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TTF-1 and c-MYC-defined Phenotypes of Large Cell Neuroendocrine Carcinoma and Delta-like Protein 3 Expression for Treatment Selection

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Abstract: The standard treatment regimen has not yet been established for advanced pulmonary large cell neuroendocrine carcinoma (LCNEC) because of its rarity. LCNEC can be subdivided into 2 mutually exclusive molecular subgroups: *STK11*/*KEAP1* and *TP53* mutated with high neuroendocrine expression and transcriptional profile of ASCL1^{high}/DLL3^{high}/NOTCH^{low} (non–small cell lung carcinoma, NSCLC-like) or *RB1* and *TP53* mutated with reduced neuroendocrine markers and transcriptional pattern of ASCL1^{low}/DLL3^{low}/NOTCH^{high} (small cell lung cancer, SCLC-like). Model-based clustering shows that SCLC has subdivided into 2 major proteomic subsets defined by either TTF-1^{high}/c-MYC^{low} or TTF-1^{low}/c-MYC^{high}, which may correspond to 2 mutually exclusive molecular subgroups: NSCLC-like or SCLC-like, respectively. We herein investigated whether TTF-1 and c-MYC could be applied to LCNEC to identify distinct subsets immunohistochemically and assessed DLL3 expression in these subsets. The protein expression profile may be useful to select patients for potential efficacy of targeted therapies including aurora kinase inhibitors for MYC alterations or anti-DLL3 antibody-drug conjugates. TTF-1 and c-MYC expression was mutually exclusive in 25 of 27 (93%) cases; TTF-1^{+}/c-MYC^{-} in 10, TTF-1^{-}/c-MYC^{+} in 15, and TTF-1^{+}/c-MYC^{+} in 2. DLL3 expression was seen in 15 of 27 cases (56%). All 12 TTF-1^{+} LCNEC cases were positive for DLL3. Three of 15 (20%) TTF-1^{-}/c-MYC^{+} cases showed DLL3 positivity. LCNEC could be separated into 2 subsets proteomically defined by TTF-1 and c-MYC expression, which may be suitable to guide treatment selection including aurora kinase inhibitors for c-MYC^{+} cases. TTF-1 positivity can serve as a surrogate marker for DLL3, but caution is necessary as 20% of TTF-1^{-} cases showed DLL3 positivity.

Key Words: aurora kinase A inhibitor, c-MYC, high-grade neuroendocrine lung tumor, RB protein, Rova-T

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Large cell neuroendocrine carcinoma (LCNEC) is a non–small cell lung cancer (NSCLC) that shows neuroendocrine morphology including organoid nesting and peripheral palisading and expresses neuroendocrine markers immunohistochemically. It classifies under the category of neuroendocrine lung tumors, which include small cell lung cancer (SCLC) and pulmonary carcinoid and accounts for 2% to 3% of lung cancer with rarest neuroendocrine lung tumors.[1] LCNEC is an aggressive tumor that occurs in elderly smokers with 5-year survival rates below 15% to 25%, which is second only to SCLC (5-y survival rates of 5%). The standard treatment regimen has not yet been established in advanced LCNEC. Although patients with advanced LCNEC are often treated with chemotherapy used for SCLC, the efficacy is limited.[2]

Recent next-generation sequencing data segregated LCNEC into 2 mutually exclusive molecular subtypes: NSCLC-like and SCLC-like subsets. NSCLC-like LCNEC was characterized by biallelic *TP53* and *STK11*/*KEAP1* alterations and SCLC-like characterized by biallelic alterations of *TP53* and *RB1*.[3–5] Further, transcriptomic analysis revealed that NSCLC-like LCNEC exhibits a neuroendocrine profile of ASCL1^{high}/DLL3^{high}/NOTCH^{low}, while SCLC-like LCNEC shows reduced neuroendocrine markers and transcriptional pattern of ASCL1^{low}/DLL3^{low}/NOTCH^{high}.[4] SCLC-like LCNEC shows significantly higher levels of a repressor of neuroendocrine markers, *REST*, which may explain the low level of neuroendocrine phenotypes.[4] In addition, a recent report showed that LCNEC with wild-type *RB1* gene had a better prognosis when treated with NSCLC-type chemotherapy (platinum-gemcitabine or paclitaxel) than with

