Genomic Profiling of Large-Cell Neuroendocrine Carcinoma of the Lung

Tomohiro Miyoshi¹², Shigeki Umemura¹², Yuki Matsumura², Sachiyu Mimaki¹, Satoshi Tada¹, Hideki Makinoshima¹, Genichiro Ishii⁴, Hibiki Udagawa¹², Shingo Matsumoto¹², Kiyotaka Yoh², Seiji Niho², Hironobu Ohmatsu², Keiju Aokage⁵, Tomoyuki Hishida², Junji Yoshida³, Kanji Nagai², Koichi Goto², Masahiro Tsuboi⁷, and Katsuya Tsuchihara¹

Abstract

Purpose: Although large-cell neuroendocrine carcinoma (LCNEC) of the lung shares many clinical characteristics with small-cell lung cancer (SCLC), little is known about its molecular features. We analyzed lung LCNECs to identify biologically relevant genomic alterations.

Experimental Design: We performed targeted capture sequencing of all the coding exons of 244 cancer-related genes on 78 LCNEC samples [65 surgically resected cases, including 101LCNECs combined with non–small-cell lung cancer (NSCLC) types analyzed separately, and biopsies of 13 advanced cases]. Frequencies of genetic alterations were compared with those of 141 SCLCs (50 surgically resected cases and biopsies of 91 advanced cases).

Results: We found a relatively high prevalence of inactivating mutations in TP53 (71%) and RB1 (26%), but the mutation frequency in RB1 was lower than that in SCLCs (40%, P = 0.039). In addition, genetic alterations in the PI3K/AKT/mTOR pathway were detected in 12 (15%) of the tumors: PIK3CA 3%, PTEN 4%, AKT2 4%, RICTOR 5%, and mTOR 1%. Other activating alterations were detected in KRAS (6%), FGFR1 (5%), KIT (4%), ERBB2 (4%), HRAS (1%), and EGFR (1%). Five of 10 cases of LCNECs combined with NSCLCs harbored previously reported driver gene alterations, all of which were shared between the two components. The median concordance rate of candidate somatic mutations between the two components was 71% (range, 60%–100%).

Conclusions: LCNECs have a similar genomic profile to SCLC, including promising therapeutic targets, such as the PI3K/AKT/mTOR pathway and other gene alterations. Sequencing-based molecular profiling is warranted in LCNEC for targeted therapies.

Introduction

Large-cell neuroendocrine carcinoma (LCNEC) of the lung is a highly malignant tumor with a poor prognosis. It is a rare tumor, diagnosed in approximately 3% of patients with lung cancer who undergo surgical resection (1). LCNEC is classified as a neuroendocrine tumor along with small-cell lung cancer (SCLC) in the 4th edition of the World Health Organization Classification of Lung Tumors (2). LCNEC is distinguished from SCLC based on histologic criteria, including larger cell size, abundant cytoplasm, prominent nucleoli, vesicular nuclei or coarse chromatin, and a polygonal rather than a fusiform shape (3). Asamura and colleagues (4) demonstrated similar clinicopathologic characteristics and prognoses between 141 surgically resected LCNECs and 113 SCLCs in a large-scale Japanese multi-institutional study.

As LCNEC shares many similarities with SCLC in terms of histologic structure, immunohistochemical staining characteristics, and molecular biology (5–7), SCLC-based chemotherapy was expected to show similar effectiveness in patients with LCNEC (8). In a multicenter prospective phase II study, however, combination chemotherapy with irinotecan and cisplatin resulted in inferior overall survival (OS) among patients with LCNEC than those with SCLC (9), suggesting a metabolic distinction between LCNEC and SCLC.

Genomic analyses have revealed therapeutic targets in lung adenocarcinoma, such as EGFR mutations, and molecularly targeted therapies have achieved significant improvement in patient outcomes. Recent studies featuring comprehensive genomic analysis of SCLC have demonstrated a high prevalence of inactivating mutations in TP53 and RB1 (10–13). Genetic alterations in the PI3K/AKT/mTOR pathway were identified as possible therapeutic targets (12, 13). Because of its rarity, however, information about therapeutically relevant genetic alteration in LCNEC is insufficient.

