

Programmed Death-Ligand 1 Expression in Lymphovascular Tumor Emboli in Lung Cancer



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Received 4 May 2022; revised 25 May 2022; accepted 27 May 2022

Available online - 8 June 2022

ABSTRACT

Introduction: Programmed death-ligand 1 (PD-L1) expression determined by immunohistochemistry is the most widely used biomarker for predicting response to immune checkpoint inhibitors. The characteristics of PD-L1 expression in tumor cells inside lymphovascular spaces are largely unknown. Although PD-L1 expression in circulating tumor cells within vascular spaces had been studied, results were conflicting due to lack of standardized PD-L1 expression assessment.

Methods: We investigated PD-L1 expression in lung cancer primary tumor tissue, lymphovascular tumor emboli, and lymph node metastasis using the standard PD-L1 immunohistochemistry 22C3 pharmDx assay. PD-L1 expression was scored in the primary tumor, lymphovascular emboli, and lymph node metastasis by a pathologist using the tumor proportion score (TPS).

Results: We collected and analyzed surgical specimens from 36 patients with lung cancer with lymph node metastasis. In the primary tumor, 64% of cases were PD-L1 negative (TPS < 1%), 25% were PD-L1 low (TPS 1%–49%), and 11% were PD-L1 high (TPS ≥ 50%). In contrast, in lymphovascular tumor emboli, 89% of cases were PD-L1 negative, 11% were PD-L1 low, and none were PD-L1 high. In lymph node metastases, 72% of cases were PD-L1 negative, 17% were PD-L1 low, and 11% were PD-L1 high.

Conclusions: We observed a significant decrease of PD-L1 expression in lymphovascular tumor emboli compared with that in primary tumors ($p = 0.002$). Whether such differences are related to intrinsic tumor cell heterogeneity or extrinsic factors such as the microenvironment warrants further investigation.

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Keywords: Lung cancer; PD-L1; Immunohistochemistry; Lymphovascular tumor emboli

Introduction

Programmed death-ligand 1 (PD-L1) expression is the most widely used biomarker for gauging NSCLC response to immune checkpoint inhibitor therapy. Extensive studies have been conducted to investigate PD-L1 expression in primary tumors and metastatic sites. In the metastatic cascade, a critical step before establishing metastasis in the lymph nodes or distant organs is intravasation of tumor cells into the

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Disclosure: The authors declare no conflict of interest.

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Cite this article as: Yeh YC, Ma HH, Chu PY, Ho HL, Chou TY. Brief report: programmed death-ligand 1 expression in lymphovascular tumor emboli in lung cancer. *JTO Clin Res Rep.* 2022;3:100349.

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ISSN: 2666-3643

<https://doi.org/10.1016/j.jtocr.2022.100349>

vasculature or lymphatic system. Nevertheless, little is known about the characteristics of PD-L1 expression in tumor cells inside lymphovascular spaces.

Recently, substantial efforts have been made to study PD-L1 expression in circulating tumor cells (CTCs) inside the blood vessels.¹⁻⁷ Nevertheless, the results have been conflicting. For example, in the two largest series, Ilić et al.⁴ observed 80% concordance between blood and tissue, whereas Guibert et al.³ observed no correlation between tissue and CTC PD-L1 expression ($r = 0.04$, $p = 0.77$). Regarding the predictive value of PD-L1 expression in CTCs, it has been found that PD-L1 expression in CTCs was either not predictive of treatment response to programmed cell death protein-1 (PD-1) or PD-L1 inhibitors, or associated with a worse prognosis in patients treated with PD-1 inhibitors.^{3,7} These results contradict those observed based on tissue PD-L1 expression.