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SCLC-type therapy (platinum-etoposide).[5] These studies suggest that the distinction of LCNEC representing as NSCLC-like or SCLC-like is important for the efficacy of targeted therapeutics, including NOTCH pathway and immune checkpoint inhibitors.[6]

NOTCH ligand is a downstream target of ASCL1 and is overexpressed in many neuroendocrine cancers.[7] NOTCH signaling is a conserved cell signaling system in multicellular organisms that plays an important role in developmental cell fate decisions.[8] The cell surface NOTCH ligand delta-like 3 (DLL3) is an atypical member of the NOTCH receptor-ligand family that is located in the Golgi apparatus and inhibits NOTCH signaling, unlike other ligands of NOTCH receptors. DLL3 overexpression can promote cell proliferation and tumor growth in murine lung cancer cells by PI3K/Akt signaling through inhibiting NOTCH signaling.[9] DLL3 is expressed on the cell surface membrane of pulmonary neuroendocrine tumors,[7] and, in particular, DLL3 is expressed in more than 80% of SCLC cases.[10–14] Because normal lung tissue does not exhibit DLL3, DLL3 is thought to be a tumor-associated antigen and a DLL-3-targeting antibody-drug conjugate, rovalpituzumab tesirine (Rova-T), was developed.[11] Eight LCNEC cases were included in a phase 1 study of Rova-T for recurrent SCLC, however, because these cases comprised a small proportion of the study population, LCNEC patients were excluded from the analysis.[12]

Recent model-based clustering has shown that SCLC has subdivided into 2 proteomic subsets defined by either thyroid transcription factor-1 (TTF-1)^{high}/c-MYC^{low} or TTF-1^{low}/c-MYC^{high} expression.[13] TTF-1 and DLL3 levels are highly correlated and TTF-1 could be used as a surrogate marker of DLL3.[13] Based on these studies, LCNEC may cluster into 2 proteomic subsets defined by TTF-1 and c-MYC, similar to SCLC. In addition, MYC is a transcriptional regulator of aurora kinases A and B, which provide cell growth advantage in the absence of p53.[14] SCLC with *MYC* alterations is sensitive to aurora kinase inhibitors (ie, alisertib).[15] Thus, we examined TTF-1 and c-MYC protein expression profiles in LCNEC patients to determine whether there were distinct subtypes in LCNEC and assessed DLL3 expression in these LCNEC subgroups. The protein expression profile could predict the response to targeted therapies including aurora kinases and DLL3 and may be useful in routine clinical practice to rapidly select subsequent therapies.[13] Therefore, we accessed whether DLL3 expression is associated with LCNEC subgroups to guide selection of targeted therapies.

MATERIALS AND METHODS

Patient Selection and Histologic Definition of LCNEC

Twenty-seven patients who had been diagnosed with lung LCNEC in the pathology database at Kyoto Prefectural University of Medicine during 2007 to 2019 were included in this study. Neuroendocrine morphology including organoid nesting, rosette-like structures, and peripheral palisading was required for the diagnosis of LCNEC. Neuroendocrine differentiation was confirmed by at least one of the following neuroendocrine markers: chromogranin A, synaptophysin, and CD56 in more than 10% of the tumor cells.[1] LCNEC with high neuroendocrine expression was defined as LCNEC that was positive for 2 or more neuroendocrine markers. LCNEC with reduced neuroendocrine markers was defined as LCNEC that was positive for only 1 of the 3 neuroendocrine markers tested.

Clinical Data and Treatment

Clinical data were collected for all patients from electronic medical records. Clinical and pathologic stages were determined by the eighth edition of the Union for International Cancer Control/American Joint Committee on Cancer staging system for lung cancer.[16] Patients with stage I LCNEC received surgery alone. Patients with stage II and IIIA LCNEC, as a rule, received adjuvant platinum-based chemotherapy: cisplatin (CDDP) and etoposide (ETP) or carboplatin (CBDCA) and ETP, which is the standard treatment for SCLC.[17,18] Patients with advanced stage LCNEC were given CDDP-based chemotherapy and, if applicable, immune checkpoint inhibitors or EGFR tyrosine kinase inhibitors (TKIs).