In this report, we examined surgically resected LCNEC for biologically relevant genomic alterations using a next-generation sequencing–based genomic profiling analysis. Biopsied samples of advanced stage LCNEC, which are the major subject of molecular targeted therapies in clinical practice, were also analyzed to investigate the changes in genetic profiles with cancer progression.
We compared the genomic profiles of LCNECs with those of SCLCs. Combined LCNECs with additional components of other non–small cell lung cancer (NSCLC) were analyzed for intratumoral heterogeneity to investigate the origin of LCNEC.

Materials and Methods
This study was approved by the Institutional Review Board (IRB) of the National Cancer Center, Japan (IRB number: 2011-201 and 2013-294). All clinicopathologic data extracted in this study were obtained from our database.

Samples
We collected 78 LCNEC samples (65 surgically resected and 13 biopsied from patients with advanced disease) from 1992 through 2014 and 90 advanced SCLC samples obtained by biopsy from 1995 through 2013 at the National Cancer Center Hospital East, Japan. All patients were chemotherapy naïve. All specimens had been fixed with 10% formalin (52 LCNECs and 90 SCLCs) or 100% methyl alcohol (26 LCNECs) and were embedded in paraffin. Serial 4-μm sections, including the largest diameter of the primary tumor, were stained using hematoxylin and eosin and were reviewed and classified by two pathologists (T. Miyoshi and G. Ishii) according to the 4th edition of the World Health Organization Classification of Lung Tumors. For LCNEC, neuroendocrine features were confirmed by at least one of the following neuroendocrine markers: CD56, chromogranin A, or synaptophysin. Specimens containing a minimum of 50% tumor cells were used for analysis.

We cored the tumor component out of the paraffin-embedded block with a 2-mm-diameter punch for tissue microarray (TMA) and used the core for DNA extraction. In cases of combined LCNEC and NSCLC, both components were identified using light microscopy and cored out separately. A representative example of LCNEC combined with adenocarcinoma is illustrated in Fig. 1, and histopathologic images of TMA cored out from each component (LCNEC and adenocarcinoma) are shown in Supplementary Fig. S1.

Procedures to extract DNA and target sequencing
DNA was prepared [Absolutely RNA FFPE Kit (modified protocol for DNA extraction), Agilent Technologies] and quantified (Quant-iT PicoGreen dsDNA Reagent and Kits, Life Technologies). Target-sequencing libraries were prepared using up to 1 μg of dsDNA. Targeted regions were captured using a custom target-capturing panel (SureSelect XT custom 0.5–2.9 Mb, Agilent Technologies) containing all the coding exons of 244 genes selected on...
the basis of the results of previous genomic analyses of SCLC (Supplementary Table S1; ref. 12), giving a final capture size of 1.499 Mb. The target capture libraries were sequenced (HiSeq 1500, Illumina) to generate 100-bp paired-end data. The results of the sequencing run are shown in Supplementary Tables S2 and S3.

Mutation call
The sequencing reads were mapped to the UCSC human reference genome (GRCh37/hg19) using Burrows-Wheeler Aligner (BWA) software (14) set to the default parameters. The SNVs and insertions and deletions (indels) were called using the Genome Analysis Toolkit (GATK version 1.6, Broad Institute, Cambridge, MA; refs. 15, 16). Systematic errors in sequencing and mapping were filtered out using custom filters (GATK confidence score ≥ 50, number of variant reads in each direction ≥ 1, and variant allele frequency ≥ 10%). Known germline variations represented in the National Center for Biotechnology Information Database (dbSNP build 131; ref. 17) were excluded. In addition, rare germline SNVs were discarded using exomes provided from the 1000 Genomes Project (the phase I exome data, 20110521) and 274 in-house Japanese exomes. False-positive calls (residual errors after this filtering) were excluded through visual inspection. To reduce contamination of the germline variants as much as possible and to extract more probable variants as candidate somatic mutations, we defined candidate somatic mutations as follows: (i) nonsense, in-frame, and frame-shift insertions or deletions (indels); and (ii) missense mutations registered in the Catalogue of Somatic Mutations in Cancer (COSMIC, released ver. 72) database as "confirmed somatic" or "previously reported." We defined mutations with more than 10 cases registered in the COSMIC database as hotspot mutations.

