Comprehensive Biomarkers for Personalized Treatment in Pulmonary Large Cell Neuroendocrine Carcinoma: A Comparative Analysis With Adenocarcinoma

Takashi Makino, MD, Tetuo Mikami, MD, PhD, Yoshinobu Hata, MD, PhD, Hajime Otsuka, MD, PhD, Satoshi Koezuka, MD, Kazutoshi Isobe, MD, PhD, Naobumi Tochigi, MD, PhD, Kazutoshi Shibuya, MD, PhD, Sakae Homma, MD, PhD, and Akira Iyoda, MD, PhD

Division of Chest Surgery, Department of Pathology, Division of Respiratory Medicine, and Department of Surgical Pathology, Toho University School of Medicine, Tokyo, Japan

Background. The prognosis for patients with large cell neuroendocrine carcinoma (LCNEC) of the lung is extremely poor, and optimal treatment strategies have not yet been established. To improve prognoses in patients with LCNEC, this study analyzed immunohistochemical expression and gene mutations of several known molecular targets in LCNECs and compared the expression levels of these targets with those in lung adenocarcinomas.

Methods. Twenty-six patients with primary LCNEC and 40 patients with adenocarcinoma were analyzed. Excision repair cross-complementation group 1 (ERCC1), class III β-tubulin, topoisomerase I, topoisomerase II, epidermal growth factor receptor (EGFR)–L858R, and somatostatin receptor expression were evaluated by immunohistochemistry, and *EGFR* mutations were evaluated using direct DNA sequencing and the Scorpion-amplified refractory mutation system.

Results. In patients with LCNEC and adenocarcinoma, positive rates of topoisomerase I, topoisomerase II, ERCC1, class III β-tubulin, EGFR-L858R, and somatostatin were 100.0% and 100.0%, 65.4% and 15.0% ($p < 0.0001$), 42.3% and 17.5% ($p = 0.0462$), 46.2% and 62.5%, 0.0% and 20.0% ($p = 0.0182$), and 50.0% and 5.0% ($p < 0.0001$), respectively. The frequencies of *EGFR* mutations were 0.0% and 37.5% in LCNEC and adenocarcinoma ($p = 0.0002$), respectively. Five-year overall survival rates were 64% in LCNEC and 91% in adenocarcinoma in stage I ($p = 0.0132$). Multivariate analysis showed that LCNEC histologic type was an independent prognostic factor in stage I.

Conclusions. LCNEC showed overexpression of topoisomerase II, somatostatin, and ERCC1. These findings suggested that it was possible to have good response to treatment with etoposide and octreotide and that LCNEC may be resistant to platinum-based therapy compared with adenocarcinoma. *EGFR* mutations were not observed in LCNEC. These results may indicate a favorable response to adjuvant treatments that are not typically prescribed for non-small cell lung cancer.

(Ann Thorac Surg 2016;102:1694–701)

Large cell neuroendocrine carcinoma (LCNEC) of the lung is an aggressive tumor exhibiting features of high-grade neuroendocrine tumors, a poor clinical prognosis [1], and a biologic behavior similar to that of small cell lung carcinoma (SCLC) [2, 3]. Several studies have shown that LCNEC responds to cisplatin-based chemotherapeutic regimens similar to those used for SCLC [4–6]. To investigate suitable personalized therapy for patients with LCNEC, with a view to developing a clinical trial in the future, the identification of biomarkers that may predict the prognosis and chemotherapeutic response of patients should be essential.

Taxanes are among the most active antitumor agents in the treatment of non-small cell lung carcinoma (NSCLC). Taxanes bind to β-tubulin, which is one of the major components of microtubules, and these agents exert their growth-inhibitory effects by blocking microtubule dynamics, thus resulting in growth arrest of tumor cells at the G_2 to M phase [7]. The isotype composition of β_1-tubulins has been shown to be related to taxane-based chemotherapy responsiveness [8]. One review summarized evidence showing that high levels of class III β-tubulin (TUBB3) expression are associated with taxane resistance in advanced cases of NSCLC [9]. Several studies have demonstrated that high TUBB3 expression

Accepted for publication April 28, 2016.

Address correspondence to Dr Iyoda, Division of Chest Surgery, Department of Surgery, Toho University School of Medicine, 6-11-1 Omori-nishi, Ota-ku, Tokyo, 1438541 Japan; email: aiyoda@med.toho-u.ac.jp.

0003-4975/$36.00
http://dx.doi.org/10.1016/j.athoracsur.2016.04.100

Abbreviations and Acronyms

AC = adenocarcinoma
EGFR = epidermal growth factor receptor
ERCC1 = Excision repair cross-complementation group 1
LCNEC = large cell neuroendocrine carcinoma
NSCLC = non-small cell lung carcinoma
OS = overall survival
SCLC = small cell lung carcinoma
SST = somatostatin
SSTR = somatostatin receptor
TKI = tyrosine kinase inhibitor
TUBB3 = class III β-tubulin
Topo1 = topoisomerase-I
Topo2 = topoisomerase-II

predicts a poorer outcome in patients with advanced NSCLC who are treated with taxane-based regimens [10, 11].

Cisplatin causes monoadducts and intrastrand or interstrand cross-links in DNA [12]. Nucleotide excision repair has been shown to be a factor in the repair of platinum-induced DNA damage. Excision repair cross-complementation group 1 (ERCC1) is involved in the nucleotide excision repair system and has been shown to be associated with resistance to platinum-based chemotherapy.

