CASE REPORT

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

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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.

Keywords: EGFR-mutated lung adenocarcinoma; Small cell lung cancer; Transformation; *TP53* mutation; Loss of heterozygosity; Case report

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

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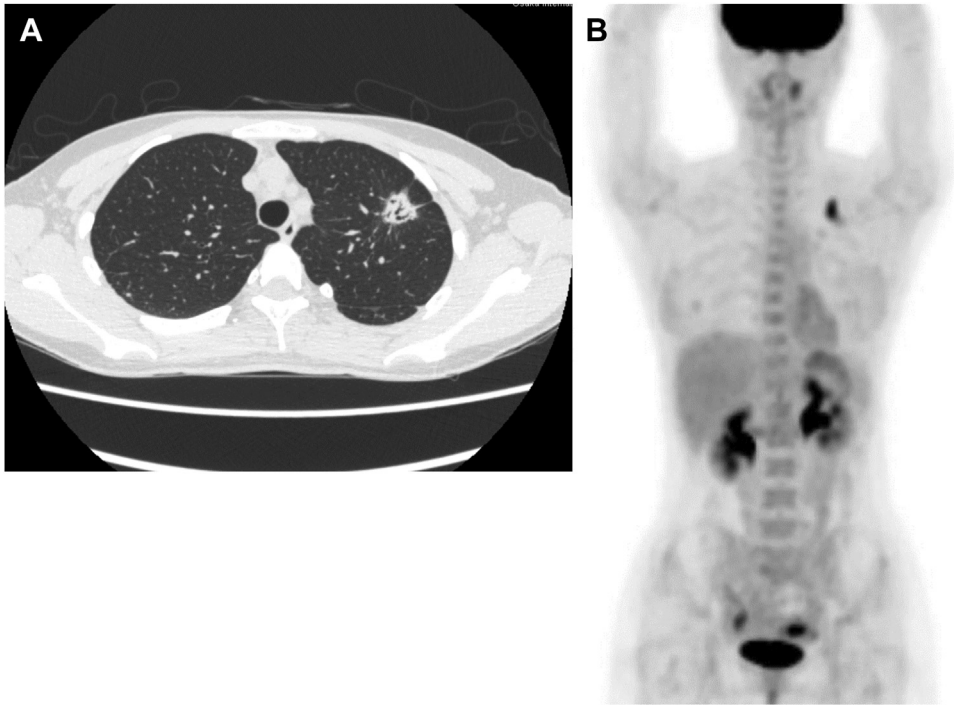

Figure 1. (*A*) Plain chest CT at the first visit revealed a lung nodule in the left upper lobe. (*B*) FDG-PET scan revealed FDG uptake in the nodule and no uptake in any other organs except for physiological uptakes. CT, computed tomography; FDG, fluorodeoxyglucose; PET, positron emission tomography.

doubling and APOBEC mutation signatures have been implicated in SCLC transformation.[1] 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 1*A*. 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. 1*B*). *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. 2*A–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/m^2) in addition to cisplatin (80 mg/m^2) 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. 2*D*), 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