Immunohistochemistry

Lobectomy and biopsy specimens were fixed in 10% buffered formalin and embedded in paraffin. Tissue sections of 4-μm thickness were stained with hematoxylin and eosin and immunohistochemistry was conducted for CD56 (NCAM, Clone 1B6, Novocastra Leica Biosystems, Newcastle, UK), chromogranin A (DAK-A3, Dako, Glostrup, Denmark), synaptophysin (27G12, Leica Biosystems, Nussloch, Germany), TTF-1 (NKX2-1, Clone 8G7G3/1, Dako), c-MYC (Y69, Ventana Medical Systems Inc., Tucson, AZ, USA), DLL3 (SP347, Ventana Medical Systems Inc.), and RB protein (13A10, Leica Biosystems) using an autoimmunostainer (Ventana XT System Benchmark; Ventana Medical Systems Inc.).

Definition of Positivity and Tumor Proportion Score for DLL3

Because SCLC patients with high DLL3 expression (at least 50% of cancer cells by immunohistochemistry) showed better response to Rova-T than those with low DLL3 expression (fewer than 50% of cells),[10,12] the percentage of positive tumor cells at any staining intensity in all tumor cells (tumor proportion score, TPS) was calculated for DLL3 by 2 experienced pathologists (A.M.-H. and N. T.-M.). TPS $\geq$75% was regarded as high expression level, 1% to 74% as low expression level, and <1% as negative for DLL3.[19,20] Positivity for c-MYC and TTF-1 expression was defined $\geq$40% and >10% of the tumor cells reacted with any intensity, respectively.[21,22] For RB protein, diffuse and strong staining in tumor cells was regarded as high expression and focal and weak staining as low expression.[23]

TABLE 1. Clinical and Pathologic Summary of LCNEC Patients in Order of TTF-1 Positivity and Tumor Stage

Case No.	Age (y)	Sex	Specimen	Histology	TTF-1	c-MYC	Stage (8th ed)	Adjuvant Chemotherapy	*EGFR* Mutation Status, ALK IHC	Outcome
1	78	F	Lobectomy	LCNEC	P	N	I A1	(−)	NA	Alive, 5 y
2	77	M	Lobectomy	LCNEC	P	N	I B	(−)	NA	Alive, 5 y
3	69	M	Lobectomy	Combined LCNEC (90%) and Ad (10%)	P	N	I B	(−)	NA	Dead, 7 mo (due to gastric cancer, no recurrence of LCNEC)
4	71	M	Lobectomy	LCNEC	P	N	I B	(−)	NA	Alive, 6 y
5	72	M	Lobectomy	Combined LCNEC (10%), SCLC (40%), and Sq (50%)	P	N	I B	CDDP+ETP	NA	Alive, 8 mo
6	58	M	Lobectomy	LCNEC	P	N	II B	(−) due to patient refusal	NA	Dead, 7 mo
7	79	M	Lobectomy	LCNEC	P	N	II B	CDDP+ETP	NA	Dead, 1.8 y
8	64	M	Lobectomy	Combined LCNEC (40%) and Ad (60%)	P	N	III A	Adjuvant chemotherapy	NA	Dead, 5 y
9	78	M	Mediastinal lymph node	Metastatic LCNEC	P	N	cIV A	CBDCA+ETP, AMR, CPT, ATZ	NA	Dead, 9 mo
10	46	M	Mediastinal lymph node	Metastatic LCNEC	P	N	cIV B	CDDP+ETP, EGFR TKI (osimertinib, afatinib), AMR	Mutation in *EGFR* (L858R), ALK IHC (−)	Dead, 9 mo
11	70	M	Lobectomy	LCNEC	P	P	I A2	(−)	NA	Alive, 2.5 y
12	56	M	Lobectomy	Combined LCNEC (80%) and Ad (20%)	P	P	III A	CDDP+ETP	*EGFR* mutation (−), ALK IHC (−)	Alive, 10 mo
13	71	M	Lobectomy	LCNEC	N	P	I A2	CDDP+ETP	NA	Alive, 1.3 y
14	61	M	Lobectomy	LCNEC	N	P	I A2	(−)	NA	Alive, 2 mo
15	60	M	Lobectomy	LCNEC	N	P	I B	(−)	NA	Alive, 12 y
16	74	M	Lobectomy	LCNEC	N	P	I B	Radiation+CDDP+ETP (due to positive margins)	NA	Dead, 6 y
17	72	M	Lobectomy	LCNEC	N	P	I B	(−)	NA	Alive, 2.2 y
18	71	M	Lobectomy	LCNEC	N	P	II B	CDDP+ETP	NA	Alive, 5 y
19	51	M	Lobectomy	LCNEC	N	P	II B	CDDP+ETP	*EGFR* mutation (−)	Alive, 5 y
20	70	M	Lobectomy	LCNEC	N	P	II B	CDDP+ETP	NA	Dead, 2 y
21	72	M	Lobectomy	LCNEC	N	P	II B	Adjuvant chemotherapy +radiation	NA	Dead, 9 y (due to other disease, no recurrence of LCNEC)
22	62	M	Lobectomy	LCNEC	N	P	III A	Ajuvant chemotherapy	*EGFR* mutation (−)	Alive, 1.7 y with multiple liver metastasis
23	72	M	Lobectomy	Combined LCNEC (90%) and Sq (10%)	N	P	III A	CDDP+ETP	NA	Alive, 3.1 y
24	77	M	Mediastinal lymph node	Metastatic LCNEC	N	P	cIII B→ypI A1	Preoperative CDDP+ETP +radiation→ lobectomy	NA	Alive, 7 mo
25	72	M	Axillary lymph node	Metastatic LCNEC	N	P	cIV A	Radiation+CDDP+PD	NA	Dead, 7 mo
26	75	M	Lung biopsy	LCNEC	N	P	cIV A	CBDCA+ETP	NA	Dead, 5 mo
27	75	M	Lung biopsy	LCNEC	N	P	cIV B	CDDP+PD	*EGFR* mutation (−), ALK IHC (−)	Dead, 8 mo