Irinotecan is a topoisomerase I (Topo1) inhibitor that is active in the treatment of chemotherapy-naive and chemotherapy-sensitive patients with recurrent SCLC.

Topo1 is a plausible predictive marker for irinotecan. Topoisomerase IIα (Topo2) is a nuclear enzyme often expressed in cells with high proliferative activity and has been shown to catalyze the conversion to different DNA topologic isomers. Topo2-inhibiting chemotherapeutic agents, including etoposide, are commonly used for the treatment of SCLC, but they are rarely used for the treatment of NSCLC. Low levels of Topo2 expression may be associated with resistance to Topo2 inhibitors [13].

Epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs) have been shown to be effective for NSCLC, particularly in patients with lung adenocarcinoma (AC), patients with specific *EGFR* mutations in exon 19 or exon 21, and Asian patients. The *EGFR* mutation status can be used as a good predictor of the clinical benefit of EGFR TKIs [14].

Somatostatin (SST) receptor (SSTR) expression has been analyzed to evaluate potential future diagnostic or therapeutic approaches similar to those shown for low-grade neuroendocrine cancers. The SST analogue octreotide is used to treat patients with pancreatic neuroendocrine tumors, particularly patients with high SSTR type 2A expression [15].

In this study, we analyzed gene expression profiles and mutations in samples from patients with LCNEC and discussed the possibility of personalized therapy in the management of patients with LCNEC.

Patients and Methods

Patients

We analyzed 26 patients diagnosed with LCNEC according to the World Health Organization classification at Toho University School of Medicine in Tokyo. Additionally, as a comparative cohort we analyzed 40 consecutive patients with ACs. This study was reviewed and approved by the Institutional Review Board of Toho University (26-41).

Immunohistochemical Staining

Formalin-fixed paraffin-embedded tissues were sectioned to 4-μm thickness. Immunoperoxidase staining was carried out with the antibodies described in Table 1 by using a Ventana BenchMark XT automated slide staining system (Ventana Medical Systems, Tucson, AZ). Sections were deparaffinized, pretreated with Cell Conditioning 1 (CC1; Ventana Medical Systems), reacted with primary antibodies for 32 minutes at room temperature, and visualized using an iVIEW DAB detection kit or OptiView DAB detection kit (Ventana Medical Systems). For SSTR, heat treatment rinsed with citrate buffer (pH 6.0) was performed for antigen retrieval before incubation with primary antibody. Counterstaining with Hematoxylin II (Ventana Medical Systems) and Bluing Reagent (Ventana Medical Systems) was performed (Table 1). For all antigens, negative controls were conducted by adding REAL Antibody Diluent (Dako, Glostrup, Denmark) instead of the primary antibody.

Table 1. Antibodies and Working Dilution

	Clone	Supplier	Working Dilution
Topoisomerase I	Rabbit monoclonal antibody clone EPR5375	Abcam, Cambridge, United Kingdom	1/100
Topoisomerase II	Mouse monoclonal antibody clone Ki-S1	Dako, Glostrup, Denmark	1/100
Class III β-Tubulin	Rabbit monoclonal antibody clone EP1569Y	Abcam, Cambridge, United Kingdom	1/100
ERCC1	Mouse monoclonal antibody clone 4F9	Dako, Glostrup, Denmark	1/50
EGFR-L858R	Rabbit monoclonal antibody clone 43B2	Cell Signaling Technology, Danvers, MA	1/100
SSTR	Polyclonal anti-SSTR type 2A antibody	Gramsch Laboratories, Schwabhausen, Germany	1/1,000

EGFR = epidermal growth factor receptor; ERCC1 = expression of excision repair cross-complementation group 1; SSTR = somatostatin receptor.

The numbers of Topo1- and Topo2-positive cells among 500 tumor cells within 10 microscopic fields at ×200 magnification were counted and scored as follows: 0 (negative, <25%), 1 (focal, 25% to 49%), 2 (moderate, 50% to 74%), or 3 (diffuse, ≥75%). Samples with scores of 0 and 1 were considered to have low expression, whereas samples with scores of 2 and 3 were considered to have high expression [16].

ERCC1 was assessed semiquantitatively by estimating the percentage of tumor cells with positive nuclear or cytoplasmic staining, or both, on whole tumor slides (0, no staining; 0.1, positive staining in 1% to 9% of the tumor cells; 0.5, positive staining in 10% to 49% of the tumor cells; 1, positive staining in ≥50% of the tumor cells). The staining intensity was also evaluated semiquantitatively (0, no staining; 1, weak staining; 2, moderate staining; 3, strong staining). The proportion and intensity scores were then multiplied to obtain a total score, which ranged from 0 to 3 (H-score) [17]. As previously described, tumors with an ERCC1 H-score higher than 1 were classified as high ERCC1.

TUBB3 was also assessed using the semiquantitative H-score. TUBB3 tumor staining (cytoplasm) intensity was graded on a scale of 0 to 2 by using adjacent nonmalignant cells as a reference. The percentage of positive tumor cells was evaluated, and an area proportion score was determined (0 if 0%, 0.5 if 1% to 9%, 1 if 10% to 24%, 2 if 25% to 49%, 3 if 50% to 74%, and 4 if ≥75%). This proportion score was then multiplied by the staining intensity to obtain a final semiquantitative H-score with a range of 0 to 8 [18]. As previously described, tumors with a TUBB3 H-score higher than the median H-score were classified as having high TUBB3 expression.