Copy number analysis
We calculated the gene copy number using the total depth on the covered region of each of the 244 targeted genes. The copy number of a gene (gene: t) of a sample (sample: i) was calculated as follows:

\[ Ct,i = \frac{R_{t,i}}{R_{\text{standard},i}} \]

The \( P \) values of the \( R_{t,i} \) were calculated using the binomial probability function. We assumed the \( R_{t,i} \) of gene t to be under \( X \sim B(n, p) \) where \( X \) is the integer of \( n R_{t,i} \), setting \( n = 1,000 \) for convenience and \( P = \text{mean}(R_{t,i}) \), respectively. Samples with \( P \) values of the \( R_{t,i} < 0.05 \) were excluded from calculation of the standard coverage rate of gene t (\( R_{\text{standard},i} \)). If the number of samples whose \( P \) values of the \( R_{t,i} \) was ≥ 0.05 was \( m \), \( R_{\text{standard},i} \) was calculated as follows:

\[ R_{\text{standard},i} = \frac{\sum_{k=1}^{m} R_{t,i}}{m} \]

The predicted copy number of gene t of a sample i (\( C_{t,i} \)) was calculated as follows:

\[ C_{t,i} = 2 \times \frac{R_{t,i}}{R_{\text{standard},i}} \]

The \( P \) values of the \( C_{t,i} \) were also calculated using the binomial probability function.
We defined copy number gain if the \( C_{t,i} \) was ≥ 4 and the \( P \) value of the \( R_{t,i} \) was < 0.05. Then, we defined copy number amplified if the \( C_{t,i} \) was ≥ 10, and the \( P \) value of the \( R_{t,i} \) was < 0.05.

Comparison with SCLC
We performed target capture sequencing on 90 biopsy samples of advanced SCLC. The genomic alteration data for 50 surgically resected SCLC tumors and one advanced SCLC tumor (biopsied by mediastinoscopy) were extracted from our previous work (12). For SCLC cases with combined histology, only the SCLC component was selectively cored out and used for the analysis. Mutation call and copy number analyses were performed in the same manner as in the LCNEC samples. Combining genomic alteration data of these 141 SCLC samples, they were compared with the genomic profiles of the 78 LCNECs.

Validation of the result of target sequence and copy number analysis
We validated the accuracy of the results of the target sequencing employed in this study using a sequencing panel [Oncomine Cancer Research Panel (OCP), Fisher Scientific]. All 20 mutations detected in seven LCNEC mutations, and 13 SCLC mutations were validated with the OCP (Supplementary Table S4). We then performed Sanger sequencing for five mutations on the PI3K/AKT/mTOR pathway detected in LCNEC, and all of them were validated (Supplementary Fig. S2; Supplementary Tables S4 and S5). The methods of Sanger sequencing are summarized in the Supplementary Methods. In addition, one EGFR mutation (L858R) detected in a SCLC biopsy sample (No. 198) was validated by the PCR clamp method.
To validate the accuracy of copy number calculation using total depth on the covered region of each of the 244 targeted genes, we made a comparison with whole-exome sequencing for surgically resected SCLCs performed previously in our institution, in which an SNP array was employed to analyze the genotype and DNA copy number in 47 primary–normal paired samples (12). In the SNP array, a gene was considered copy number amplified if the calculated copy number was ≥ 24. When we compared the results of the SNP array for surgically resected SCLC samples, the sensitivity, specificity, positive predictive value, and negative predictive value of the estimated copy number employed in this study were 43.6%, 99.9%, 85.0%, and 99.5%, respectively. Furthermore, we selected 10 genes in seven samples (four LCNEC samples and three SCLC biopsy samples) whose copy number was ≥ 4 and validated them using OCP. In OCP, a gene was considered as "amplification suspected" if the copy number obtained was ≥ 4 and "amplified" if the copy number obtained was ≥ 7. There was a high correlation between the estimated copy number calculated in this study and that obtained by OCP (Supplementary Fig. S3). In addition, we validated 13 samples (eight LCNEC samples, one SCLC biopsy sample, and four SCLC surgically resected samples), which had copy number gains in MYCL1, MYC, or FGFR1 by a qPCR (Supplementary Fig. S4). The methods are summarized in the Supplementary Methods.
Eventually, all 18 copy number gains for MYCL1, MYC, or FGFR1 subject to validation were confirmed via Oncomine and/or qPCR.