"Dead" indicates the patient died due to LCNEC unless otherwise specified.

Ad indicates adenocarcinoma; AMR, amrubicin; ATZ, atezolizumab; CBDCA, carboplatin; CDDP, cisplatin; CPT, irinotecan; M, male; N, negative; NA, not available; P, positive; PD, pemetrexed disodium; SCLC, small cell lung carcinoma; Sq, squamous cell carcinoma; TKI, tyrosine kinase inhibitors.

Statistical Analysis

Analyses were performed using GraphPad Prism 6 (MDF Co. Ltd, Tokyo, Japan). Differences in categorical variables between 2 groups were evaluated by the Fisher exact test. The significance level was set at $P < 0.05$.

Ethical Approval

This study was conducted according to the principles expressed in the Declaration of Helsinki. The Ethics Committee of Kyoto Prefectural University of Medicine approved the study (ERB-C-1452).

RESULTS

Patient Demographics

Twenty-seven patients were diagnosed with LCNEC at Kyoto Prefectural University of Medicine. Of these, 26 were male and 1 was female with the median age of 71 years (range, 46 to 79). All patients were Japanese. All except 1 patient (case 10) had a history of smoking (96%). Disease stage at initial diagnosis was as follows: IA1 in 1, IA2 in 3, IB in 7, IIB in 6, IIIA in 4, IIIB in 1, IVA in 3, and IVB in 2 patients. Transbronchial lung biopsy was available in 2 cases, lymph node biopsy in 4 cases, and lobectomy specimens in 21 cases. The details of patients included in the study are summarized in Table 1. Twenty-two patients had LCNEC and 5 patients had LCNEC combined with adenocarcinoma (n=3), squamous cell carcinoma (n=1), and SCLC and squamous cell carcinoma (n=1).

Immunohistochemical Staining for Neuroendocrine Markers, TTF-1, c-MYC, and RB1 Protein

The results of immunohistochemistry in LCNEC components are shown in Figure 1.

Ten cases were positive for TTF-1 and negative for c-MYC (Fig. 2A) and 15 cases were negative for TTF-1 and positive for c-MYC (Fig. 2B). The remaining 2 cases were positive for both TTF-1 and c-MYC (Fig. 2C).

All TTF-1^+ LCNEC cases (n=12) showed positivity for at least 2 neuroendocrine markers, indicating high neuroendocrine expression. Of 15 TTF-1^-/c-MYC^+ cases, 9 showed reduced neuroendocrine markers (CD56 only) and the other 6 showed high neuroendocrine expression. TTF-1^+ LCNEC cases expressed higher levels of neuroendocrine markers than TTF-1^- LCNEC cases ($P < 0.05$).

RB protein was negative in 16 cases (59%) and decreased expression of RB protein was seen in 4 cases (15%). The expression was retained in the remaining 7 cases (27%). Loss or decreased RB protein expression was not statistically significant in TTF-1^+/c-MYC^- (9/10, 90%) or TTF-1^-/c-MYC^+ groups (9/15, 60%) ($P > 0.05$).