EGFR-L858R was assessed as previously described, with scoring based on the cytoplasmic or membrane staining intensity, or both, as follows: 0, no or weak staining intensity in less than 10% of tumor cells at high levels of magnification (40× objective lens); 1, weak staining in 10% or greater of tumor cells at high levels of magnification (40× objective lens); 2, moderate staining in 10% or greater of tumor cells at intermediate levels of magnification (10× or 20× objective lens); and 3, strong staining in 10% or greater of tumor cells at low levels of magnification (5× objective lens). Samples with scores of 0 were considered to have low expression, whereas samples with scores of 1 to 3 were considered to have high expression [19].

For SSTR type 2A, a semiquantitative scoring system was designed, taking into consideration both the subcellular localization and the extent of the staining, as follows: 0, absence of immunoreactivity; 1, pure cytoplasmic immunoreactivity, either focal or diffuse; 2, membranous reactivity, irrespective of the presence of cytoplasmic staining; 3, circumferential membranous reactivity, irrespective of the presence of cytoplasmic staining. Samples with scores of 0 were considered to have low expression, whereas samples with scores of 1 to 3 were considered to have high expression [20].

Two researchers (Drs Makino and Mikami), blinded to the patients' clinical data, independently assessed the scoring by microscopy, and interobserver reproducibility was analyzed.

EGFR Gene Mutation Analysis

Sections stained with hematoxylin and eosin were reviewed to identify regions of tissue composed of tumor cells. Genomic DNA was isolated from paraffin-embedded sections, and mutations were analyzed using the direct DNA sequencing method and the Scorpion-amplified refractory mutation system method.

Statistical Analysis

Patients' characteristics were compared using Student's *t* tests for continuous variables and Pearson's χ^2 tests for categorical variables. Immunohistochemical staining scores were compared using the Wilcoxon rank-sum test. Differences were considered to be statistically significant when the *p* value was less than 0.05. Overall survival (OS) was defined as the time from the date of operation to the date of the last follow-up for living patients or until death. Survival curves were prepared using the Kaplan-Meier method and were compared univariately using the log-rank test. To determine which factors were significantly associated with survival, a multivariate analysis using a Cox proportional hazards model was performed. All statistical analyses were performed using JMP version 11.0 (SAS Institute Inc, Cary, NC).

Table 2. Patients' Characteristics

Variable	Number		
	LCNEC (n = 26)	AC (n = 40)	*p* Value
Age (mean ± SD) (years)	69.6 ± 7.2	68.1 ± 9.0	0.73
Sex			
Male	20	28	…
Female	6	12	0.53
Smoking habits			
Nonsmoker	1	5	…
Current or former smoker	25	35	0.12
Tumor location			
Right	13	19	…
Left	13	21	0.84
Tumor size (mean ± SD) (cm)	4.2 ± 2.5	3.7 ± 2.0	0.55
Pathologic stage			
Stage I	12	25	…
Stage II	9	9	…
Stage III	5	6	0.41
Performance status			
0–1	26	40	…
2	0	0	1.00
Pulmonary function			
%VC (mean ± SD) (%)	99.8 ± 3.2	103.0 ± 2.6	0.44
$\%FEV_1$ (mean ± SD) (%)	93.6 ± 4.8	103.8 ± 3.9	0.11

AC = adenocarcinoma; FEV = forced expiratory volume; LCNEC = large cell neuroendocrine carcinoma; VC = vital capacity.

