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Reliability of Small Biopsy Samples Compared With Resected Specimens for the Determination of Programmed Death-Ligand 1 Expression in Non—Small-Cell Lung Cancer

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Abstract

Expression of programmed death-ligand 1 (PD-L1) in non—small-cell lung cancer (NSCLC) has mainly been examined using surgically resected specimens. We retrospectively evaluated the expression of PD-L1 using immunohistochemistry in 79 paired small biopsy and resected specimens of NSCLC. The concordance between the samples was good, with a concordance rate of 92.4% and κ value of 0.8366.

Background: Several studies have assessed the expression of programmed death-ligand 1 (PD-L1) in resected surgical specimens of non—small-cell lung cancer (NSCLC). However, the expression of PD-L1 in smaller biopsy samples of advanced NSCLC has not been reported. **Patients and Methods:** A total of 79 patients with NSCLC at our institution with available biopsy samples and resected specimens were retrospectively enrolled in the present study. PD-L1 expression was assessed by immunohistochemistry and scored using the hybrid scoring method. The concordance rates for the expression of PD-L1 between the 2 samples were analyzed. **Results:** The pathologic stage of the patients (51 men, 28 women; median age, 68 years) was stage I in 37, stage II in 18, and stage III in 24. The diagnostic procedures included transbronchial biopsy in 59, transbronchial needle aspiration biopsy in 14, and computed tomography (CT)-guided needle biopsy in 6. The positivity rate of PD-L1 in these samples was 38.0% (27 transbronchial biopsies, 6 transbronchial needle aspiration biopsies, 3 CT-guided needle biopsies) versus 35.4% in the resected specimens. The median hybrid score was 0 (range, 0-170), and the mean score was 28.7 ± 43.4. Comparing the biopsy samples and resected specimens with a score of ≥ 1 as positive for PD-L1 staining, 6 tumors were discordant for PD-L1 expression and 73 were concordant, for a concordance rate of 92.4% and κ value of 0.8366. **Conclusion:** PD-L1 status showed good concordance between the biopsy samples and resected specimens. These small samples, even those derived from transbronchial needle aspiration biopsies, appear adequate for the assessment of PD-L1 expression.

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Introduction

The development of personalized medicine has refined the treatment of non–small-lung cancer (NSCLC). Several clinical trials have demonstrated that epidermal growth factor receptor (EGFR) gene mutation and anaplastic lymphoma kinase (ALK) gene rearrangement are strong predictive markers of a better outcome with EGFR tyrosine kinase inhibitors[1-4] and ALK inhibitors.[5] The assessment of validated biomarkers such as these is essential to determining the therapeutic strategies for NSCLC.[6,7] Optimal care requires sufficient samples to provide a precise pathologic diagnosis, including the assessment of biomarkers. Although surgical specimens are generally considered of a suitable size for biomarker analysis, most patients with NSCLC present with unresectable or advanced disease and are often investigated with noninvasive procedures, including bronchoscopic biopsy, endobronchial ultrasound-guided transbronchial needle biopsy, and computed tomography (CT)-guided needle biopsy. These procedures generally provide only small biopsy and cytology specimens.

Programmed death 1 (PD-1) has 2 known ligands, PD-L1 (B7-H1)[8,9] and PD-L2 (B7-DC).[10,11] PD-1 is a key immune checkpoint receptor expressed by activated T cells and mediates immunosuppression.[12,13] PD-L1 is selectively expressed by many tumor cells and by cells within the tumor microenvironment in response to inflammatory stimuli. Blockade of the interaction between PD-1 and PD-L1 enhances the T-cell response in vitro and mediates preclinical antitumor activity.[14,15] Several studies have investigated its expression using immunohistochemistry (IHC) in surgically resected specimens of NSCLC and assessed the relationship between PD-L1 IHC expression and the clinicopathologic variables or survival.[16,17] In recent early-phase clinical trials, anti–PD-1 antibody and anti–PD-L1 antibody demonstrated durable objective responses.[18,19] Preliminary data have suggested a relationship between the PD-L1 expression of tumor cells and the objective response.[18,20]

The aim of the present study was to retrospectively investigate the reliability of small biopsy samples of NSCLC in determining the PD-L1 expression status. We subsequently analyzed the relationship between PD-L1 expression and the clinicopathologic variables or postoperative survival.