Immunohistochemical staining for ALK, RB, and p16
We performed immunohistochemical staining of ALK, RB, and p16 protein for all 65 resected LCNECs. The companies from which we purchased the antibodies and details of the IHC procedures are shown in Supplementary Table S6. The procedure for immunohistochemical staining of RB and p16 is summarized in the Supplementary Methods.

Statistical analysis
Differences in categorical variables between two groups for statistical significance were evaluated using the $\chi^2$ test or Fisher exact test. All reported $P$ values were two sided, and the significance level was set at $P < 0.05$. Analyses were performed using commercial software (SPSS 22 SPSS II for Windows, SPSS Inc.).

Results

Patient demographics
Demographics of the 78 patients with LCNEC are summarized in Table 1. Median age at the time of surgical resection or biopsy was 70 years (range, 22–84). Sixty-seven (86%) patients were male, and 76 (97%) patients had a history of smoking. Pathologic stages were distributed as follows: I/II/III = 38/14/13 for 65 surgically resected patients, and clinical stages were distributed as follows: II/III/IV = 1/4/8 for 13 advanced biopsy patients.

For the 91 SCLC biopsy cases, median age at the time of biopsy was 67 years (range, 37–85). Seventy-one (78%) patients were male, and 88 (97%) patients had a history of smoking. Clinical stages were distributed as follows: II/III/IV = 1/26/64.

Frequent genetic alterations and comparison between LCNEC and SCLC
The profile of frequent genetic alterations in LCNECs and SCLCs is in Fig. 2, and the entire list of mutations is provided in the

![Figure 2](image_url)

Profile of frequent genetic alterations in LCNEC compared with SCLC. A, The mutation frequency of RB1 in LCNECs (20 cases, 26%) was lower than that in the SCLCs (40%, $P = 0.039$). The mutations of LAMA1, PCLO, MEGFR, and RICTOR were significantly more frequent in LCNEC than SCLC. B, Copy number gains or amplifications of ERBB2 and SETBP1 were significantly more frequent in LCNEC than SCLC.
Supplementary Tables S7 and S8. In all LCNECs, both resected and biopsy cases, we identified a relatively high prevalence of inactivating mutations in TP53 (55 cases, 71%) and RB1 (20 cases, 26%). The mutation frequency of RB1 in the LCNECs was lower than that in the SCLCs (40%, \( P = 0.039 \)). Other mutations that were significantly more frequent in LCNEC were as follows: LAMA1 (10% in LCNEC and 2% in SCLC, \( P = 0.019 \)), PCLO (6% in LCNEC and 1% in SCLC, \( P = 0.023 \)), MEGF8 (5% in LCNEC and 0% in SCLC, \( P = 0.015 \)), and RICTOR (3% in LCNEC and 0% in SCLC, \( P = 0.044 \)). Copy number gain in ERBB2 and SETBP1 was significantly more frequent in LCNEC (4% in LCNEC and 0% in SCLC, \( P = 0.044 \)).