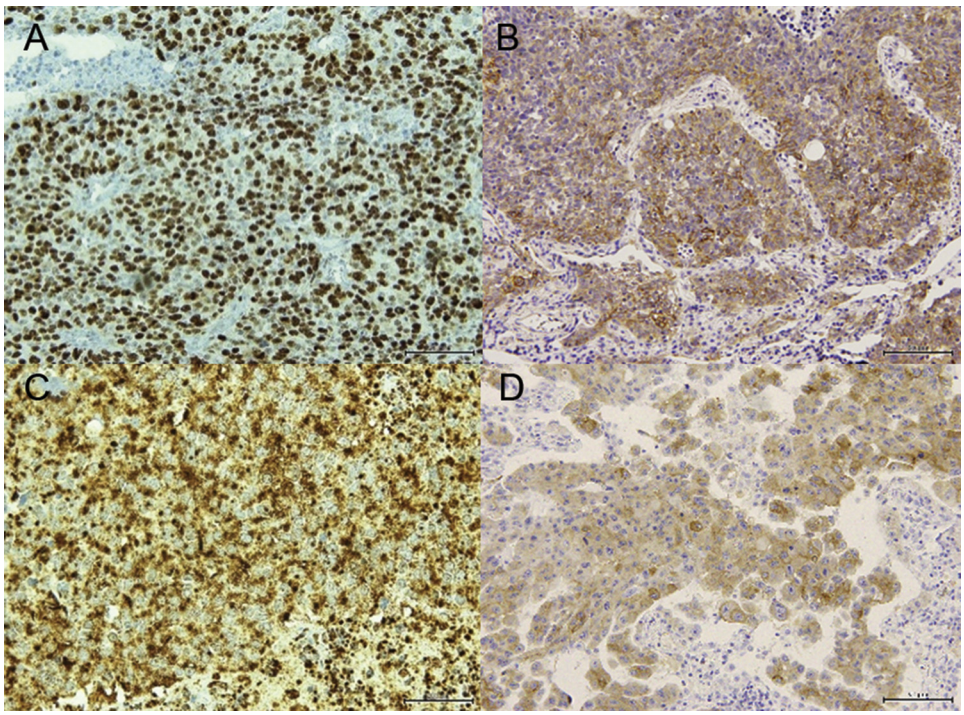

Fig 1. Immunohistochemical staining of topoisomerase II (Topo2), somatostatin receptor (SSTR), excision repair cross-complementation group 1 (ERCC1) and epidermal growth factor receptor (EGFR)–L858R. (A) Topo2 in large cell neuroendocrine carcinoma (LCNEC) (score 3); (B) SSTR in LCNEC (score 3); (C) ERCC1 in LCNEC (score 3); (D) EGFR-L858R in adenocarcinoma (score 3).

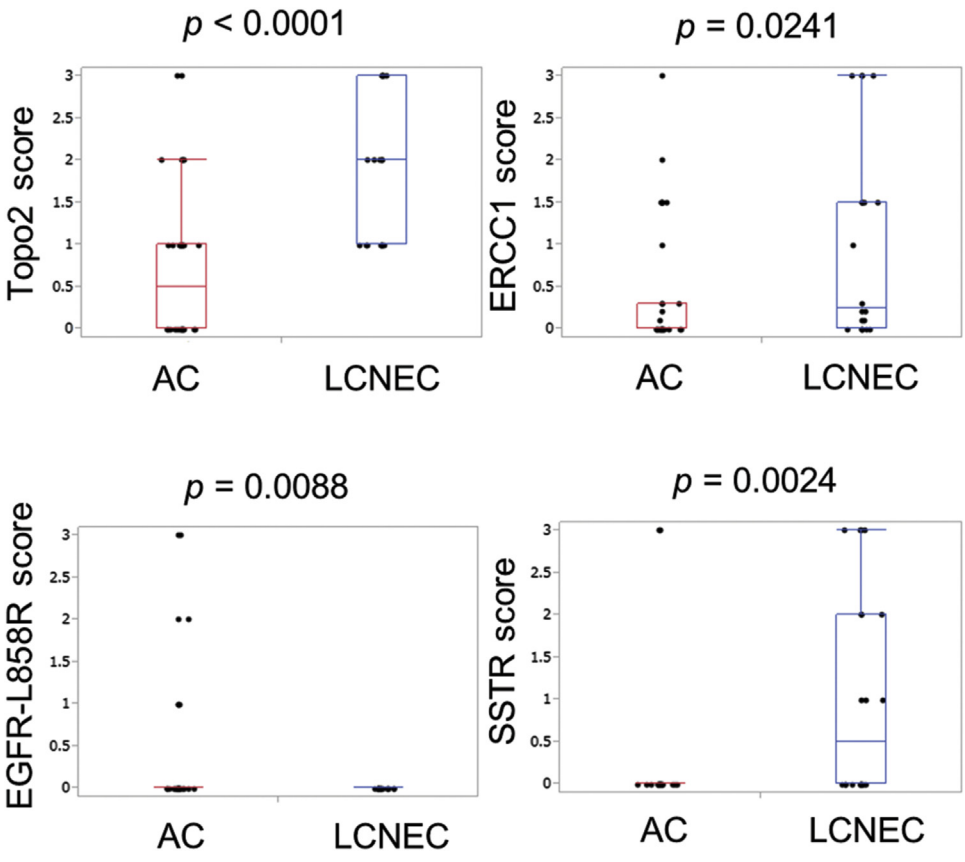

Fig 2. Significant association of topoisomerase II (Topo2), excision repair cross-complementation group 1 (ERCC1), epidermal growth factor receptor (EGFR)–L858R, and somatostatin receptor (SSTR) staining scores between adenocarcinoma (AC) and large cell neuroendocrine carcinoma (LCNEC). Data are presented as box-and-whisker plots; p values were determined using Wilcoxon rank-sum tests.

Results

Patients' Characteristics

There was no significant difference between patients with LCNEC and AC with regard to age, sex, smoking status, pathologic stage, performance status, and pulmonary function (Table 2). No patients received neoadjuvant treatment, and 17 patients received adjuvant treatment for stage II/III disease: carboplatin in combination with etoposide (n = 6), carboplatin in combination with gemcitabine (n = 4), tegafur, gimeracil, and oteracil in combination (n = 3), tegafur in combination with uracil (n = 3), and carboplatin in combination with docetaxel (n = 1). The main surgical procedures were lobectomies (n = 33), and sublobar resections were performed for patients with stage I disease (n = 4).

