CASE REPORT

Composite Clonal Analysis Reveals Transition of NSCLC Subtypes Through Accumulation of Gene Mutations: A Case Report

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ABSTRACT

We analyzed an EGFR-mutated lung cancer with a pathologic diagnosis of combined large cell neuroendocrine carcinoma with mixed adenocarcinoma subtypes. Targeted next-generation sequencing of each component suggested that mutations in *RB1, TP53,* and *SMAD4* and apparent loss of heterozygosity of *TP53* and *SMAD4* accompanied the transition of different adenocarcinoma subtypes. Additional gene mutations including *PTEN, MST1R,* and *PIK3CA* were noted during transdifferentiation from acinar adenocarcinoma to large cell neuroendocrine carcinoma. Combined DNA and RNA analysis using Todai OncoPanel revealed that transdifferentiation to different pathologic subtypes occurred in a single tumor through the accumulation of gene mutations.

Keywords: Clonal analysis; NSCLC; EGFR; Gene mutations; Pathologic subtypes; Case report

Introduction

Lung cancer is histologically classified as NSCLC or SCLC. Large cell neuroendocrine carcinoma (LCNEC) is biologically similar to SCLC, and they are classified as neuroendocrine carcinomas. The transformation from adenocarcinoma to neuroendocrine carcinoma is an established mechanism of acquired resistance to EGFR tyrosine kinase inhibitors.[1] Inactivation of *RB1* and *TP53* is known to associate with the transformation from EGFR-mutated adenocarcinoma to SCLC.[2] In addition, transformation can occur without tyrosine kinase inhibitor treatment.[3,4] We report an EGFR-mutated lung cancer that spontaneously transdifferentiated to different pathologic subtypes within a single tumor through the

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accumulation of gene mutations in *RB1, TP53, PTEN*, and *SMAD4.*

Case Presentation

A 67-year-old man with an 18-pack-year smoking history was referred for a lung nodule of the left upper lobe. He had no history of malignant diseases. Adenocarcinoma was suspected by bronchoscopy, and the left upper lobe was resected. The primary tumor was combined LCNEC with mixed adenocarcinoma subtypes including papillary (35%), LCNEC (20%), acinar (20%), lepidic (20%), micropapillary (<5%), and solid adenocarcinoma (<5%) (Fig. 1*A–E*). The LCNEC component was diffusely positive for synaptophysin, CD56, TTF1, and p53, and negative for chromogranin A, RB1, and PTEN (Fig. 1*F* and Supplementary Fig. 1). The adenocarcinoma components were diffusely positive for TTF1, focally positive for p53, and negative for synaptophysin, CD56, and chromogranin A (Supplementary Fig. 1). The lepidic component was positive for RB1 and PTEN, and the acinar and papillary components were heterogeneously positive for PTEN and negative for RB1 (Fig. 1*F*). The lymph node metastasis was positive for the micropapillary component. The pathologic stage was determined as pT1bN2M0, stage IIIA. cobas *EGFR* Mutation Test v2 (Roche Diagnostics K.K., Tokyo, Japan), a commercial companion diagnostic, detected exon 19 deletion.

We performed targeted next-generation sequencing to compare molecular changes in each component of the adenocarcinoma and the LCNEC subtypes. The tissue of each histologic component was obtained by macrodissecting the formalin-fixed paraffin-embedded sections. After obtaining informed consent, genomic DNA was extracted from each pathologic component and peripheral blood lymphocytes as matched normal control and was subjected to targeted sequencing of 464 cancer-related genes with the use of Todai OncoPanel.[3] The tumor content of micropapillary and solid adenocarcinoma components and lymph node metastasis was too low to be analyzed. We detected 3, 5, 6, and 9 nonsynonymous somatic mutations in the lepidic, acinar, papillary, and LCNEC components, respectively (Table 1). We also detected 1 and 3 synonymous somatic mutations in the adenocarcinoma and LCNEC components. Copy number graph revealed loss of chromosome 13 in papillary and LCNEC components, consistent with *RB1* loss (Supplementary Fig. 2). In addition, loss of chromosome 10 in papillary and LCNEC components was consistent with PTEN loss. RNA expression analysis was performed using the Todai OncoPanel RNA panel for each component, and hierarchical clustering confirmed that the lepidic and acinar subtypes clustered, and the papillary and LCNEC subtypes (Fig. 2*A*). Gene Set Enrichment Analysis revealed down-regulation of EGFR signaling, and, RB1 targets, SOX4 targets, and cell cycle genes were enriched in the papillary and LCNEC subtypes. No pathogenic germline mutations were detected.

