Correlation Between Programmed Death Receptor-1 Expression in Tumor-Infiltrating Lymphocytes and Programmed Death Ligand-1 Expression in Non–Small Cell Lung Carcinoma

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Context.—The interaction between programmed death ligand-1 (PD-L1) and programmed death receptor-1 (PD-1) on activated T cells sends an inhibitory signal that dampens the immune response. Tumors can express PD-L1 and evade the immune system. In advanced non–small cell lung carcinoma, expression of PD-1 in tumor-infiltrating lymphocytes (TILs) correlates with PD-L1 expression in tumor cells (TCs). However, this relationship has not been thoroughly explored in early disease.

Objective.—To investigate the correlation of PD-1 and PD-L1 in non–small cell lung carcinoma tumor samples, with emphasis on stage I disease.

Design.—Whole tissue sections from non–small cell lung carcinoma tumors were retrospectively evaluated by immunohistochemistry for PD-1 and PD-L1 expression. The scoring was based on the percentage of cells positive for PD-1 in TILs and PD-L1 in TCs and tumor-infiltrating immune cells (ICs).

Results.—Expression of PD-1 in TILs was observed in 147 of 161 non–small cell lung carcinoma cases (91%). The majority of cases negative for PD-1 also lacked PD-L1 in TCs. The 68 cases with highest PD-1 expression in TILs included 33 (49%) with expression of PD-L1 in TCs and ICs. Strong correlations were observed in patients with elevated PD-1 expression in TILs and PD-L1 in TCs and ICs (P = .01) and ICs (P = .003). Expression of PD-1 also correlated with increased PD-L1 in TCs and ICs when the 2 were grouped together (P < .001). Finally, stage I patients with negative PD-1 and PD-L1 expression showed trends toward increased disease-specific survival.

Conclusions.—Expression of PD-1 in TILs correlates with PD-L1 expression in both TCs and ICs. Furthermore, negative expression of PD-1 and PD-L1 suggest trends toward disease-specific survival, even in early disease stages.

response rates of the underlying physiology within the tumor microenvironment remain to be elucidated.

Recently, He et al. investigated NSCLC tumor microenvironment by analyzing PD-1 and PD-L1 protein expression and their correlation with tumor-infiltrating lymphocytes (TILs). Their data set included surgically resected specimens from NSCLC patients, the majority with advanced-stage and nodal disease, including patients with diagnosed metastasis. Their cohort had all histotypes (squamous cell carcinoma, adenocarcinoma, large cell carcinoma, NSCLC not otherwise specified, and others), the majority of which were squamous cell carcinoma. Their results showed that high expression of PD-1 in TCs significantly correlated with high expression of PD-L1 in a high percentage of TILs. Furthermore, they found that patients whose TCs were PD-L1 negative had a tendency toward longer relapse-free survival compared to patients whose TCs were PD-L1 positive.

To date, there are no current studies focusing on the correlations of PD-1 and PD-L1 proteins in stage I disease, which can be treated and theoretically cured by surgery. Moreover, advancements in imaging technologies and patient screening are allowing early detection of lung cancer, and the prognostic relevance of PD-1/PD-L1 is not known for this patient group. Thus, in this study we evaluated the expression of PD-1 and PD-L1 in NSCLC patients with emphasis on the most common type, adenocarcinoma, but also including some squamous cell carcinomas. Most of the patients we selected had a diagnosis of an early/limited disease stage, few had evidence of nodal metastasis, and only one had distant metastasis. All of our patients were treated by surgical resection with no adjuvant therapy. We then investigated the expression of PD-1/PD-L1 using previously established categories for immunohistochemistry scoring, and assessed the correlation between the expression of PD-1 in TILs and PD-L1 in TCs and tumor-infiltrating immune cells (ICs). We also evaluated the prognostic significance of the PD-1/PD-L1 immune checkpoint in NSCLC patients of all disease stages.

MATERIALS AND METHODS

Study Samples

Institutional review board approval of research protocols for this project was obtained through Houston Methodist Hospital Research Institute (Houston, Texas). Surgical pathology blocks were obtained from a series of NSCLCs from attempted curative surgical resections of lung cancers between 1975 and 1991 at Houston Methodist Hospital for which 5-year or greater survival data were available. Because these were attempted curative surgical resection specimens, the great majority were expected to represent early-stage NSCLC. Retrospective chart review was performed to obtain pathology reports, including original cell type diagnosis, and clinical data, including patient age, sex, smoking history, and survival status. Routine hematoxylin-eosin sections were obtained to aid in the diagnosis.