Immunohistochemical Analysis of Protein Expression in Tumor Specimens

Immunohistochemical staining for Topo1 (for irinotecan), Topo2 (for etoposide), ERCC1 (resistant to platinum), TUBB3 (resistant to taxanes), EGFR-L858R (for EGFR TKI), and SSTR (for octreotide) was evaluated in 66 primary lesions (Fig 1). Immunohistochemical staining scores for Topo2 ($p < 0.0001$), ERCC1 ($p = 0.0086$), and SSTR ($p < 0.0001$) were significantly higher in LCNEC than in AC, whereas that of EGFR-L858R ($p = 0.0165$) was significantly higher in AC than in LCNEC (Fig 2). For Topo1, Topo2, ERCC1, TUBB3, EGFR-L858R, and SSTR, markers with significant differences between LCNEC and AC in expression included Topo2 ($p < 0.0001$), ERCC1 ($p = 0.0462$), EGFR ($p = 0.0182$), and SSTR ($p < 0.0001$) (Table 3). The frequency of *EGFR* gene mutations was higher in AC ($p = 0.0002$), with no *EGFR* mutations identified in LCNEC (Table 3).

Prognostic Analysis

The median follow-up in the study group as a whole was 60.5 months (range, 2 to 137 months). The median follow-up in AC group was 70.0 months, and in LCNEC group it was 23.0 months. The 5-year OS rates were 47% and 68% in patients with LCNEC and AC, respectively ($p = 0.0195$). The 5-year OS rates were 64% and 91% in patients with stage I LCNEC and stage I AC, respectively ($p = 0.0132$) (Fig 3).

Univariate and multivariate analyses were performed in patients with all stages of disease. Univariate analysis showed that LCNEC histologic type and pathologic stage predicted poorer OS (Table 4), and multivariate analysis showed that pathologic stage was an independent prognostic factor (Table 5).

Because of the small numbers of patients with stage II or stage III disease, univariate and multivariate analyses were performed in the patients with stage I disease. Univariate analysis showed that LCNEC histologic type predicted poorer OS (Table 6), and multivariate analysis

Table 3. Comparison of Different Variables Between Patients With LCNEC and Patients With AC

Variable	Number			
	Total (n = 66)	LCNEC (n = 26)	AC (n = 40)	*p* Value
Topo1 (for irinotecan)				
High	66 (100.0%)	26 (100.0%)	40 (100.0%)	…
Low	0 (0.0%)	0 (0.0%)	0 (0.0%)	1.0000
Topo2 (for etoposide)				
High	23 (34.8%)	17 (65.4%)	6 (15.0%)	…
Low	43 (65.2%)	9 (34.6%)	34 (85.0%)	<0.0001
ERCC1 (resistant to platinum)				
High	18 (27.3%)	11 (42.3%)	7 (17.5%)	…
Low	48 (72.7%)	15 (57.7%)	33 (82.5%)	0.0462
TUBB3 (resistant to taxanes)				
High	37 (56.1%)	12 (46.2%)	25 (62.5%)	…
Low	29 (43.9%)	14 (53.8%)	15 (37.5%)	0.1911
EGFR-L858R (for EGFR TKI)				
High	8 (12.1%)	0 (0.0%)	8 (20.0%)	…
Low	58 (87.9%)	26 (100.0%)	32 (80.0%)	0.0182
EGFR mutation (for EGFR TKI)				
Positive	15 (22.7%)	0 (0.0%)	15 (37.5%)	…
Negative	51 (77.3%)	26 (100.0%)	25 (62.5%)	0.0002
SSTR (for octreotide)				
High	15 (22.7%)	13 (50.0%)	2 (5.0%)	…
Low	51 (77.3%)	13 (50.0%)	38 (95.0%)	<0.0001

AC = adenocarcinoma; EGFR = epidermal growth factor receptor; ERCC1 = excision repair cross-complementation group 1; LCNEC = large cell neuroendocrine carcinoma; SSTR = somatostatin receptor; TKI = tyrosine kinase inhibitor; Topo = topoisomerase; TUBB3 = class III β-tubulin.

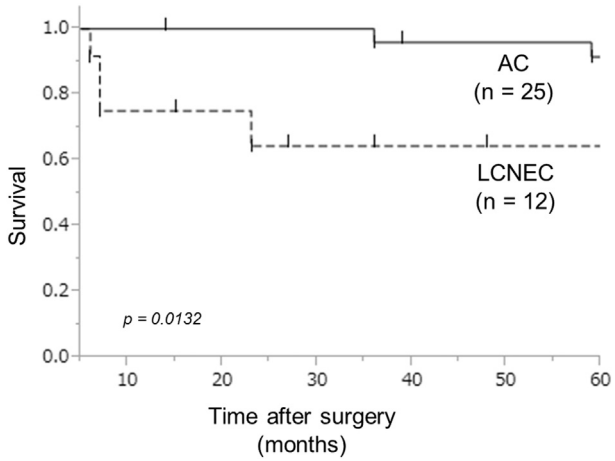

Fig 3. Five-year overall survival rates in patients with stage I large cell neuroendocrine carcinoma (LCNEC) and adenocarcinoma (AC).

showed that LCNEC histologic type was an independent prognostic factor (Table 7).