A major limitation of previous studies on PD-L1 expression in CTCs is that the detection methods of PD-L1 expression in CTCs are not standardized. Most studies used PD-L1 detection methods that are different from those used for tissue PD-L1 expression. The result of PD-L1 expression can differ significantly depending on the detection method used.^{8,9} Currently, five U.S. Food and Drug Administration–approved immunohistochemical PD-L1 assays exist, including Dako 22C3, Dako 28-8, Dako 73-10, Ventana SP142, and Ventana SP263 assays. It has been found that the results of these standardized PD-L1 assays are highly consistent and reproducible, provided that staining protocols are strictly followed.⁹ Nevertheless, all standardized assays are intended for use in tissue sections only, and they are not applicable to CTCs isolated from the peripheral blood. Currently, there is no standardized method for detecting PD-L1 expression in CTCs. It is, therefore, difficult to judge whether the differences in PD-L1 expression observed between CTCs and tumor tissue are due to intrinsic differences in PD-L1 expression level or to technical influences.

A fair comparison of PD-L1 expression between tumor tissues and tumor cells inside lymphovascular spaces can only be achieved when the same PD-L1 detection methods are applied to both. In tissue sections, clusters of tumor cells inside lymphovascular spaces, which are known as lymphovascular tumor emboli, can be observed in approximately 30% of surgically resected lung cancer.¹⁰ Because these lymphovascular tumor emboli are present in tissue sections, it is possible to use a standard tissue PD-L1 assay to evaluate PD-L1 expression. In this study, we performed a standard PD-L1 immunohistochemical assay on cancer tissue containing lymphovascular emboli and specifically examined PD-L1 expression in cancer tissue and

lymphovascular tumor emboli on the same tissue slide. Through this approach, we were able to perform a direct comparison of PD-L1 expression between tumor cells in the tissue and CTCs inside lymphovascular spaces, using the same PD-L1 assay and staining protocols.

Materials and Methods

This study included 36 patients with lung cancer and lymph node metastases who underwent surgical resection at the Taipei Veterans General Hospital between 2004 and 2019. This study was approved by the institutional review board, and the requirement for informed consent was waived. The pathologic slides were reviewed by a pathologist, and only tumors with more than 100 tumor cells in the lymphovascular spaces per slide were included.

Four-micrometer-thick slides were cut from the formalin-fixed paraffin-embedded (FFPE) blocks. PD-L1 immunohistochemical staining was performed following the protocols of the Dako PD-L1 immunohistochemistry 22C3 pharmDx assay (Dako, Carpinteria, CA). PD-L1 expression was evaluated by a pathologist using the tumor proportion score (TPS), which is the percentage of viable tumor cells having partial or complete membrane staining with any intensity. PD-L1 expression was further divided into the following three categories: PD-L1 high (TPS $\geq 50\%$), PD-L1 low (TPS 1%–49%), and PD-L1 negative (TPS $< 1\%$). In selected cases, additional immunohistochemical staining with CD34, an endothelial cell marker, was used to better visualize the lymphovascular spaces. Comparison of PD-L1 expression TPS scores between the primary tumor tissue, tumor cells in the lymphovascular spaces, and lymph node metastasis was assessed using the Wilcoxon signed rank test. The agreement of PD-L1 expression categories between tumor cells at different sites was measured using the κ -statistic.

Results

The clinical characteristics of the patients are summarized in [Table 1](#). Most cases (86.1%) were adenocarcinomas. PD-L1 expression in primary tumors, lymphovascular tumor emboli, and lymph node metastases is summarized in [Figure 1A](#). We observed a significant decrease in PD-L1 expression in lymphovascular tumor emboli compared with that in primary tumors ($p = 0.002$). There was also a trend toward a decrease in PD-L1 expression in lymphovascular tumor emboli compared with lymph node metastasis, although this was not statistically significant ($p = 0.065$). There was no significant difference in PD-L1 expression between the primary tumors and lymph node metastases ($p = 0.944$). There was no significant difference in PD-L1