Tumors lacking both adenocarcinoma and squamous differentiation were reclassified according to 2015 World Health Organization criteria. Tumors with sarcomatoid or neuroendocrine morphology were also excluded. Subtypes were recorded for adenocarcinoma cases according to 2015 World Health Organization criteria. Tumor staging was modified if necessary to be consistent with the 7th American Joint Committee on Cancer Staging Manual.

Immunohistochemistry

Tissue sections on glass slides were cut to 4 to 5 μm, deparaffinized, and hydrated in a series of gradient alcohols. A peroxidase block was performed, and tissue sections were then retrieved in a modified citrate buffer using a pressure cooker (Decloaking Chamber, Biocare Medical, Pacheco, California) at 110°C for 15 minutes. Tissue sections were then cooled for 20 minutes and placed in buffer.

Immunohistochemistry for PD-L1 was carried out with an automated stainer (Leica Bond III, Leica Biosystems, Buffalo Grove, Illinois) using anti–PD-L1 (clone SP142) obtained from Spring Bioscience (Pleasanton, California). Incubation time was 30 minutes and dilution was 1:1000. Expression of PD-L1 was scored semiquantitatively according to percentage of tumor area with PD-L1–positive ICs (IC0 for <1%, IC1 for 1%–4%, IC2 for 5%–49%, and IC3 for ≥50%) and percentage of tumor area with PD-L1–positive TCs (TC0 for <1%, TC1 for 1%–4%, TC2 for 5%–49%, and TC3 for ≥50%) and percentage of tumor area with PD-L1–positive ICs (IC0 for <1%, IC1 for 1%–4%, IC2 for 5%–9%, IC3 for ≥10%) as previously described. Immunochemistry for PD-1 was performed using a PD-1 antibody (NAT105, Biocare), which was diluted at 1:50 and incubated for 1 hour at room temperature, followed by a 2-step
Abbreviation: IHC, immunohistochemistry score.

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Table 2. Programmed Death Receptor-1 (PD-1) Is Significantly Expressed in Non–Small Cell Lung Cancer (NSCLC)

<table>
<thead>
<tr>
<th>PD-1 Expression</th>
<th>NSCLC (N = 161)</th>
<th>Adenocarcinoma (n = 124)</th>
<th>Squamous Cell Carcinoma (n = 37)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IHC0, No. (%)</td>
<td>14 (9)</td>
<td>10 (8)</td>
<td>4 (11)</td>
</tr>
<tr>
<td>IHC1, No. (%)</td>
<td>39 (24)</td>
<td>30 (24)</td>
<td>9 (24)</td>
</tr>
<tr>
<td>IHC2, No. (%)</td>
<td>40 (25)</td>
<td>29 (23)</td>
<td>11 (30)</td>
</tr>
<tr>
<td>IHC3, No. (%)</td>
<td>68 (42)</td>
<td>55 (44)</td>
<td>13 (35)</td>
</tr>
</tbody>
</table>

Abbreviation: IHC, immunohistochemistry score.

horseradish peroxidase polymer detection system and visualization by 3,3’-diaminobenzidine. An automated Intellipath (Biocare), was used. Immunohistochemistry score for PD-1 was scored semiquantitatively according to percentage of tumor area with PD-1–positive TILs (IHC0 for <1% IHC1 for 1%–4%, IHC2 for 5%–9%, IHC3 for ≥10%). All immunohistochemistry results were evaluated against a negative control.

Statistics

Statistical calculations were performed using GraphPad Prism version 7 software (GraphPad Software, Inc, La Jolla, California). The χ² test was used to compare PD-1 and PD-L1 expression among all tumor samples. The correlation between the expression of PD-1 and PD-L1 was analyzed by Spearman rank correlation. Disease-specific survival was analyzed using the log-rank (Mantel-Cox) test. Hazard ratios (log rank) and 95% confidence intervals were also obtained. Patients who died for reasons other than lung cancer and patients with an unknown cause of death were excluded from the disease-specific survival analysis. All statistics were 2-sided, and P values less than .05 were considered statistically significant.

