Lung Cancer 116 (2018) 1–6

Contents lists available at ScienceDirect

Lung Cancer

journal homepage: www.elsevier.com/locate/lungcan

PD-L1 expression according to the *EGFR* status in primary lung adenocarcinoma

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ARTICLE INFO

Keywords:
PD-L1
EGFR
Lung adenocarcinoma

ABSTRACT

Objectives: It was reported that programmed cell death-ligand 1 (PD-L1) expression is associated with smoking and wild-type *epidermal growth factor receptor* (*EGFR*) in lung adenocarcinoma. However, the association between PD-L1 expression and *EGFR* mutation site in *EGFR* mutation-positive lung adenocarcinoma is unclear.

Materials and methods: We retrospectively examined the relationship between PD-L1 expression and *EGFR* status in 441 surgically resected primary lung adenocarcinomas. Membrane PD-L1 expression on tumor cells was evaluated by immunohistochemical analysis using a PD-L1 antibody (clone SP142) and defined by tumor proportion scores (TPSs) of 0%, 1–4%, 5–49%, and ≥50%, respectively.

Results: Two hundred and eighteen (49.4%) patients had wild-type *EGFR*, and 223 (50.6%) had mutant *EGFR*—98 (44.0%) with exon 19 deletion, 116 (52.0%) with exon 21 L858R point mutation, and nine (4.0%) with another *EGFR* mutation. Overall, Fisher's exact test showed that PD-L1 positivity was associated with wild-type *EGFR*, and there was only one case with PD-L1 TPS ≥50% among the cases with mutant *EGFR*. The analysis of cases with mutant *EGFR* indicated no significant association between *EGFR* mutation site and PD-L1 expression. However, the prevalence of PD-L1 TPS 5–49% was higher among patients with *EGFR* exon 19 deletion than with *EGFR* exon 21 L858R point mutation.

Conclusions: PD-L1 expression was significantly associated with wild-type *EGFR*, and PD-L1 TPS ≥50% seldom overlaps with presence of driver oncogene *EGFR*. There was no significant difference in PD-L1 expression among the *EGFR* mutation sites

1. Introduction

Immune checkpoint inhibitors have been developed to target programmed cell death-1 (PD-1) and programmed cell death-ligand 1 (PD-L1). PD-1 inhibitors such as nivolumab in the CheckMate study and pembrolizumab in the KEYNOTE study, and PD-L1 inhibitors including atezolizumab in the POPLAR and OAK studies, have provided survival benefit in non-small cell lung cancer (NSCLC) compared with conventional standard therapy [1–6]. Given these results, PD-1 inhibitors have become the standard treatment for advanced-stage NSCLC patients. Currently, there is no ideal biomarker for the response to immune checkpoint inhibitors; however, the immunohistochemical analysis of PD-L1 expression on tumor cells, which is associated with the response to immune checkpoint inhibitors, is used as a predictive biomarker for the outcome of immunotherapy. For example, patients with smoking history and wild-type *epidermal growth factor receptor* (*EGFR*) associated with high PD-L1 expression in lung adenocarcinoma, had a higher sensitivity to immune checkpoint inhibitors in some clinical trials [2,3,6–8]. However, some patients with *EGFR* mutation-positive lung adenocarcinoma also responded to immunotherapy. However, why there is a difference in benefit by immunotherapy among cases with mutant *EGFR* is not clear.

Recently, a difference in the therapeutic effect of an EGFR-tyrosine kinase inhibitor (TKI) according to the mutation site in cases with mutant *EGFR* was reported [9]. Therefore, it is necessary to consider the presence of mutant *EGFR* and the mutation site when managing the therapeutic strategy for lung adenocarcinoma. If there is a difference in benefit by immunotherapy among cases with mutant *EGFR* because of

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https://doi.org/10.1016/j.lungcan.2017.12.003
Received 16 June 2017; Received in revised form 22 October 2017; Accepted 4 December 2017