Table 1. Clinicopathologic Characteristics of the Patients

Variables	Total (N = 36)
Age (y)	
Median (range)	62 (38-78)
Sex, n (%)	
Female	18 (50.0)
Male	18 (50.0)
Stage, n (%)	
IIB	2 (5.6)
IIIA	28 (77.8)
IIIB	6 (16.7)
T category, n (%)	
T1	6 (16.7)
T2	19 (52.8)
T3	9 (25.0)
T4	2 (5.6)
N category, n (%)	
N1	7 (19.4)
N2	29 (80.6)
Location, n (%)	
Right upper lobe	13 (36.1)
Right middle lobe	2 (5.6)
Right lower lobe	7 (19.4)
Left upper lobe	5 (13.9)
Left lower lobe	8 (22.2)
Left upper lobe and left lower lobe	1 (2.8)
Tumor size (cm)	
Median (range)	3.3 (1.5-7.2)
Tumor type, n (%)	
Adenocarcinoma	31 (86.1)
Squamous cell carcinoma	3 (8.3)
Small cell carcinoma	1 (2.8)
Pleomorphic carcinoma	1 (2.8)
EGFR mutation status, ^a n (%)	
Wild type	7 (28.0)
Mutated	18 (72.0)
ALK immunohistochemistry, ^b (%)	
Positive	0 (0)
Negative	10 (100)

^aEGFR mutation status was available in 25 patients.

^bALK immunohistochemistry results were available in 10 patients.

expression between *EGFR* wild type and *EGFR*-mutated tumors ($p = 0.623$).

The PD-L1 expression categories (PD-L1 negative, low, and high) for each patient are illustrated in Figure 1C. The PD-L1 expression categories were discordant between primary tumors and lymphovascular emboli in 11 of 36 patients (30.6%). All cases had downgraded PD-L1 expression categories (i.e., changed from high expression to low expression or from low expression to negative expression). There was only fair agreement between PD-L1 expression in the primary tumor tissue and lymphovascular tumor emboli ($\kappa = 0.244$). In contrast, there was a moderate agreement between PD-L1 expression in the primary tumor tissue and lymph node metastasis ($\kappa = 0.599$).

Although most tumors had similar PD-L1 expression between the primary tumors and lymph node metastases, a subset of lymph node metastases had significant change of PD-L1 expression (>10% difference in TPS) compared with their primary tumors. We identified four patients with increased PD-L1 expression and three patients with decreased PD-L1 expression in the lymph node metastasis. The patient characteristics are summarized in Supplementary Table 1. Although the number of patients was very limited, there was a trend toward younger age, female sex, solid pattern, and wild-type *EGFR* in the group with increased PD-L1 expression in lymph node metastasis.

The pathologic images of PD-L1 expression in a representative case are shown in Figure 2A and B. In this patient, the primary tumor had high PD-L1 expression (TPS $\geq 50\%$); however, there was no PD-L1 expression in the lymphovascular tumor emboli (TPS = 0%).

Discussion

To our knowledge, this study is the first to directly compare PD-L1 expression between primary tumor tissue, tumor cells inside lymphovascular spaces, and lymph node metastasis using the same PD-L1 assay and staining protocols. Our study revealed a significant difference in PD-L1 expression between primary tumors and lymphovascular tumor emboli. Tumor cells in the lymphovascular emboli tended to have lower PD-L1 expression than those in primary tumors.

There are several possible explanations for these differences. First, although we intentionally chose tumors with extensive lymphovascular tumor emboli in this study, the number of tumor cells in lymphovascular spaces was still far less than that in primary tumors. Therefore, it is possible that lower PD-L1 expression in lymphovascular tumor emboli could be related to sampling issues. Nevertheless, this does not fully explain the cases with high PD-L1 expression (TPS $\geq 50\%$) in the primary tumor but negative PD-L1 expression in the lymphovascular tumor emboli.

Another possibility is that the tumor cells in the lymphovascular spaces represent a subclone with lower PD-L1 expression. Considerable intratumoral heterogeneity in PD-L1 expression can be detected between different tumor subclones.¹¹ This hypothesis implies that there is a positive selection pressure favoring tumor subclones with low PD-L1 expression to enter or survive in lymphovascular spaces, which warrants further investigation.