Comment

The frequency of *EGFR* mutations has been reported to be 30% in AC and 2% in non-AC, as well as 32% in Asian and 7% in non-Asian patients [21]. Previous studies reported that the frequency of *EGFR* mutations in LCNEC ranges from 7.7% to 41% [22–24]. De Pas and colleagues [25] reported a case of LCNEC in a patient with an exon 19 deletion in *EGFR*; this patient showed good response to treatment with an EGFR TKI. In the present study, we confirmed *EGFR* mutations in 37.5% of ACs. Because the rates of *EGFR* mutation in this population are different from those in other populations and because this finding may reflect a unique aspect of lung cancer in Japan, these data may apply only to patient populations in Japan. No mutations were observed in 26 LCNEC samples, thus suggesting that EGFR TKIs are not likely to be effective in the therapy of LCNEC.

SSTR is distributed throughout the nervous system and is expressed in many neuroendocrine carcinomas. SST analogues have been used to treat patients exhibiting hormone overexpression and related symptoms [26]. The currently available SST analogue octreotide

Table 4. Univariate Analyses of Survival

	Overall Survival		
	Hazard Ratio	95% CI	*p* Value
Histologic type[a]			
LCNEC	2.489	1.116–5.602	0.026
Age[b]	1.025	0.981–1.075	0.275
Tumor size[c]	1.183	0.991–1.380	0.062
Stage[d]			<0.001
Stage III	8.175	2.919–24.610	<0.001
Stage II	4.421	1.639–13.014	0.004
Operation[e] sublobar resection	1.991	0.316–6.928	0.399
Location[f] right	1.350	0.611–3.006	0.455
Pulmonary function			
%VC[g]	0.976	0.951–1.000	0.052
$\%FEV_1$[h]	0.988	0.973–1.005	0.163

[a] Histologic type (LCNEC vs adenocarcinoma). [b] Age continuous variable. [c] Tumor size continuous variable. [d] Stage (stage III vs stage I, stage II vs stage I). [e] Operation (sublobar resection vs lobectomy). [f] Location (right vs left). [g] %VC continuous variable. [h] $\%FEV_1$ continuous variable.

CI = confidence interval; FEV_1 = forced expiratory volume in 1 second; LCNEC = large cell neuroendocrine carcinoma; VC = vital capacity.

Table 5. Multivariate Analyses of Survival

	Overall Survival		
	Hazard Ratio	95% CI	*p* Value
Histologic type[a]			
LCNEC	1.992	0.831–4.829	0.122
Tumor size[b]	1.034	0.868–1.221	0.695
Stage[c]			<0.001
Stage III	8.056	2.745–25.196	<0.001
Stage II	3.710	1.226–12.330	0.020
Pulmonary function			
%VC[d]	0.968	0.936–1.001	0.054
$\%FEV_1$[e]	0.999	0.976–1.022	0.945

[a] Histologic type (LCNEC vs adenocarcinoma). [b] Tumor size continuous variable. [c] Stage (stage III vs stage I, stage II vs stage I). [d] %VC continuous variable. [e] $\%FEV_1$ continuous variable.

CI = confidence interval; FEV_1 = forced expiratory volume in 1 second; LCNEC = large cell neuroendocrine carcinoma; VC = vital capacity.

Table 6. Univariate Analyses of Survival in Patient With Pathologic Stage I

	Overall Survival		
	Hazard Ratio	95% CI	*p* Value
Histologic type[a]			
LCNEC	6.818	1.278–50.753	0.025
Age[b]	1.056	0.963–1.185	0.264
Tumor size[c]	0.557	0.180–1.310	0.196
Operation[d] sublobar resection	2.404	0.123–15.629	0.473
Location[e] right	2.332	0.456–16.833	0.312
Pulmonary function			
%VC[f]	0.957	0.909–1.002	0.061
$\%FEV_1$[g]	0.976	0.947–1.004	0.093

[a] Histologic type (LCNEC vs adenocarcinoma). [b] Age continuous variable. [c] Tumor size continuous variable. [d] Operation (sublobar resection vs lobectomy). [e] Location (right vs left). [f] %VC continuous variable. [g] $\%FEV_1$ continuous variable.

CI = confidence interval; FEV_1 = forced expiratory volume in 1 second; LCNEC = large cell neuroendocrine carcinoma; VC = vital capacity.

Table 7. Multivariate Analyses of Survival in Patient With Pathologic Stage I

	Overall Survival		
	Hazard Ratio	95% CI	*p* Value
Histologic type[a]			
LCNEC	9.527	1.498–98.292	0.017
Age[b]	1.201	0.960–1.734	0.127
Pulmonary function			
%VC[c]	0.985	0.914–1.058	0.671
$\%FEV_1$[d]	0.964	0.901–1.014	0.162

[a] Histologic type (LCNEC vs adenocarcinoma). [b] Age continuous variable. [c] %VC continuous variable. [d] $\%FEV_1$ continuous variable.

CI = confidence interval; FEV_1 = forced expiratory volume in 1 second; LCNEC = large cell neuroendocrine carcinoma; VC = vital capacity.

binds preferentially to SSTR-2. Moreover, octreotide, which is used for the treatment of carcinoid syndrome, is effective for patients with LCNEC [27]. Previous studies reported that the frequency of SSTR-2 expression in LCNEC ranges from 21% to 65% [26, 28, 29], with raised expression observed with increasing differentiation status from poorly differentiated (LCNEC and SCLC) to well differentiated (atypical carcinoid and typical carcinoid) [28]. We found positive staining for SSTR-2 in 13 (50.0%) of our patients with LCNEC, and expression of SSTR-2 was significantly higher in LCNEC than in AC. These results indicated that SST analogues may have therapeutic effects and that SSTR expression may be a predictive marker for personalized treatment in LCNEC.

