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Differences in PD-L1 expression on tumor and immune cells between lung metastases and corresponding primary tumors

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ABSTRACT

Background: It has been reported that the tumor microenvironment, including tumor-associated immune cells (ICs) and programmed cell death-ligand 1 (PD-L1) expression, differs between primary and metastatic tumors. This study aimed to elucidate the differences in PD-L1 expression on tumor cells (TCs) and ICs between lung metastases and corresponding primary tumors.

Methods: We analyzed paired lesions from 44 patients diagnosed with lung metastases between 2005 and 2017 at Kyushu University. The percentages of PD-L1-positive TCs and ICs in lung metastases and the primary tumor were classified into five categories (0: < 1%; 1: 1%–4%; 2: 5%–9%; 3: 10%–49%; and 4: ≥50%). Lesions in which ≥1% of the TCs and ICs were PD-L1-positive were considered positive.

Results: The primary cancers included rectal (n = 19), colon (n = 10), liver (n = 10), bile duct (n = 2), stomach (n = 1), gall bladder (n = 1) and breast (n = 1). Discrepancies in PD-L1 expression on TCs and ICs between lung metastases and primary lesions were observed in 5 (11.4%, κ = 0.23) and 9 (20.5%, κ = 0.11) of the 44 cases, respectively. PD-L1 expression on ICs was higher in lung metastases than paired primary tumors ($p = 0.026$), although the percentage of PD-L1-positive TCs was not significantly different between lung metastases and primary tumors ($p = 0.767$).

Conclusions: There were significant differences in PD-L1 expression on TCs and ICs between lung metastases and primary tumors. Clinicians should be aware of these differences in the tumor microenvironment when treating patients with immunotherapy.

1. Introduction

Since its superiority to chemotherapy was reported, immunotherapy targeting programmed cell death-1 (PD-1) and/or programmed cell death-ligand 1 (PD-L1) has become the standard of care for lung cancer treatment [1–4]. PD-1 is a cell surface receptor on T cells that plays an important role in downregulating immune responses; PD-L1, which is expressed not only on T cells but also tumor cells (TCs), is a PD-1 ligand [5]. The efficacy of anti-PD-1/PD-L1 therapies derives from their inhibitory effects on the PD-1/PD-L1 interaction, which attenuates immune-modulatory T cell activation and facilitates tumor progression [6,7]. Substantial effort has been devoted to finding biomarkers that can predict prognoses and responses to immune checkpoint inhibitors [8]. Previous reports have suggested that PD-L1 expression on TCs and/or immune cells (ICs) could provide clinicians with useful information regarding prognoses and responses to immunotherapy in patients with various types of malignancies, including non-small cell lung cancer (NSCLC) [9–13].

PD-L1 expression on TCs and ICs has been reported to differ between primary tumors and metastatic lesions [14,15]. This is partly due to differences in the tumor microenvironment (TME), which is different based on the target organ [15]. Our previous study revealed that 24% of NSCLC cases showed differential PD-L1 expression on TCs between the primary and distant metastatic lesions [14]. Mansfield et al. found that 14% of NSCLC cases showed discordant rates of PD-L1 expression between primary tumors and corresponding brain metastases [15]. These findings indicate differences in the TME, including PD-L1 positivity, between primary and metastatic sites in NSCLC patients. However,

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relationships in the TME between metastatic lung tumors and corresponding primary cancers has not been investigated. Thus, the aim of this study was to analyze differences in PD-L1 expression on TCs and ICs between lung metastases and primary tumors of different origins.

2. Materials and methods

2.1. Patients

Between January 2005 and December 2017, 43 patients were diagnosed with cancer that had metastasized to the lung and underwent surgical resection of both the metastases and the corresponding primary cancer at the Department of Surgery and Science, Graduate School of Medical Sciences, Kyushu University (Fukuoka, Japan). One patient was diagnosed with pulmonary metastatic breast cancer and underwent surgical resection of the lung metastasis and only a biopsy of the primary breast cancer. In total, 44 patients with lung metastases were enrolled in this retrospective study. The variables investigated in this study included age, sex and smoking status (pack year index). This study was approved by our institution's review board.

2.2. Immunohistochemical analyses

Surgical or biopsy specimens of the primary tumors and corresponding lung metastases from the 44 patients were subjected to immunohistochemical analyses. Formalin-fixed tissue sections were embedded in paraffin and cut into 4-μm-thick slices, which were dewaxed with xylene and rehydrated through a graded series of ethanol. Endogenous peroxidase activity was inhibited for 30 min with 3% H_2O_2 in methanol. The sections were pretreated with Target Retrieval Solution (Dako, Glostrup, Denmark) in a decloaking chamber for 15 min at 110 °C, and then incubated with rabbit anti-human PD-L1 monoclonal antibody (dilution 1:100; clone SP142, Ventana, Tucson, AZ, USA) at 4 °C overnight. Immune complexes were detected with the Dako EnVision Detection System (Dako). The sections were then incubated with 3,3′-diaminobenzidine and counterstained with hematoxylin. Finally, the slides were dehydrated in a graded series of alcohol and xylene, and then cover slip-mounted.