DLL3 Expression in Association With LCNEC Subtypes

DLL3 expression localized to the plasma membrane and at the Golgi apparatus in 15 of 27 LCNEC cases (56%). DLL3 staining was negative in 12 cases. Concordant staining, either positive or negative for both TTF-1 and DLL3, was found in 24/27 (89%) cases; 12 cases were positive and 12 cases were negative for both markers. Thus, a significant association was found between DLL3 and TTF-1 expression ($P < 0.05$). Among cases that were positive for both markers, 9 cases showed high DLL3 expression (TPS $\geq$75%) and 3 cases showed low DLL3 expression (TPS 40% to 60%). Three cases (11%) exhibited discordant staining for TTF-1 and DLL3 (all TTF-1^-/$DLL3^+$). Two surgical cases (cases 15 and 27) showed low DLL3 expression (TPS 20% and 50%, respectively) and negative TTF-1 expression. The other surgical case (case 26) exhibited high DLL3 expression and negative TTF-1 expression. For 3 cases with negative TTF-1 (clone 8G7G3/1, Dako) and positive DLL3, TTF-1 staining was reassessed by TTF-1 clone SPT24 (Leica) because the clone SPT24 had higher sensitivity than the clone 8G7G3/1.[24] However, all 3 cases were negative for the clone SPT24.

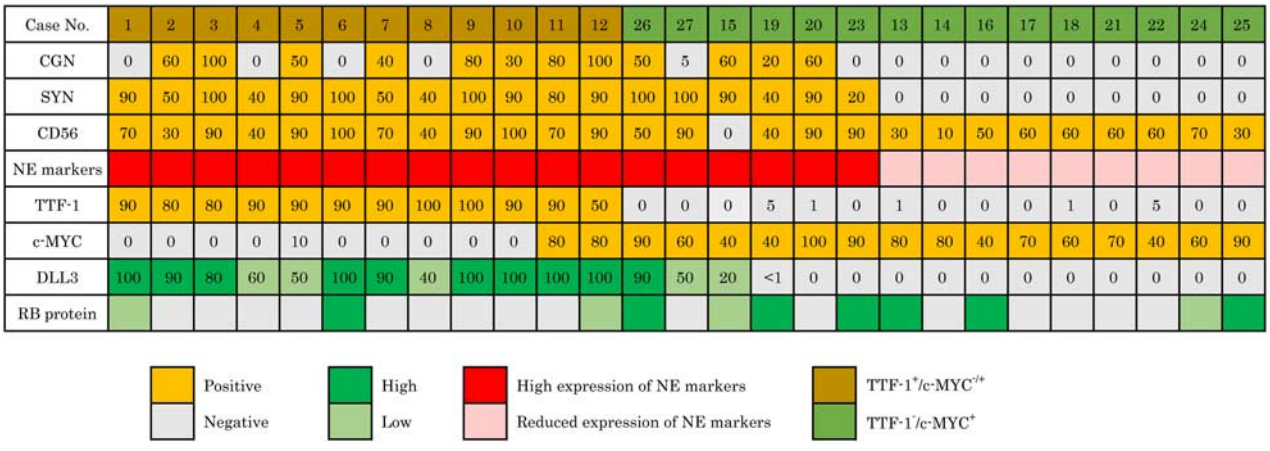

FIGURE 1. Immunohistochemical summary of large cell neuroendocrine carcinoma (LCNEC) patients in order of TTF-1 positivity and high expression of DLL3 and neuroendocrine markers. LCNEC clusters into 2 subsets defined by TTF-1 and c-MYC expression. Numeric data indicate percentage of positive tumor cells for each protein. Tumor proportion score $\geq$75% was regarded as high expression level, 1% to 74% as low expression level, and <1% as negative for DLL3. CGN indicates chromogranin A; NE, neuroendocrine; SYN, synaptophysin.

