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

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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.**

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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

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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.