A third possibility is that PD-L1 expression may be down-regulated when tumor cells enter the lymphovascular spaces. PD-L1 expression is dynamically regulated by the tumor microenvironment. For example, interferon- γ secreted by inflammatory cells in the tumor

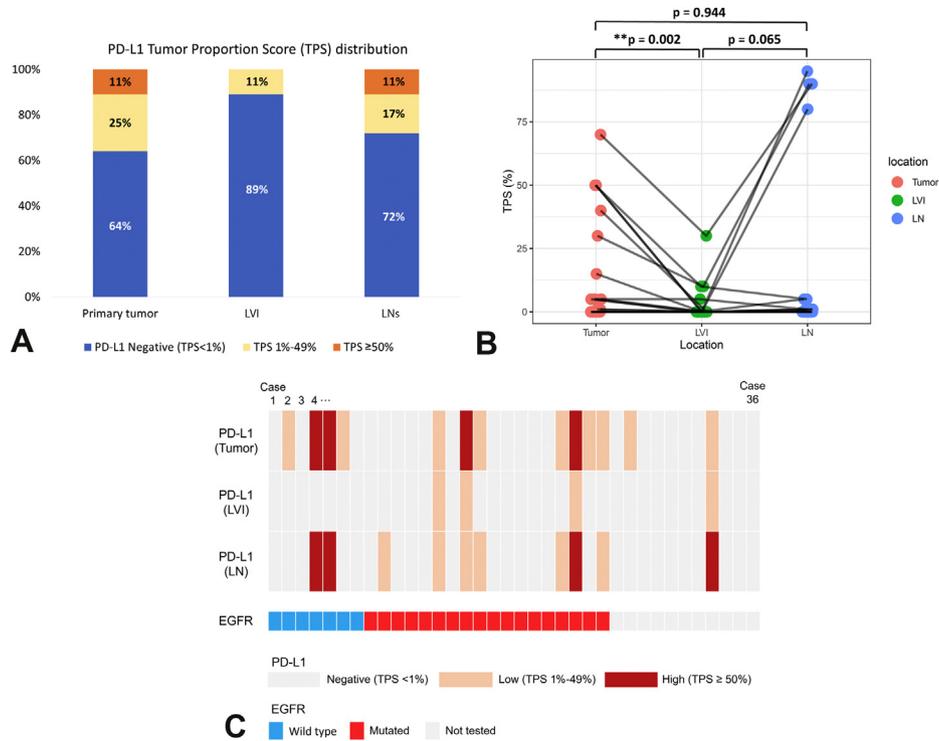


Figure 1. PD-L1 expression in primary tumors, LVI, and LNs. (A) Distribution of PD-L1 expression categories. (B) PD-L1 expression TPS in each patient. Each dot represents one patient. (C) PD-L1 expression categories in each patient. LN, lymph node metastasis; LVI, lymphovascular tumor emboli; PD-L1, programmed death-ligand 1; TPS, tumor proportion score. (** $p < 0.005$)

microenvironment is a prominent stimulator that can induce PD-L1 expression in the tumor cells.¹² Besides, a recent study revealed that PD-L1 expression varied substantially across different anatomical sites, which was possibly attributed to the immune microenvironment in different organs.¹³ Because the microenvironment in blood and lymphatics is significantly different from tumor tissue, it is not surprising to observe changes in PD-L1 expression when tumor cells move from tissue to lymphovascular spaces.

In our study, there were no significant differences in PD-L1 expression between primary tumors and lymph node metastases, which was in concordance with previous reports.¹³ Although decreased PD-L1 expression was observed in the tumor cells in the lymphovascular spaces, some of them had elevated PD-L1 expression in the lymph node metastases, as revealed in [Figure 1B](#) and [Supplementary Table 1](#). The underlying mechanism for such variation remains unclear, but the differences in immune microenvironment between

