several mutations were annotated as pathogenic in the Catalog of Somatic Mutations in Cancer database, but the contribution of these mutations to the carcinogenesis of lung cancer was unclear for many mutations.
Conclusions

Using laser microdissection and WES, TP53 LOH was found to be potentially involved in SCLC transformation of EGFR-mutated lung adenocarcinoma. Because SCLC in the present case represents a small component, this finding is valuable in that it captures the early changes in the transformation process from EGFR-mutated lung adenocarcinoma to SCLC.

CRediT Authorship Contribution Statement

Kei Kunimasa: Formal analysis, Investigation, Writing - original draft.

Yosuke Hirotsu: Conceptualization, Investigation, Methodology.

Kenji Amemiya: Investigation.

Harumi Nakamura: Formal analysis, Investigation.

Kazumi Nishino, Keiichiro Honma, Jiro Okami: Investigation.

Masao Omata: Funding acquisition, Supervision.

Toru Kumagai: Supervision.

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Table 1. Result of WES of Adenocarcinoma and SCLC Components

| Gene | Mutation   | OncoKB | COSMIC       | VAF (%) | SCLC |
|------|------------|--------|--------------|---------|------|
| EGFR | p.E746_A750del1 | O      | Pathogenic   | 42.5    | 50.9 |
| TP53 | p.H168P    | LO     | Pathogenic   | 50.0    | 98.5 |
| FAM83E| p.W397*    | NA     | Pathogenic   | 32.6    | 59.4 |
| EYS  | p.D1816H   | NA     | Neutral      | 16.5    |      |
| PKD1L1| p.T1887M   | NA     | Neutral      | 19.2    |      |
| MUC17| p.G970V    | NA     | Neutral      | 23.5    |      |
| VIM  | p.E230K    | NA     | Pathogenic   | 20.7    |      |
| ZNF502| p.A336T   | NA     | Neutral      | 31.2    |      |
| OTOL1| p.P169L    | NA     | Neutral      | 58.3    |      |
| PARP8| p.E147K    | NA     | Neutral      | 19.4    |      |
| STAG3| p.R83C1    | NA     | Neutral      | 41.9    |      |
| KLF10| p.P94A     | NA     | Pathogenic   | 12.9    |      |
| MUC6 | p.P1656L   | NA     | Neutral      | 12.1    |      |
| OR5L2| p.E207K    | NA     | Neutral      | 21.4    |      |
| AKAP3| p.R609C    | NA     | Neutral      | 37.1    |      |
| TMEM117| p.M1621   | NA     | Pathogenic   | 27.0    | 100  |
| GUPR1| p.R39*     | NA     | Neutral      | 36.8    | 80   |
| NOS1 | p.E1334Q   | NA     | Pathogenic   | 45.5    | 60   |
| IRX6 | p.C427*    | NA     | Pathogenic   | 23.3    | 40   |
| SCR2 | p.G96D     | NA     | Pathogenic   | 21.7    | 20   |
| LRRC3| p.A32T     | NA     | Neutral      | 21.3    | 10   |
| PAS D1| v.E650M    | NA     | Neutral      | 21.3    | 10   |

VAF: variant allele frequency

COSMIC, Catalogue of Somatic Mutations in Cancer; LO, likely oncogenic, NA, not annotated, O, oncogenic, VAF, variant allele frequency, WES, whole-exome sequencing.

Supplementary Data

Note: To access the supplementary material accompanying this article, visit the online version of the JTO Clinical and Research Reports at www.jtocrr.org/ and at https://doi.org/10.1016/j.jtocrr.2022.100305.

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