**Comparison between resected and biopsy cases**

In LCNEC, the number of patients with copy number gain in MYC family genes was significantly higher in advanced stage biopsy cases (MYC/MYCL1/MYC/N, 15%/23%/8%) than in early-stage surgically resected cases (MYC/MYCL1/MYC/N, 0%/8%/0%; \( P = 0.002 \)). Other genomic alterations did not differ among disease stages.

**Key driver mutations**

As for key driver mutations, we focused on the mutations that occurred in the receptor tyrosine kinases (RTK)/RAS signaling pathway genes listed in two previous comprehensive genomic studies of lung cancer (13, 18). An overview of the key driver alterations and other activating alterations in LCNEC is shown in Fig 3. Genetic mutations or copy number gains in the PI3K/AKT/mTOR pathway were detected in 12 (15%) tumors. Other activating alterations were detected in KRAS (6%), FGFR1 (5%), KIT (4%), ERBB2 (4%), HRAS (5%), and EGFR (1%). Among the 18 cases with mutations in targetable RTK genes, three cases had an activating mutation in the protein tyrosine kinase domain: one in EGFR (E746_A750 del), one in KIT (D816H), and one in ERBB2 (V842I). Hotspot mutations detected in this study are listed in Table 2.

For the 141 SCLC patients, including surgically resected and biopsy cases, Supplementary Fig. S5 shows an overview of the key driver alterations and other activating alterations. Genetic alterations in the PI3K/AKT/mTOR pathway were detected in 24 (17%) of the tumors: PIK3CA (4%), Pten (6%), AKT2 (2%), and RICTOR (6%). Other known activating alterations were also detected in FGFR1 (3%), KRAS (2%), KIT (1%), ERBB2 (1%), and EGFR (1%). Among 91 biopsy cases, genetic alterations in the
PI3K/AKT/mTOR pathway were detected in 15 (16%) of the tumors: 
PISKCA (4%), PTEN (7%), AKT2 (1%), and RICTOR (4%). Other activating alterations were also detected in FGFR1 (3%), KRAAS (2%), KIT (1%), and EGFR (1%). Hotspot mutations detected in the biopsy cases of SCLC are listed in the Supplementary Table S9.

Immunohistochemical staining for ALK, RB1, and p16
In the 65 resected LCNECs, no sample was positive for ALK staining, suggesting that these cases did not possess any fusion of this gene. Decreased expression of RB protein was detected in 74% (48/65) of the samples by IHC. Most of the RB1-mutated samples (93%, 13/14) were negative for RB staining, and the mutual exclusivity of protein expression between RB and p16 was distinct (Supplementary Fig. S6).

Relationship between genomic alteration of RTKs and protein expression in LCNEC
Supplementary Fig. S7 shows the relationship between RTK overexpression and genomic alterations in 51 resected LCNECs. The protein expression data for these LCNEC tumors are from our previous report (7). In this analysis, we saw no significant correlation between strong positivity for RTK expression and genomic alterations.

Overall survival of surgically resected LCNEC cases
The median follow-up period was 37 months. The 3-year OS rate for the 65 surgically resected LCNEC patients was 65%. We classified cases with/without PI3K/AKT/mTOR pathway alteration, copy number gain in MYC family genes, RB expression, and p16 expression, but no prognostic significance was determined (data not shown).

Combined LCNEC
In one case of LCNEC combined with adenocarcinoma, both components shared a well-established in-frame deletion in EGFR: protein tyrosine kinase domain in Exon 19 (E746_A750 del). A case of LCNEC combined with adenocarcinoma had an activating mutation in KRAAS: ras family domain in codon 12 (G12V) in both components, and one LCNEC combined with squamous cell carcinoma (SCC) had an activating mutation in PI3KCA: the catalytic subunit of phosphoinositide-3 kinase (E545K) in both components. In total, five of 10 LCNECs combined with another tumor type harbored the same key driver mutations in both components. An overview of the individual mutations occurring in each component is listed in Table 1.