Discussion

Mutations in *EGFR* and *ARHGEF12* and *TERT* gene amplification were detected as trunk mutations, common among all four lesions, indicating each subtype had the same clonal origin. The results of targeted next-generation sequencing in each pathologic subtype suggest that mutations in *RB1, TP53*, and *SMAD4* and apparent loss of heterozygosity of *TP53* and *SMAD4* accompanied the transition of different adenocarcinoma subtypes (Table 1 and Fig. 2*B*). Furthermore, additional gene mutations including *PTEN*, *MST1R*, and *PIK3CA* were noted during transdifferentiation from acinar adenocarcinoma to LCNEC.

The association between the specific histologic pattern of lung adenocarcinoma and gene mutations has been unclear. Whereas invasive mucinous adenocarcinoma has been associated with *KRAS* mutations and *NRG1* fusions,[5] associations between genetic changes and other histologic types of lung cancer have not been reported. Inactivation of *RB1* and *TP53* is known to associate with the transformation from *EGFR*-mutated adenocarcinoma to neuroendocrine carcinoma. In our case, inactivation of *RB1* and *TP53* was necessary, but not sufficient, for the transformation to neuroendocrine carcinoma. Currently, unknown additional factors are needed for the transformation.

We, here, report targeted next-generation sequencing of four lung cancer pathologic subtypes within a single tumor and found that accumulation of genetic mutations can lead to the transition of pathologic subtypes.

Conclusions

Composite clonal analysis revealed transdifferentiation to different pathologic subtypes occurred in a single tumor through the accumulation of gene mutations.

CRediT Authorship Contribution Statement

Takahiro Ando: Writing - original draft, Visualization.

Hidenori Kage: Conceptualization, Writing - review & editing, Project administration.

Aya Shinozaki-Ushiku: Visualization, Investigation.

Kenji Tatsuno, Shuichi Tsutsumi: Data curation, Visualization, Formal analysis.

Kazuhiro Nagayama: Data curation, Resources.

Jun Nakajima: Data curation, Resources, Writing - review & editing.