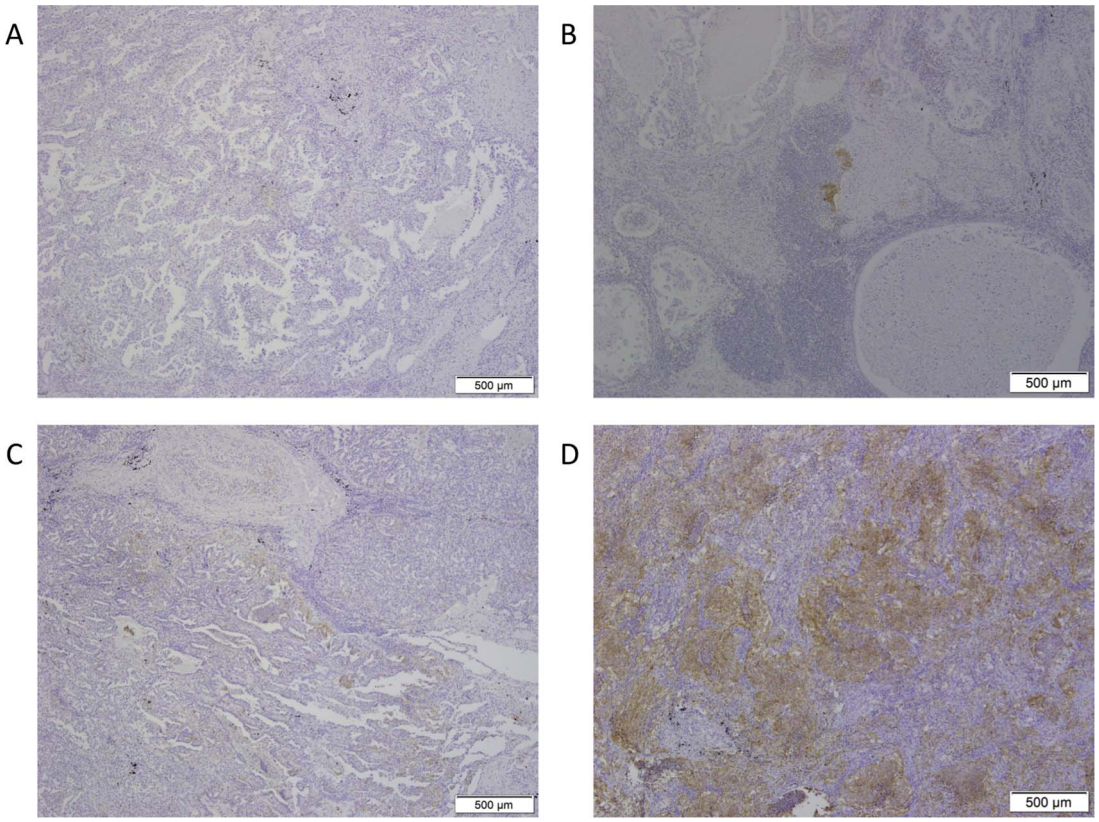

Fig. 1. Representative images of immunohistochemical staining for PD-L1 in surgically resected tumors from patients with primary lung adenocarcinoma. (A) PD-L1 TPS 0%. (B) PD-L1 TPS 1–4%. (C) PD-L1 TPS 5–49%. (D) PD-L1 TPS ≥50%. PD-L1: programmed cell death-ligand 1, TPS: tumor proportion score. Scale bar: 500 μm.

differential PD-L1 expression according to the mutation site, then information regarding the mutation site as well as the presence of mutant *EGFR* should be considered when using immune checkpoint inhibitors. However, the relationship between PD-L1 expression and *EGFR* mutation site in lung adenocarcinoma harboring *EGFR* mutation is unclear.

In this translational study, we examined the expression of PD-L1 protein in 441 surgically resected primary lung adenocarcinoma patients, and investigated the association between PD-L1 expression and *EGFR* status. Furthermore, we discuss the therapeutic strategy for the management of advanced lung adenocarcinoma harboring *EGFR* mutation.

2. Materials and methods

2.1. Patients and samples

We retrospectively examined patients who underwent surgical resection of their primary lung adenocarcinoma between January 2005 and December 2015 at the Department of Surgery and Science, Graduate School of Medical Sciences, Kyushu University. Of these, only those cases with available information regarding the *EGFR* mutation site and presence of mutant *EGFR* were selected. Finally, 441 paraffin-embedded specimens were available and retrieved from the registry of the Department of Anatomic Pathology, Graduate School of Medical Sciences, Kyushu University. *EGFR* status was determined in tumor tissues using the peptide nucleic acid-locked nucleic acid (PNA-LNA) polymerase chain reaction clamp method (Mitsubishi Chemical Medience, Tokyo, Japan). All clinical information was obtained from medical records. This study was approved by our institutional review board (Kyushu University, IRB No. 28-100).

2.2. Immunohistochemical analysis

Immunohistochemistry was performed in 441 cases of surgically resected primary lung adenocarcinoma using formalin-fixed tissue sections according to our PD-L1 immunohistochemistry protocol as described previously [7,8,10–13].

The primary antibody was an anti-human PD-L1 rabbit monoclonal antibody (clone SP142, dilution 1:100; Spring Bioscience, Ventana, Tucson, AZ). Carcinoma cells showing membranous staining for PD-L1 were evaluated as positive cells. The proportion of PD-L1-positive cells was independently estimated as the percentage of total carcinoma cells in whole sections by three investigators (K.T., K.K., and S.T.). If the independent assessments did not agree, the slides were reviewed by all three investigators together to achieve consensus. The consensus judgments were adopted as the final results. In this study, membrane PD-L1 expression on tumor cells was defined by tumor proportion scores (TPSs) of 0%, 1–4%, 5–49%, and ≥50%, respectively, consistent with the cut-offs used in atezolizumab clinical trials [1,4]. Fig. 1 shows the representative images of (A) PD-L1 TPS 0%, (B) PD-L1 TPS 1–4%, (C) PD-L1 TPS 5–49%, and (D) PD-L1 TPS ≥50%, respectively.

2.3. Statistical analysis

Associations between *EGFR* status, its mutation site, and smoking status and PD-L1 expression were analyzed using Fisher's exact test. We examined the association between *EGFR* status and smoking status using the Student's *t*-test. All statistical analyses were performed by JMP Statistical Discovery Software (v11.0; SAS Institute, Cary, NC, USA). *P* values < 0.05 were statistically significant.