Plasma membrane PD-L1 expression on TCs and ICs was independently evaluated by a surgeon (S.T.) and a pathologist (K.T.). The percentages of PD-L1-positive TCs and ICs were then classified into one of five categories (0: < 1%, 1: 1%–4%, 2: 5%–9%, 3: 10%–49%, and 4: ≥50%). Lesions in which ≥1% of the TCs and ICs were PD-L1-positive were considered positive [4,16]. Human placenta sections were used as positive controls.

2.3. Statistical analyses

Kappa statistics were used to analyze differences in PD-L1 positivity between lung metastases and corresponding primary lesions. The Wilcoxon matched-pairs test was used to compare the PD-L1 positivity on TCs and ICs between lung metastases and primary tumors. Differences were considered statistically significant when the *p*-value was less than 0.05. All analyses were performed using JMP 13 software (SAS Institute Inc., Cary, NC, USA).

3. Results

3.1. Patient characteristics

Table 1 shows the clinical characteristics of the patients included in this study. The median age was 64 (range: 42–83) years old, and 33 (75.0%) were male. Thirty patients (68.2%) were current or former smokers, and the median pack-year was 20 (range: 0–115). The primary cancers included rectal (n = 19, 43.2%), colon (n = 10, 22.7%), liver (n = 10, 22.7%), bile duct (n = 2, 4.5%), stomach (n = 1, 2.3%), gall bladder (n = 1, 2.3%) and breast (n = 1, 2.3%). Three (6.8%) and four (9.1%) patients presented with ≥1% of TCs showing PD-L1 expression in the lung metastases and primary tumors, respectively, while 40 (90.9%) and 39 (88.6%) patients presented with ≥1% of ICs showing PD-L1 expression in the lung metastases and primary tumors, respectively.

Table 1
Clinical characteristics of the 44 patients with pulmonary metastases.

Factors		Value or no. of patients
Age (years)	Median	64
	Range	42–83
Sex, n (%)	Male	33 (75.0%)
	Female	11 (25.0%)
Smoking status, n (%)	Never-smoker	14 (31.8%)
	Smoker	30 (68.2%)
Pack year	Median	20
	Range	0–115
Primary cancer, n (%)	Rectum	19 (43.2%)
	Colon	10 (22.7%)
	Liver	10 (22.7%)
	Bile duct	2 (4.5%)
	Stomach	1 (2.3%)
	Gall bladder	1 (2.3%)
	Breast	1 (2.3%)
PD-L1 on TCs in metastatic lung tumor, n (%)	< 1%	41 (93.2%)
	1–4%	2 (4.5%)
	5–9%	1 (2.3%)
	10–49%	0 (0.0%)
	≥50%	0 (0.0%)
PD-L1 on TCs in primary cancer, n (%)	< 1%	40 (90.9%)
	1–4%	3 (6.8%)
	5–9%	1 (2.3%)
	10–49%	0 (0.0%)
	≥50%	0 (0.0%)
PD-L1 on ICs in metastatic lung tumor, n (%)	< 1%	4 (9.1%)
	1–4%	2 (4.5%)
	5–9%	8 (18.2%)
	10–49%	18 (40.9%)
	≥50%	12 (27.3%)
PD-L1 on ICs in primary cancer, n (%)	< 1%	5 (11.4%)
	1–4%	8 (18.2%)
	5–9%	10 (22.7%)
	10–49%	15 (34.1%)
	≥50%	6 (13.6%)

PD-L1, programmed cell death-ligand 1; TC, tumor cell; IC, immune cell.

3.2. Differences in PD-L1 expression on TCs between lung metastases and corresponding primary tumors

Fig. 1 shows the rate of positive or negative PD-L1 staining on TCs from the 44 metastatic lung tumors and their corresponding primary lesions with a cut-off value of 1%. Table 2 shows the PD-L1 positivity of lung metastases and their corresponding primary lesions. Four of the 44 primary tumors (9.1%) were positive for PD-L1. Among the four patients with PD-L1-positive primary tumors, the corresponding lung metastases were PD-L1-negative in three cases (75.0%). Among the 40 cases (90.9%) in which the primary tumors were PD-L1-negative, two corresponding metastatic lesions (5.0%) were PD-L1-positive. Thus, discrepancies in PD-L1 expression were observed between primary tumors and lung metastases in 5 of 44 cases (11.4%). While there were differences in PD-L1 expression on TCs between primary and metastatic tumors ($\kappa = 0.23$), as shown in Table 3, no significant differences in the PD-L1 positivity scores for TCs was found between primary and metastatic lesions ($p = 0.767$).