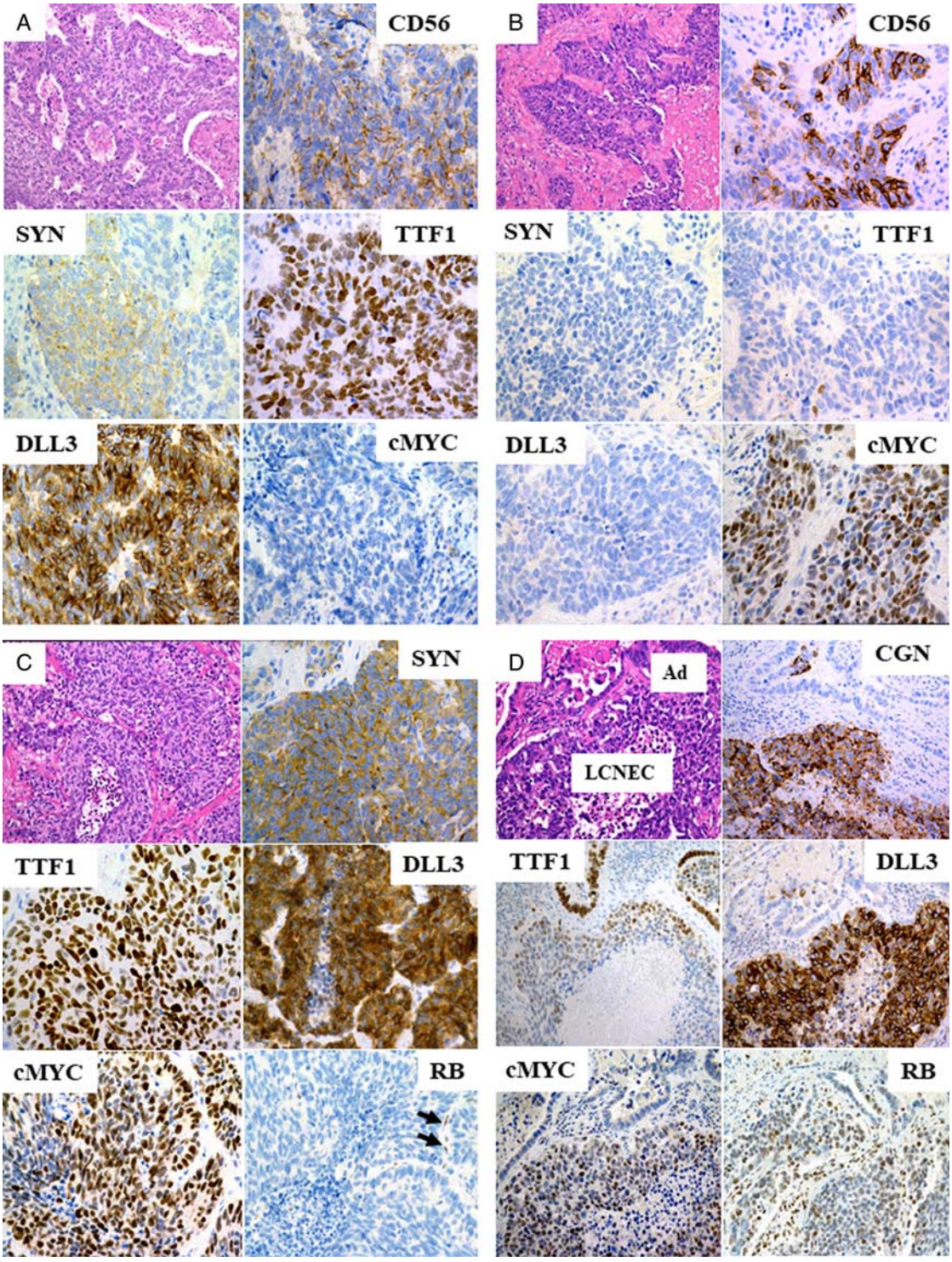

FIGURE 2. Representative staining pattern of large cell neuroendocrine carcinoma (LCNEC). A, Representative case of TTF-1^{+}/c-MYC^{-} immunophenotype (case 4). This case shows high neuroendocrine expression, and DLL3. B, Representative case of TTF-1^{-}/c-MYC^{+} immunophenotype (case 13). This case shows reduced neuroendocrine markers (focal CD56 staining only) with negative DLL3 expression. C, Representative case of LCNEC with TTF-1^{+}/c-MYC^{+} (case 11). This case shows high expression of neuroendocrine markers and DLL3. RB protein was lost (intact RB protein in stromal cells as internal control, arrows). D, Representative case of combined LCNEC (lower two-thirds) and adenocarcinoma (upper one-third) (case 12). Chromogranin A, DLL3, TTF-1, and c-MYC are positive in the LCNEC component, while TTF-1 is positive and the other markers are negative in the adenocarcinoma component. RB protein staining was weak and focal in both components. A–C, Original magnification is ×200 for HE and ×400 for immunostaining. D, Original magnification for all images is ×200.