Table 1. Patient characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No. of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LCNEC (n = 78)</td>
</tr>
<tr>
<td>Gender: male/female</td>
<td>67/11</td>
</tr>
<tr>
<td>Age, years: median (range)</td>
<td>70 (22-84)</td>
</tr>
<tr>
<td>Smoking status: never/ever</td>
<td>2/76</td>
</tr>
<tr>
<td>Pack years: median (range)</td>
<td>51 (0-208)</td>
</tr>
<tr>
<td>Histology: pure/combined type</td>
<td>68/10</td>
</tr>
<tr>
<td>Surgically resected/biopsy</td>
<td>65/13</td>
</tr>
<tr>
<td>Surgically resected sample</td>
<td>65</td>
</tr>
<tr>
<td>Pathologic stage I/Il/III</td>
<td>38/14/33</td>
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<tr>
<td>Vascular invasion: present/absent</td>
<td>52/13</td>
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<tr>
<td>Lymphatic invasion: present/absent/unknown</td>
<td>23/42/0</td>
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<tr>
<td>Pleural invasion: present/absent/unknown</td>
<td>25/40/0</td>
</tr>
<tr>
<td>Biopsy sample</td>
<td>13</td>
</tr>
<tr>
<td>Clinical stage I/Il/IV</td>
<td>1/4/8</td>
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<tr>
<td>Biopsy procedure: TBLB/lymph node biopsy/others</td>
<td>9/2/2</td>
</tr>
<tr>
<td>First-line treatment: CRT/chemotherapy alone/RT alone</td>
<td>4/7/1</td>
</tr>
<tr>
<td>BSC unknown</td>
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</tbody>
</table>

Abbreviations: BSC, best supportive care; CRT, chemoradiotherapy; RT, radiotherapy; TBLB, transbronchial lung biopsy.
Table 2. Hotspot mutations detected in LCNEC

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Case No.</th>
<th>Mutation type</th>
<th>Mutation site</th>
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<tbody>
<tr>
<td>PIK3CA</td>
<td>LCNEC_66c</td>
<td>E545K</td>
<td>PIK helical domain</td>
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<tr>
<td>KRAS</td>
<td>LCNEC_39c</td>
<td>G12D</td>
<td>Ras family domain</td>
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<tr>
<td></td>
<td>LCNEC_62c</td>
<td>G12V</td>
<td>Ras family domain</td>
</tr>
<tr>
<td></td>
<td>LCNEC_46c</td>
<td>Q66R</td>
<td>Ras family domain</td>
</tr>
<tr>
<td>HRAS</td>
<td>LCNEC_3c</td>
<td>G12V</td>
<td>Ras family domain</td>
</tr>
<tr>
<td>KIT</td>
<td>LCNEC_26c</td>
<td>D816H</td>
<td>Protein tyrosine kinase domain</td>
</tr>
<tr>
<td>ERBB2</td>
<td>LCNEC_40c</td>
<td>V842I</td>
<td>Protein tyrosine kinase domain</td>
</tr>
<tr>
<td>EGFR</td>
<td>LCNEC_60c</td>
<td>E746-A750 del</td>
<td>Protein tyrosine kinase domain</td>
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</tbody>
</table>

Overview of individual mutations in each component of 10 LCNECs combined with another cell type

Table 3. Overview of individual mutations in each component of 10 LCNECs combined with another cell type