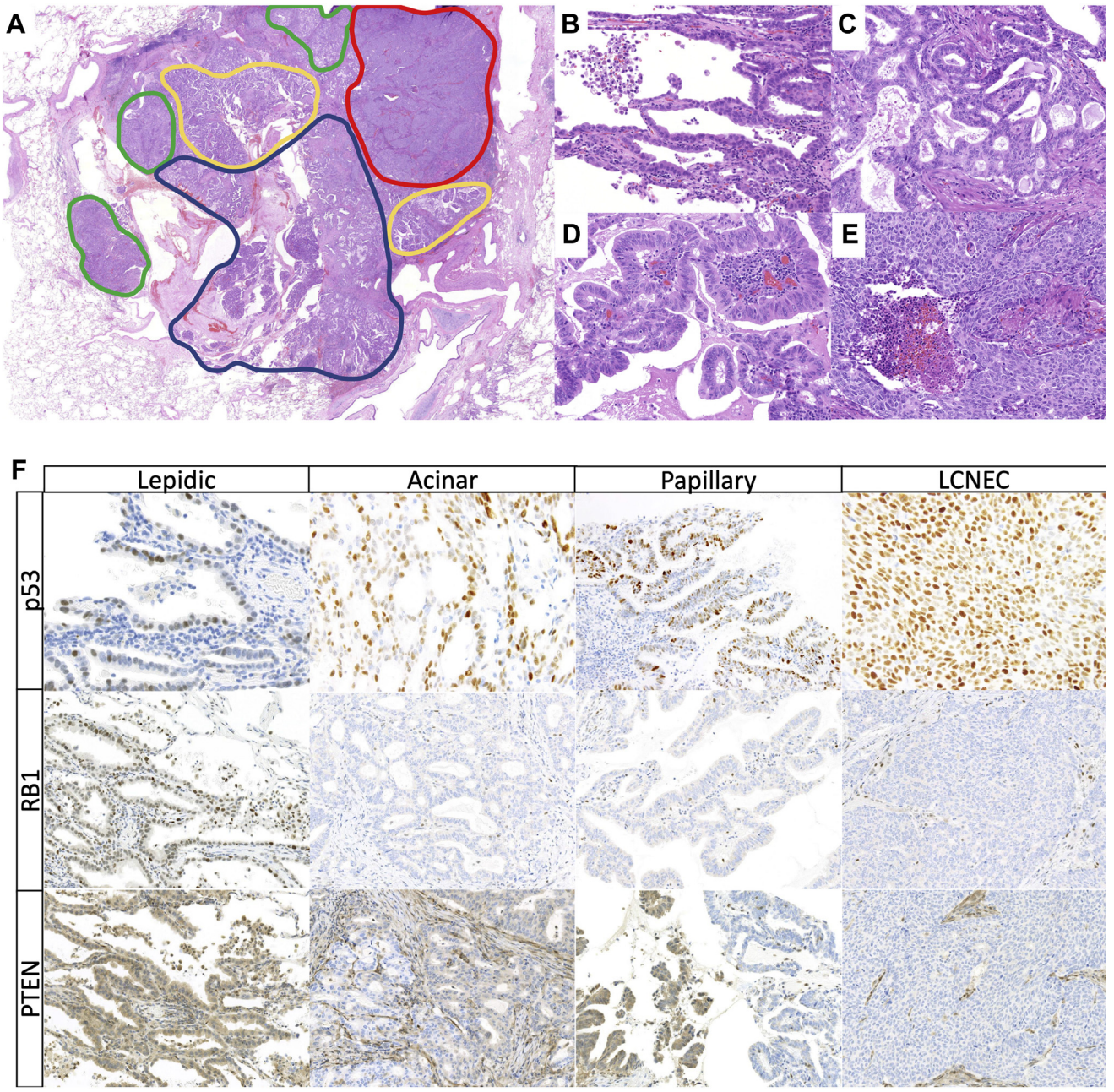

Figure 1. (*A*) Combined LCNEC and adenocarcinoma with mixed histologic pattern resected from a 67-year-old man (hematoxylin and eosin stain; overview). Acinar component (green), papillary component (yellow), both acinar and papillary components (blue), LCNEC (red). The lepidic component was obtained from different sections (not shown). Higher magnification of (*B*) lepidic component, (*C*) acinar component, (*D*) papillary component, and (*E*) LCNEC component (hematoxylin and eosin stain; original magnifications: ×200). (*F*) Immunohistochemical staining of the adenocarcinoma components and the LCNEC component. The adenocarcinoma components were focally positive for p53. The lepidic component was positive for RB1 and PTEN; the acinar and papillary components were heterogeneously positive for PTEN and negative for RB1. The LCNEC component was diffusely positive for p53 and negative for RB1 and PTEN. LCNEC, large cell neuroendocrine carcinoma.

Shinji Kohsaka: Data curation, Investigation.

Kiyoshi Miyagawa, Hiroyuki Mano, Takahide Nagase: Writing - review & editing, Supervision.

Hiroyuki Aburatani: Data curation, Investigation, Formal analysis, Writing - review & editing, Supervision.

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Table 1. Nonsynonymous and Synonymous Gene Mutations from the Primary Tumor Detected by Targeted Sequencing

Gene Mutation Type	Gene	Amino Acid Change	Histologic Subtype			
			Lepidic Adenocarcinoma, %[a]	Acinar Adenocarcinoma, %[b]	Papillary Adenocarcinoma, %[c]	Large Cell Neuroendocrine Carcinoma, %[d]
Nonsynonymous gene mutations						
	EGFR	**p.E746_P753delinsVS**	**11**	**9**	**20**	**25**
	ARHGEF12	**p.Y391C**	**11**	**13**	**34**	**55**
	CDKN2A	p.D74N	6			
	FMN2	p.E1615Dfs*8		5		10
	FMN2	p.Q584*				13
	SMAD4	p.R361C		12	43	55
	TP53	p.V173E		12	46	61
	CCND1	p.E69			6	
	ZFHX3	p.S3638C			39	
	BRCA1	p.E1526K				9
	MST1R	p.E1121K				60
	PIK3CA	p.E545Q				36
Synonymous gene mutations						
	BCL6	**c.1677C>G**	**7**	**13**	**35**	**41**
	ALK	c.450C>T				40
	DDR2	c.1914C>T				33

Note: The values in the table represent detected nonsynonymous or synonymous somatic mutations and allele frequency. Gene mutations shared between large cell neuroendocrine carcinoma and each component of adenocarcinoma are in boldface type.
[a]Estimated tumor purity of the lepidic component was 27.0%.
[b]Estimated tumor purity of the acinar component was 33.3%.
[c]Estimated tumor purity of the papillary component was 63.0%.
[d]Estimated tumor purity of the large cell neuroendocrine carcinoma component was 76.5%.