TABLE 2. Staining Results of other Histologic Subtypes With Combined LCNEC

Case No.	Histology	TTF-1		c-MYC		DLL3		RB protein	
		LCNEC	Other Components	LCNEC	Other Components	LCNEC	Other Components	LCNEC	Other Components
3	LCNEC (90%), Ad (10%)	P	P (Ad)	N	N (Ad)	High	N (Ad)	N	N (Ad)
5	LCNEC (10%), SCLC (40%) Sq (50%)	P	N (Sq), P (SCLC)	N	P (Sq), N (SCLC)	Low	N (Sq), Low (SCLC)	N	High (Sq), N (SCLC)
8	LCNEC (40%), Ad (60%)	P	P (Ad)	N	N (Ad)	Low	N (Ad)	N	Low (Ad)
12	LCNEC (80%), Ad (20%)	P	P (Ad)	P	N (Ad)	High	N (Ad)	Low	Low (Ad)
23	LCNEC (90%), Sq (10%)	N	N (Sq)	P	P (Sq)	N	N (Sq)	High	Low (Sq)

Ad indicates adenocarcinoma; LCNEC, large cell neuroendocrine carcinoma; N, negative; P, positive; SCLC, small cell lung carcinoma; Sq, squamous cell carcinoma.

TTF-1, c-MYC, and DLL3 Staining in other Histologic Components in Combined LCNEC

Five patients had LCNEC combined with other histologic subtypes (combined LCNEC). Three cases were combined with adenocarcinoma components (cases 3, 8, and 12), 1 squamous cell carcinoma (case 23), and the remaining 1 with SCLC and squamous cell carcinoma (case 5). Among these 5 cases, DLL3 was negative in adenocarcinoma and squamous cell carcinoma components, while TTF-1 and DLL3 were positive in the SCLC component, similar to LCNEC (Table 2). In 3 cases combined with adenocarcinoma, all adenocarcinoma components were TTF-1-positive and c-MYC-negative (Fig. 2D). For 2 combined cases with squamous cell carcinoma, both were TTF-1-negative and c-MYC-positive in squamous cell carcinoma components. RB protein was retained in adenocarcinoma and squamous cell carcinoma components except for case 3. For case 3, RB protein loss was not only confined to the LCNEC component, but also in the adenocarcinoma component.[4] The SCLC component showed RB protein loss as the LCNEC component.

Therapy and Outcome

Patients with stage I LCNEC received surgery alone except for 2 patients with pathologic stage IB LCNEC who also received chemotherapy after surgery. For these 2 patients, one received radiotherapy plus chemotherapy because of positive surgical margins (case 16) and the other (case 5) received adjuvant chemotherapy because of the combined component of SCLC with LCNEC. All patients with stage II and IIIA LCNEC received adjuvant chemotherapy of platinum-based chemotherapy (Table 1).

One patient (case 24) diagnosed with metastatic LCNEC on mediastinal lymph node biopsy received lobectomy after induction therapy including chemotherapy (CDDP+ETP) and radiotherapy under the clinical diagnosis of stage IIIB LCNEC. Lobectomy specimens revealed a residual 5-mm LCNEC and no lymph node metastasis (ypIA1).

Outcome was likely to depend on stage. Fifteen patients survived and 12 patients died. Ten patients died because of metastatic LCNEC and the other 2 died from other diseases with no recurrence of LCNEC. There was no significant difference in patient outcome between LCNEC subtypes ($P > 0.05$).

All 5 patients with clinical stage IV LCNEC received chemotherapy. All died at a median of 8 months after the diagnosis (range, 5 to 9 mo). One recent case received anti-PD-L1 antibody (atezolizumab) after CDDP and ETP therapy followed by amrubicin and irinotecan. One case with *EGFR* mutation (L858R) received EGFR TKIs osimertinib and afatinib after CDDP+ETP, however, drug-induced interstitial pneumonia was suspected with disease progression, leading to TKI cessation.