Patients and Methods

Study Population

The subjects consisted of consecutive patients with NSCLC who had undergone surgical resection at the National Cancer Center Hospital from April 2009 to March 2012. We retrospectively enrolled patients for whom both surgically resected specimens and small biopsy samples for diagnosis before surgery were available in our institute. The histologic classification of tumors was determined using the criteria of the World Health Organization and staging was done using TNM staging (7th edition).[21] The patient characteristics, including age, sex, pathologic stage, and location and size of the primary tumor and histologic diagnosis, were obtained from the medical records. All the patients had provided written informed consent for the analysis of their medical records. The institutional review board of our institution approved the present study.

Pathologic Evaluation and Immunohistochemistry Staining

At least 1 hematoxylin-eosin–stained section from both the resected tumor and the biopsy was reviewed for each case to confirm the diagnosis of NSCLC, and IHC for PD-L1 expression was performed on both samples. The formalin-fixed and paraffin-embedded sections (4-μm thick) were deparaffinized. The sections were exposed to 3% hydrogen peroxide for 15 minutes to block endogenous peroxidase activity and then washed in deionized water for a few minutes. The preparations were autoclaved in citrate buffer for antigen retrieval. The primary antibody used was a polyclonal clone (catalog no. 4059, dilution 1:1600; ProSci, Inc, Poway, CA). IHC evaluations and scoring were performed by 2 investigators (S.K. and K.K.) who were unaware of the status of the clinical data using a BX 40 microscope (Olympus, Tokyo, Japan). The PD-L1–positive (PD-L1$^+$) tumor cells were counted under high magnification (400×) in 5 random and nonoverlapping fields (100 tumor cells per field; 500 tumor cells per specimen). The staining intensity was graded as follows: 0, no discernible membranous staining; 1+, weak and/or incomplete membranous staining; 2+, weak to moderate, continuous membranous staining; and 3+, strong membranous staining that was readily apparent at low (40×) magnification. Representative images of each score are shown in Figure 1.

IHC scoring was performed using a hybrid score assessment that combines the intensity and percentage of the cells. The values were summed using the following formula: hybrid score = (percentage of tumor 1+) + 2(percentage of tumor 2+) + 3(percentage of tumor 3+). The hybrid score could range from 0 to 300. The sample was considered negative if the hybrid score was 0 and positive if the hybrid score was > 0. A minimum of 100 cells were evaluated in calculating the score. A score of 0 was regarded as PD-L1–negative (PD-L1$^-$) and > 0 as PD-L1$^+$.

Statistical Analysis

Correlations between the PD-L1 expression by tumor cells and the clinicopathologic characteristics were analyzed statistically using Student's paired t test or the χ^2 test. The 95% confidence intervals were computed using the normal approximation to the binomial distribution. The concordance levels of the results between the surgically resected specimens and biopsy samples in the individual cases were also evaluated using κ statistics. The level of concordance was classified as poor (κ < 0.00), slight (κ = 0.00-0.20), fair (κ = 0.21-0.40), moderate (κ = 0.41-0.60), substantial (κ = 0.61-0.80), and almost perfect (κ = 0.81-1.00).[22] The relapse-free and overall survival was measured from the day of surgery until relapse and death or the final day of the follow-up period. The median survival was calculated using the Kaplan-Meier method, and the 2 curves were compared using the log-rank test. All analyses were done using STATA, version 12.0 (StataCorp, College Station, TX).

Results

Patient Characteristics

During the study period, 908 patients underwent a biopsy in our institute, and 378 were diagnosed with NSCLC. We enrolled 79 patients (51 men and 28 women; median age, 68 years; range,