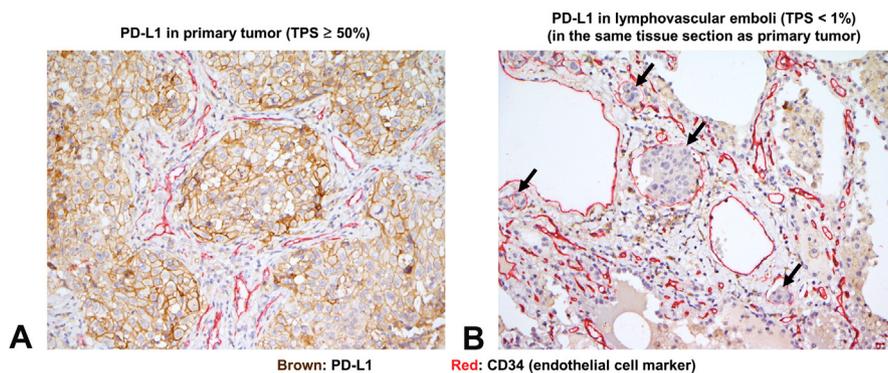


Figure 2. Pathologic images of PD-L1 expression in samples from a representative patient. (A) The primary tumor revealed high PD-L1 expression (original magnification: 200×). (B) The tumor cells in lymphovascular spaces (arrow) did not express PD-L1 (original magnification: 200×). PD-L1, programmed death-ligand 1; TPS, tumor proportion score.

lymphovascular spaces and lymph nodes might play a role.

Previous studies had revealed that preanalytic conditions could significantly affect PD-L1 expression. Delay in fixation and older FFPE block age was found to associate with decreased PD-L1 expression.^{14,15} Nevertheless, these confounding factors were unlikely to have significant effect in our study because the primary tumor tissue and tumor cells in the lymphovascular spaces were present in the same FFPE block. In addition, the lymph node metastasis specimens were obtained in the same surgery as the primary tumors, and their specimen characteristics were therefore similar.

Although the mechanisms underlying lower PD-L1 expression in tumor cells in lymphovascular spaces are currently unknown, our findings suggest that tumor cells in lymphovascular spaces may use an alternative immune escape strategy other than the PD-1/PD-L1 signaling pathway. Anti-PD-1/PD-L1 inhibitors are likely less effective against these tumor cells. A better understanding of the unique molecular characteristics of tumor cells in lymphovascular spaces is imperative for improving treatment strategies for these tumor subpopulations.

Our study had several limitations. First, the sample size is relatively small; larger studies are required to validate our findings. Second, the tumors included in this study solely consisted of surgically resected tumors which revealed extensive lymphovascular tumor emboli. It is unclear whether our findings can be extended to other types of tumors. Third, the patients included in this study did not receive anti-PD-1/PD-L1 inhibitor immunotherapy; therefore, we were unable to evaluate the association between PD-L1 expression and immunotherapy treatment response.

In conclusion, through a direct, same-tissue-section comparison of PD-L1 expression in the primary tumor and that in lymphovascular emboli, we observed that there was a significant difference in PD-L1 expression between primary tumors and lymphovascular emboli. Tumor cells in lymphovascular emboli tend to have lower PD-L1 expression than those in primary tumors. Whether such differences are related to intrinsic tumor cell heterogeneity or extrinsic factors, such as tumor microenvironment, warrants further investigation.

CRedit Authorship Contribution Statement

Yi-Chen Yeh: Conceptualization, Methodology, Investigation, Data Curation, Visualization, Writing—Original draft preparation, Funding acquisition.

Hsiu-Hsun Ma: Methodology, Investigation.

Ping-Yuan Chu: Methodology, Investigation.

Hsiang-Ling Ho: Methodology, Investigation, Supervision, Project administration.

Teh-Ying Chou: Conceptualization, Supervision, Writing—Review and Editing, Project administration, Funding acquisition.

Acknowledgments

This work was supported by the Ministry of Science and Technology, Taiwan (MOST 109-2320-B-075-004-MY3, MOST 110-2634-F-A49-005-SP5-2, MOST 109-2320-B-075-007-MY3) and Taipei Veterans General Hospital, Taiwan (V110C-196).

Supplementary Data

Note: To access the supplementary material accompanying this article, visit the online version of the *JTO Clinical and Research Reports* at www.jtocrr.org and at <https://doi.org/10.1016/j.jtocrr.2022.100349>.

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