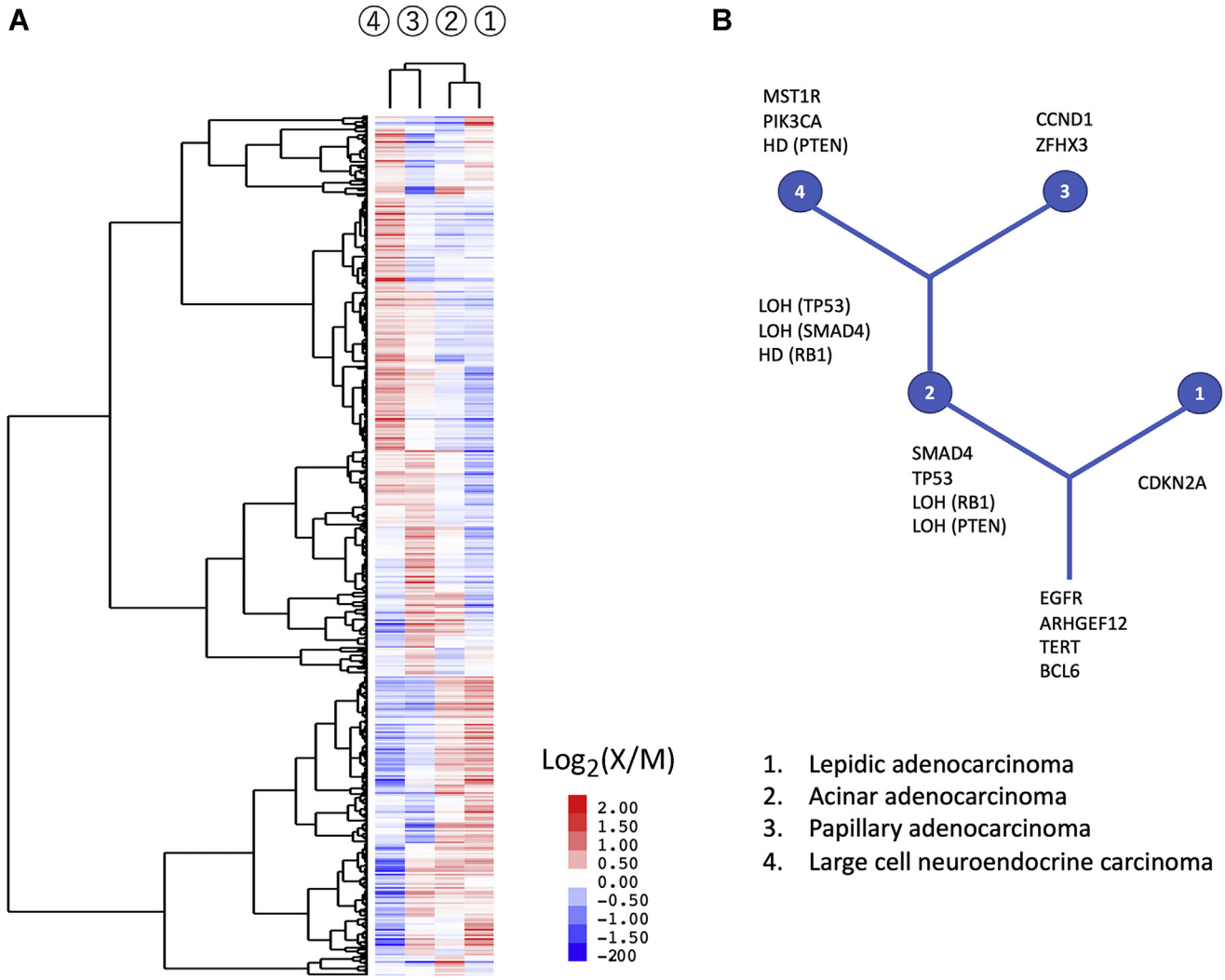

Figure 2. (*A*) Hierarchical clustering of mean-centered RNA gene expression of each component in the adenocarcinoma subtypes and the large cell neuroendocrine carcinoma subtype. We identified DEGs among the lepidic subtype and other adenocarcinoma or large cell neuroendocrine carcinoma subtypes. We performed hierarchical clustering by analyzing the difference between each FPKM value of DEGs (X) and the mean FPKM value (M). (*B*) Accumulation of genetic mutations and transition of pathologic subtypes. The relationship between genetic mutations and histologic subtype was illustrated. DEG, differentially expressed gene; FPKM, fragments per kilobase per million mapped reads; HD, homogeneous deletion; LOH, loss of heterozygosity.

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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.2022.100277.

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