ERCC1 was previously shown to be a promising biomarker in patients with NSCLC treated with platinum-based adjuvant chemotherapy; this therapy significantly prolongs survival in patients with ERCC1-negative tumors but not patients with ERCC1-positive tumors [17]. More recent studies have shown that ERCC1 expression is predictive in AC but not other types of lung cancer [30]. In this study, we found 11 (42.3%) ERCC1-positive tumors in patients with LCNEC, and the expression of ERCC1 was significantly higher in LCNEC than in AC.

High Topo2 expression may confer good responses to topoisomerase inhibitors, such as etoposide [16, 31]. Topo2 positivity was observed in 17 (65.4%) patients with LCNEC in our study, with significantly higher Topo2 expression in LCNEC than in AC. One prospective study of adjuvant chemotherapy after complete surgical resection for LCNEC revealed favorable outcomes for cisplatin in combination with etoposide compared with the historical control group [6]. Therefore our current results suggested that the etoposide regimen should be effective for patients with LCNEC.

Our results provide evidence of Topo1 expression in lung cancer and show positive reactions for both LCNEC and AC. Topo1 inhibitors are important components of standard treatments for NSCLC, SCLC, and colorectal cancer, by generating Topo1-linked DNA single-strand breaks, double-strand breaks, and cell death. The Topo1 inhibitor irinotecan has been reported to cause a response rate of 20.5% on AC [32]. There were no significant differences in Topo1 expression in this study.

Although the difference was not significant, LCNEC samples tended to have lower TUBB3 expression than AC samples, a finding suggesting the presence of taxane sensitivity. The response rate of taxane-based chemotherapy in AC has been reported to be 25% [33], and the response rate of taxane-based chemotherapy in LCNEC is expected to be higher than in AC.

Our study has several limitations. First, there were some intrinsic drawbacks to the study design because our data were collected and reviewed retrospectively. Second, the study population was small. However, we believe that these biomarkers may improve prognoses in patients with LCNEC by facilitating personalized treatment options.

In conclusion, LCNEC showed significant strong overexpression of Topo2, SSTR, and ERCC1 compared with AC. The patients with LCNEC would have been expected to respond to treatment with etoposide and octreotide and may have been resistant to platinum-based therapy compared with AC. Immunohistochemical expression for EGFR-L858R and *EGFR* mutations were not observed in the patients with LCNEC, thus suggesting the resistance to EGFR TKI. These results may indicate a favorable response to adjuvant treatments that are not typically prescribed for NSCLC. The findings of the current study should be validated with an independent study before considering developing a clinical trial.

The authors wish to thank Ms Tsukiko Sato for providing technical assistance. This study was supported in part by Grants-in-aid for Scientific Research (C) 15K10272 from the Japanese Ministry of Education, Culture, Sports, Science and Technology and the Research Promotion Grant from Toho University Graduate School of Medicine (No. 15-02 to A.I.).

References

1. Travis WD, Rush W, Flieder DB, et al. Survival analysis of 200 pulmonary neuroendocrine tumors with clarification of criteria for atypical carcinoid and its separation from typical carcinoid. Am J Surg Pathol 1998;22:934–44.
2. Iyoda A, Hiroshima K, Toyozaki T, Haga Y, Fujisawa T, Ohwada H. Clinical characterization of pulmonary large cell neuroendocrine carcinoma and large cell carcinoma with neuroendocrine morphology. Cancer 2001;91:1992–2000.
3. Iyoda A, Hiroshima K, Nakatani Y, et al. Pulmonary large cell neuroendocrine carcinoma: its place in the spectrum of pulmonary carcinoma. Ann Thorac Surg 2007;84:702–7.
4. Yamazaki S, Sekine I, Matsuno Y, et al. Clinical responses of large cell neuroendocrine carcinoma of the lung to cisplatin-based chemotherapy. Lung Cancer 2005;49:217–23.
5. Niho S, Kenmotsu H, Sekine I, et al. Combination chemotherapy with irinotecan and cisplatin for large-cell neuroendocrine carcinoma of the lung: a multicenter phase II study. J Thorac Oncol 2013;8:980–4.
6. Iyoda A, Hiroshima K, Moriya Y, et al. Prospective study of adjuvant chemotherapy for pulmonary large cell neuroendocrine carcinoma. Ann Thorac Surg 2006;82:1802–7.