RESULTS

Following reclassification by 2015 World Health Organization criteria, a total of 124 adenocarcinomas and 37 squamous cell carcinomas were included in the study (161 cases total). The clinicopathologic features of the NSCLC study population were as follows: median age 64 years (range, 35–90 years), male to female ratio of 1.48, and median smoking pack-years of 52 (range, 1–200) (Table 1). As expected, the majority of cases were T1 (52%), N0 (75%), M0 (98%), and stage I (75%). The full range of PD-1 expression in TILs was observed for adenocarcinoma and squamous cell carcinomas (Table 2). Most NSCLC cases (68 of 161; 42%) exhibited 10% or more PD-1 expression in TILs. Cases with more than 10% PD-1 expression in TILs were slightly more prevalent in adenocarcinomas than in squamous cell carcinomas (44% versus 35%).

To test if PD-1 expression in TILs correlated with PD-L1 expression in TCs, we constructed contingency tables. All 14 NSCLC cases lacking PD-1 expression in TILs concurrently lacked PD-L1 expression in TCs (Table 3). In addition, increased PD-1 expression scores correlated with increased PD-L1 expression in TCs. Approximately half of the samples having a PD-1 IHC3 score had a PD-L1 TC score of TC1, 2, or 3 (Table 3). The correlation between PD-1 expression in TILs and PD-L1 expression in TCs was statistically significant (χ²; P = .01).

The majority (13 of 14; 93%) of cases lacking PD-1 expression in TILs also lacked PD-L1 expression in ICs (Table 4). Similar to what was observed with PD-L1 expression in TCs, increased PD-1 expression scores correlated with increased PD-L1 expression in ICs, with approximately 47% of ICs having PD-L1 scores of IC1, 2, or 3. Correlation between PD-1 expression in TILs and PD-L1 expression in ICs was also statistically significant (χ²; P = .003). Additionally, high PD-1 expression strongly correlated with high PD-L1 expression in both TCs and ICs (χ²; P < .001) (Table 5). Finally, the monotonic relationship of the variables (whether linear or not) was assessed and found to be statistically significant for the percentage of PD-1 in TILs with PD-L1 in TCs (Spearman rank correlation factor = 0.29, P < .001), and PD-1 in TILs with PD-L1 in ICs (Spearman rank correlation factor = 0.31, P < .001).

For lung adenocarcinomas, PD-1 expression in TILs at a 5% cutoff was not associated with age, sex, smoking pack-years, TNM stage, or histologic subtype (data not shown).
However, lung adenocarcinomas with PD-1 expression in TILs greater than 5% were weakly associated with higher histologic grades (data not shown). For prognostic implications, we evaluated disease-specific survival according to PD-1/PD-L1 scores in the NSCLC tumor samples. A score lower than 1% was considered negative and any percentage of 1% or higher was considered positive for both PD-1 and PD-L1. Our results showed a trend toward increased disease-specific survival in NSCLC patients of all stages with PD-1 as well as PD-L1–negative scores compared with positive ones (data not shown). We further stratified the patients by disease stage and evaluated disease-specific survival in stage I patients (Figure, A and B). Our results suggest a trend toward increased disease-specific survival in stage I patients with PD-1 negative scores ($P = .21$, hazard ratio $= 0.76; 95\% \text{ CI}, 0.3758–1.557$). A limited trend was also observable in stage I patients with negative PD-L1 expression compared with positive ($P = .45$, hazard ratio $= 0.42; 95\% \text{ CI}, 0.161–1.099$). No correlation was found between PD-1 in TILs and PD-L1 in ICs.

**DISCUSSION**

Tumor cell exploitation of immune checkpoint pathways is thought to facilitate cancer tolerance and immune system evasion. The PD-1/PD-L1 receptor-ligand pair comprises a dominant immune checkpoint pathway that is known to contribute to tumor immune evasion in several cancer types, particularly NSCLC. Recent studies have shown that immune checkpoint inhibitors against proteins such as PD-1 and PD-L1 have been proven successful in clinical trials.

**Figure**

A. Negative programmed death receptor-1 (PD-1) (A) and programmed death ligand-1 (PD-L1) (B) expression in tumor cells correlates with increased disease-specific survival in patients with stage I non–small cell lung cancer (NSCLC). Disease-specific survival was evaluated according to PD-1 and PD-L1 scores in patients with disease stage I NSCLC. Increased trends toward disease-specific survival were observed in patients with negative PD-1 ($P = .21$, hazard ratio [HR] = 0.76; 95\% CI, 0.3758–1.557) and PD-L1 ($P = .45$, HR = 0.42; 95\% CI, 0.161–1.099) scores.
Survival advantages have not been fully elucidated.