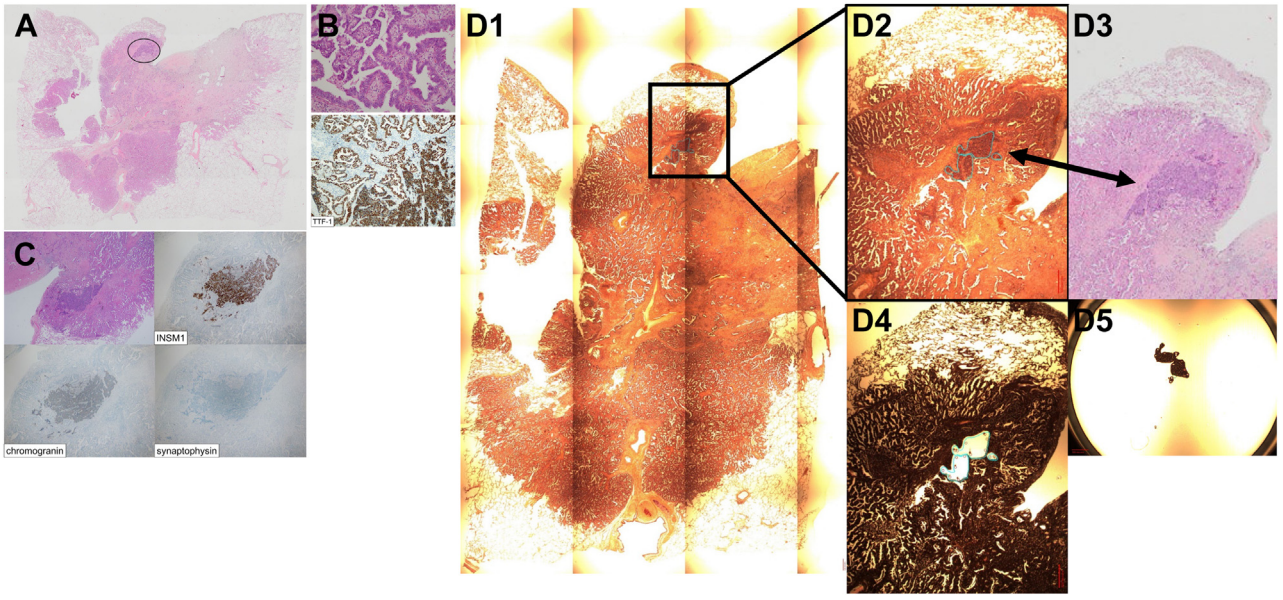

Figure 2. (*A*) Loupe view of HE stain of the dissected lung tumor. The black circle indicates the SCLC component. (*B*) The adenocarcinoma component exhibits papillary component positive for TTF-1 in immunohistochemical staining. (*C*) The SCLC component exhibits positivity for INSM1 and chromogranin and slight positivity for synaptophysin. (*D*) Laser microdissection was performed using the ArcturusXT laser-capture microdissection system (Thermo Fisher Scientific) (*D*.1) Loupe view of dissected lung tumor. The black square indicates the SCLC component. A high-power field image of the SCLC component under laser-capture microdissection (*D*.2) and HE staining (*D*.3), and the black bidirectional arrow indicate the identical SCLC components. (*D*.4) High-power field image of dissected lung tumor after laser microdissection of SCLC component. (*D*.5) SCLC component hollowed out by laser microdissection. HE, hematoxylin and *eosin*.

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.

Table 1. Result of WES of Adenocarcinoma and SCLC Components

Gene	Mutation	OncoKB	COSMIC		Adenocarcinoma VAF (%)	SCLC	
EGFR	p.E746_A750de1	O	Pathogenic	COSV51765066	42.5	50.9	
TP53	p.H168P	LO	Pathogenic	COSV52676197	50.0	98.5	
FAM83E	p.W397*	NA	Pathogenic	COSV52375426	32.6	59.4	
EYS	p.D1816H	NA	Neutral	COSV105223275	16.5		
PKD1L1	p.T1887M	NA	Neutral	COSV56961936	19.2		
MUC17	p.G970V	NA	Neutral	COSV60284427	23.5		
VIM	p.E230K	NA	Pathogenic	COSV99812970	20.7		
ZNF502	p.A336T	NA	Neutral	COSV105165073		31.2	
OTOL1	p.P169L	NA	Pathogenic	COSV60007256		58.3	
PARP8	p.E147K	NA	Pathogenic	COSV55860284		19.4	
STAG3	p.R83C1	NA	Neutral	COSV57928219		41.9	
KLF10	p.P94A	NA	Pathogenic	COSV99574981		12.9	
MUC6	p.P1656L	NA	Neutral	COSV70137259		12.1	
OR5L2	p.E207K	NA	Neutral	COSV65724118		21.4	
AKAP3	p.R609C	NA	Neutral	COSV57421584		37.1	
TMEM117	p.M1621	NA	Pathogenic	COSV56902370		27.0	VAF (%)
GUPR1	p.R39*	NA	Neutral	COSV56989959		36.8	100
NOS1	p.E1334Q	NA	Pathogenic	COSV100501584		45.5	80
IRX6	p.C427*	NA	Pathogenic	COSV51859962		23.3	60
SCRN2	p.G96D	NA	Pathogenic	COSV99274220		21.7	40
LRRC3	p.A32T	NA	Neutral	CO SV52399394		25.0	20
PAS D1	o.V650M	NA	Neutral	COSV64855178		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.

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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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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