DISCUSSION

We confirmed heterogeneity in protein expression profile among LCNEC patients by immunohistochemical analysis. Accordingly, LCNEC may be subdivided into 2 subgroups defined by TTF-1 and c-MYC protein expression. These findings are in agreement with recent model-based clustering of SCLC, which is subdivided into 2 major proteomic subsets defined by either TTF-1^{high}/c-MYC^{low} or c-MYC^{high}/TTF-1^{low}.[13] Two subgroups defined by TTF-1 and c-MYC protein expression may correspond to 2 mutually exclusive molecular subgroups: *STK11*/*KEAP1* and *TP53* mutated (NSCLC-like) or *RB1* and *TP53* mutated (SCLC-like), respectively. The detection of RB protein immunohistochemically may be helpful to subdivide LCNEC, however, RB protein loss, which may correspond to SCLC-like LCNEC, did not clearly subdivide LCNEC subtypes in our study, similar to the results of previous reports.[5,23] According to Derks et al,[5] RB protein loss was also observed in 47% of *RB1* wild-type cases. As RB protein loss may occur in the absence of genetic mutations, probably mediated by mechanisms such as promoter hypermethylation as described in SCLC, the proteomically defined subsets would be suitable to select treatment.[13] As immunohistochemistry is commonly used in routine clinical practice, TTF-1, c-MYC, and other protein markers could be used to guide rapid treatment selection for LCNEC patients.[13] The previous report suggested that TTF-1 may be a surrogate marker for DLL3 expression.[13] Because TTF-1 is used as a routine clinical diagnostic marker for lung cancer, TTF-1 could help identify patients with DLL3-positive LCNEC, which may show a response to DLL3-targeted therapy.[13] In this study, DLL3 was positive in 15 of 27 cases (56%) and concordant staining, either positive or negative for both TTF-1 and DLL3, was found

in 24 of 27 (89%) cases. However, 3 cases exhibited discordant staining with TTF-1 and DLL3 with high DLL3 expression and negative TTF-1 expression. The clear discordant result suggested that TTF-1 may not be a good surrogate marker for DLL3 expression. Unfortunately, enrollment in the phase III trial of Rova-T was stopped early due to shorter overall survival with associated treatment-emergent adverse events in the Rova-T arm compared with the topotecan arm.[19,25] However, DLL3 is still an ideal target, with high expression in pulmonary neuroendocrine carcinomas including SCLC and LCNEC and no expression in normal tissues.

Furthermore, MYC regulates the expression of aurora kinases A and B, which promote continuous cell growth in the loss of p53 function.[14] Using drug screening data, c-MYC overexpression was found to be the most sensitive marker of response to alisertib, a selective aurora kinase A inhibitor.[13] The phase II clinical trial comparing paclitaxel alone versus paclitaxel with alisertib for unselected SCLC patients has been completed with a response rate of 21%,[26] but did not meet its primary endpoint of improved progression-free survival.[27] However, if LCNEC patients with high c-MYC expression are properly selected for treatment of aurora kinase inhibitors, improved outcomes might be achieved. Biomarkers of response to alisertib need to be assessed.[26]

A recent report showed that LCNEC with wild-type *RB1* gene had a better prognosis when treated with NSCLC-type chemotherapy (platinum-gemcitabine or paclitaxel) than with SCLC-type therapy (platinum-ETP).[5] In this series, most cases who had chemotherapy received SCLC-type therapy (platinum-ETP), while 2 patients with stage IV LCNEC received NSCLC-type chemotherapy (CDDP+pemetrexed disodium). In case 24, chest computed tomography revealed a peripheral mass measuring 51 mm in diameter in the upper lobe of the left lung. Mediastinal lymph node biopsy revealed LCNEC and was graded as cT3N2M0, cStage IIIB. After chemotherapy and radiotherapy, left upper lobectomy with systemic mediastinal lymph node dissection was performed under video-assisted thoracoscopic surgery. Lobectomy specimens revealed a residual 5-mm LCNEC and no lymph node metastasis (ypT1aN0M0, pStage IA). This case showed TTF-1^{-}/c-MYC^{+} with reduced neuroendocrine expression, suggestive of the SCLC-like phenotype. SCLC-type chemotherapy (CDDP+ETP) was likely to be effective in this case. This should be verified in additional cases.

In summary, TTF-1 and c-MYC immunostaining showed clear mutual exclusivity and could be used to identify LCNEC subgroups, representing as NSCLC-like or SCLC-like. The precise distinction of LCNEC subtypes by immunohistochemical analysis in routine clinical practice may help to guide targeted treatment selection in the absence of molecular analysis.

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