In this vein, He et al.\(^\text{13}\) investigated the relationship between PD-1 expression in TILs and PD-L1 expression in surgically resected NSCLC tumors. As part of their data set, they had more patients who had a diagnosis of squamous cell carcinoma (52.3%) and fewer with a diagnosis of adenocarcinoma (28.8%). In addition, less than half of their patients were stage I (41.7%) and more patients were higher stage (stage II, 25.2%; stage III, 28.1%; stage IV, 5.0%). With their cohort, their results showed that high expression of PD-1 in TILs significantly correlated with increased expression of PD-L1 in TCs.\(^\text{13}\) Furthermore, they determined there was a trend associating negative expression of PD-1 and of PD-L1 (independently) increased relapse-free survival.\(^\text{13}\)

In this study, we investigated the correlation of PD-1 in TILs and PD-L1 in TCs. We further evaluated the correlation of PD-1 expression with PD-L1 in ICs. This is an important observation, as PD-L1 expression in ICs is thought to diminish antitumor immunity similarly to PD-L1 expression in TCs.\(^\text{26}\) In addition, we evaluated the prognostic significance of PD-1 and PD-L1 in NSCLC patients, focusing on stage I disease.

Compared with the study from He et al,\(^\text{13}\) our series includes a larger number of adenocarcinomas (77%), the most commonly diagnosed NSCLC type, and a larger percentage of early-stage cancers, particularly stage I. In our cohort, the patients had surgical resection, with no previous neoadjuvant treatment. Upon staging, most of them were disease stage I (75%), with T1 (52%); very few (18%) had nodal extension, and only one had distant metastasis. We hypothesized that PD-1 and PD-L1 could potentially be relevant in early disease such as stage I.

Our results are in good agreement with the results of the prior study by He and colleagues, despite differences in study population and study design.\(^\text{13}\) We found a significant correlation between PD-1 in TILs and PD-L1 in TCs (\(P = 0.1\)). Furthermore, our results extend the observation in NSCLC, as we evaluated and confirmed that PD-1 expression in TILs correlates with PD-L1 expression in ICs (\(P = 0.003\)). When grouped together, PD-L1 expression in TCs and ICs correlates with expression of PD-1 in TILs (\(P < 0.001\)). Additionally, we evaluated the prognostic significance of PD-1 and PD-L1 in NSCLC. In patients of all stages, we found that there was a trend toward disease-specific survival in those who were negative, compared with positive, for PD-1 expression in TILs (data not shown). Similar trends were seen in patients who were negative for PD-L1 in TCs (data not shown). Given that no previous studies had evaluated the prognostic value of this immune checkpoint in early-stage NSCLC, we further explored if these trends were seen in patients with a diagnosis of stage I disease. Our results showed that trends toward disease-specific survival were seen in patients with negative PD-1 scores compared with positive ones (\(P = 0.21\)). Similar trends were also seen toward disease-specific survival in patients negative for PD-L1 expression in TCs (\(P = 0.45\)). No trends were seen regarding PD-L1 expression in ICs. There was no difference between these results and results when our data set was stratified by lung cancer type (adenocarcinoma versus squamous cell carcinoma). We also did not see any correlation between the clinicopathologic factors of the patients evaluated and expression of PD-1 or PD-L1.

Our study design includes 2 additional strengths. First, we used the same PD-L1 immunohistochemical scoring used in the POPLAR clinical trial, and PD-1 scoring that stratifies into 4 groups rather than a single cutoff.\(^\text{14,27}\) Regarding the latter, this stratification helped to show the correlation between PD-1 expression in TILs and PD-L1 expression in the tumor. Second, we used a different antibody (compared with the study from He et al\(^\text{13}\)) to perform immunohistochemistry, and our findings confirmed their results (refer to Materials and Methods).

In summary, our study showed a trend between PD-1 in TILs and PD-L1 in TCs and in ICs. Further, in patients with stage I NSCLC, and negative PD-1 as well as PD-L1 expression in TILs, there was a trend toward disease-specific survival. The results of our study support contemplating the use of immune checkpoint blockers in patients with limited/localized disease, not only advanced disease stages. Our results are novel, because the majority of the PD-L1 clinical studies have focused on advanced-stage NSCLC.\(^\text{29}\) Further studies are needed to address these findings.

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References