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Case 1</th>
<th>Case 2</th>
<th>Case 3</th>
<th>Case 4</th>
<th>Case 5</th>
<th>Case 6</th>
<th>Case 7</th>
<th>Case 8</th>
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<th>Case 10</th>
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<tr>
<td>TP53</td>
<td>H214R</td>
<td>E258G</td>
<td>W99*</td>
<td>K120E</td>
<td>R283P</td>
<td>R158L</td>
<td>N131Y</td>
<td>E285*</td>
<td>E258G</td>
<td>V157F</td>
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<td>R158L</td>
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<tr>
<td>PIK3CA</td>
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<td>KRAF</td>
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<td>V108B</td>
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Abbreviations: A, adenocarcinoma component; aci, acinar adenocarcinoma; L, LCNEC component; pap, papillary adenocarcinoma; S, SCC component.
To further analyze the relationship between LCNEC and SCLC, we performed a Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) analysis using the STRING database (http://string-db.org/). The protein–protein interaction networks were obtained by applying the six most frequently mutated genes (TP53, RB1, MLL3, LAMA1, MLL2, and NOTCH1). The STRING network enrichment results on KEGG pathways indicated a strong association with pathways of SCLC ($P = 0.00038$; Supplementary Table S11). LAMA1, PCLO, MEGF8, and RICTOR were more mutated in LCNECs than with SCLC. It is reported that LAMA1 is associated with cancer pathways, such as integrin signaling and cell adhesion in NSCLC (39). In addition, PCLO promoted cell invasion in liver cancer cell lines (40). These four genes might be associated with the etiology of LCNEC; however, confirmation in large studies is needed, and functional experiments on these genes are required to clarify their biological role.

Patient backgrounds show that most of the biopsy cases were of advanced disease. On comparison of the resected cases and biopsy cases, copy number gain in MYC family genes was significantly more frequent in advanced cases of LCNEC. Amplified MYC family genes are reported to be negative prognostic factors in lung SCLC (41), and this may also apply to LCNEC.

In LCNECs combined with adenocarcinoma, two pairs had well-established oncogenic mutations (one in EGFR and one in KRAS), while in LCNEC combined with SCC, one pair had a PIK3CA-activating mutation and one pair had copy number gain in FGFR1. In total, up to half of the combined LCNECs had established key driver mutations in both components, suggesting that therapies targeted toward these alterations would be effective for combined LCNECs. These genetic alterations were shared in both LCNEC and NSCLC components. In addition, both components also shared genetic alterations of TP53 or RB1. These shared driver mutations between LCNEC and NSCLC components suggest that these mutations were acquired relatively early in the evolution of the combined LCNEC. In this study, the median concordance rate of genetic alterations in combined LCNEC was 71%. Zhang and colleagues (42) investigated the intratumoral heterogeneity in 11 localized lung adenocarcinomas by multi-region whole-exome sequencing and reported that 76% of all mutations and 20 of 21 known cancer genes mutations were identified in all regions of individual tumors. These concordance rates are similar, suggesting that the concordance rate of combined LCNEC is relatively high despite the morphologic differences between LCNEC and NSCLC. Further investigation is needed.

In conclusion, LCNEC and SCLC, which have been histologically categorized as HGNCEC, have similar genomic profiles, including promising therapeutic targets, such as the PI3K/AKT/MTOR pathway. LCNEC harbored other targetable activating mutations, including EGFR, ERBB2, and FGFR1. Sequencing-based molecular profiling may identify patients that will benefit from novel targeted therapies. In combined LCNECs, LCNEC and NSCLC components had highly concordant genetic alterations, including key driver mutations, despite their distinct morphologic appearances. Agents targeted to these alterations would theoretically be effective for both components.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Authors’ Contributions
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): T. Miyoshi, S. Umemura, Y. Matsumura, S. Imaihi, H. Makinoshima, G. Ishii, S. Niho, H. Ohnatsu, K. Tsuochihara
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): T. Miyoshi, S. Umemura, S. Tada, H. Makinoshima, H. Udagawa, J. Yoshida, K. Tsuochihara
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): T. Miyoshi, S. Umemura, S. Tada, H. Makinoshima, S. Matsumoto, K. Tsuochihara
Other (pathologic examination): G. Ishii

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Genomic Profiling of Large-Cell Neuroendocrine Carcinoma of the Lung

Tomohiro Miyoshi, Shigeki Umemura, Yuki Matsumura, et al.