7. Burkhart CA, Kavallaris M, Band Horwitz S. The role of beta-tubulin isotypes in resistance to antimitotic drugs. Biochim Biophys Acta 2001;1471:1–9.
8. Jordan MA, Wilson L. Microtubules as a target for anticancer drugs. Nat Rev Cancer 2004;4:253–65.
9. Seve P, Reiman T, Dumontet C. The role of betaIII tubulin in predicting chemoresistance in non-small cell lung cancer. Lung Cancer 2010;67:136–43.
10. Sève P, Mackey J, Isaac S, et al. Class III β-tubulin expression in tumor cells predicts response and outcome in patients with non–small cell lung cancer receiving paclitaxel. Mol Cancer 2005;4:2001–7.
11. Azuma K, Sasada T, Kawahara A, et al. Expression of ERCC1 and class III beta-tubulin in non-small cell lung cancer patients treated with carboplatin and paclitaxel. Lung Cancer 2009;64:326–33.
12. Chemotherapy in non-small cell lung cancer: a meta-analysis using updated data on individual patients from 52 randomised clinical trials. Non-small Cell Lung Cancer Collaborative Group. BMJ 1995;311:899–909.
13. Giaccone G, Gazdar AF, Beck H, Zunino F, Capranico G. Multidrug sensitivity phenotype of human lung cancer cells associated with topoisomerase II expression. Cancer Res 1992;52:1666–74.
14. Mitsudomi T, Kosaka T, Endoh H, et al. Mutations of the epidermal growth factor receptor gene predict prolonged survival after gefitinib treatment in patients with non-small-cell lung cancer with postoperative recurrence. J Clin Oncol 2005;23:2513–20.
15. Okuwaki K, Kida M, Mikami T, et al. Clinicopathologic characteristics of pancreatic neuroendocrine tumors and relation of somatostatin receptor type 2A to outcomes. Cancer 2013;119:4094–102.
16. Yan S, Shun-Chang J, Li C, Jie L, Ya-Li L, Ling-Xiong W. Topoisomerase II alpha expression and the benefit of adjuvant chemotherapy for postoperative patients with non-small cell lung cancer. BMC Cancer 2010;10:621.
17. Olaussen KA, Dunant A, Fouret P, et al. DNA repair by ERCC1 in non-small-cell lung cancer and cisplatin-based adjuvant chemotherapy. N Engl J Med 2006;355:983–91.
18. Zheng Z, Chen T, Li X, Haura E, Sharma A, Bepler G. DNA synthesis and repair genes RRM1 and ERCC1 in lung cancer. N Engl J Med 2007;356:800–8.
19. Brevet M, Arcila M, Ladanyi M. Assessment of EGFR mutation status in lung adenocarcinoma by immunohistochemistry using antibodies specific to the two major forms of mutant EGFR. J Mol Diagn 2010;12:169–76.
20. Volante M, Brizzi MP, Faggiano A, et al. Somatostatin receptor type 2A immunohistochemistry in neuroendocrine tumors: a proposal of scoring system correlated with somatostatin receptor scintigraphy. Mod Pathol 2007;20:1172–82.
21. Mitsudomi T, Kosaka T, Yatabe Y. Biological and clinical implications of EGFR mutations in lung cancer. Int J Clin Oncol 2006;11:190–8.
22. Matsumura Y, Umemura S, Ishii G, et al. Expression profiling of receptor tyrosine kinases in high-grade neuroendocrine carcinoma of the lung: a comparative analysis with adenocarcinoma and squamous cell carcinoma. J Cancer Res Clin Oncol 2015;141:2159–70.
23. Le X, Desai NV, Majid A, et al. De novo pulmonary small cell carcinomas and large cell neuroendocrine carcinomas harboring EGFR mutations: lack of response to EGFR inhibitors. Lung Cancer 2015;88:70–3.
24. Iyoda A, Travis WD, Sarkaria IS, et al. Expression profiling and identification of potential molecular targets for therapy in pulmonary large-cell neuroendocrine carcinoma. Exp Ther Med 2011;2:1041–5.
25. De Pas TM, Giovannini M, Manzotti M, et al. Large-cell neuroendocrine carcinoma of the lung harboring EGFR mutation and responding to gefitinib. J Clin Oncol 2011;29:819–22.
26. Righi L, Volante M, Tavaglione V, et al. Somatostatin receptor tissue distribution in lung neuroendocrine tumours: a clinicopathologic and immunohistochemical study of 218 'clinically aggressive' cases. Ann Oncol 2009;21:548–55.
27. Filosso PL, Ruffini E, Oliaro A, et al. Large-cell neuroendocrine carcinoma of the lung: a clinicopathologic study of eighteen cases and the efficacy of adjuvant treatment with octreotide. J Thorac Cardiovasc Surg 2005;129:819–24.
28. Tsuta K, Wistuba II, Moran CA. Differential expression of somatostatin receptors 1-5 in neuroendocrine carcinoma of the lung. Pathol Res Pract 2012;208:470–4.
29. Eichhorn F, Dienemann H, Muley T, Warth A, Hoffmann H. Predictors of survival after operation among patients with large cell neuroendocrine carcinoma of the lung. Ann Thorac Surg 2015;99:983–9.
30. Vilmar AC, Santoni-Rugiu E, Sørensen JB. ERCC1 and histopathology in advanced NSCLC patients randomized in a large multicenter phase III trial. Ann Oncol 2010;21:1817–24.
31. Pritchard KI, Messersmith H, Elavathil L, Trudeau M, O'Malley F, Dhesy-Thind B. HER-2 and topoisomerase II as predictors of response to chemotherapy. J Clin Oncol 2008;26:736–44.
32. Negoro S, Masuda N, Takada Y, et al. Randomised phase III trial of irinotecan combined with cisplatin for advanced non-small-cell lung cancer. Br J Cancer 2003;88:335–41.
33. Kelly K, Crowley J, Bunn PA Jr, et al. Randomized phase III trial of paclitaxel plus carboplatin versus vinorelbine plus cisplatin in the treatment of patients with advanced non–small-cell lung cancer: a Southwest Oncology Group trial. J Clin Oncol 2001;19:3210–8.