Immunohistochemistry of immune checkpoint markers PD-1 and PD-L1 in prostate cancer

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Abstract

Recent availability of immune checkpoint inhibitors has facilitated research involving programmed cell death protein 1 (PD-1) and its ligand, programmed death-ligand 1 (PD-L1). However, the incidence and clinical implication of PD-1 and PD-L1 expression in prostate cancer remain poorly understood. The current study aimed to determine the status of PD-1/PD-L1 expression in prostate cancer specimens and its prognostic significance.

We immunohistochemically stained for PD-1 and PD-L1 in our tissue microarray (TMA) consisting of radical prostatectomy specimens. The expression of PD-1/PD-L1 was designated as positive when moderate to strong staining or weak staining was seen in at least 1% or 10%, respectively, of tumor cells and/or associated immune cells. We then evaluated the relationship between the expression of each protein and clinicopathological features available for our patient cohort.

PD-1 and PD-L1 were positive in 3 (1.5%) and 1 (0.5%) of 201 non-neoplastic prostate tissues, and also in 17 (7.7%) and 29 (13.2%) of 220 prostate cancers, respectively. PD-1 and PD-L1 were also expressed in tumor-infiltrating lymphocytes/macrophages in 172 (78.2%) and 33 (15.0%) cases, respectively. PD-L1 expression in tumor cells was more often seen in high pT stage (pT2: 10.8% vs pT3/4: 20.4%; $P=.072$; pT2/3a: 11.4% vs pT3b/4: 31.6%; $P=.013$) or lymph node-positive (pN0: 10.1% vs pN1: 27.3%; $P=.086$) cases, whereas PD-1 expression in tumor cells was not significantly associated with pT/pN stage. In addition, there were no statistically significant associations between PD-1/PD-L1 expression in tumor cells or tumor-infiltrating lymphocytes/macrophages versus patient age, preoperative prostate-specific antigen level, or Gleason score. Kaplan–Meier analysis coupled with log-rank test further revealed no significant associations between PD-1/PD-L1 expression in tumor cells ($P=.619/P=.315$), tumor-infiltrating lymphocytes/macrophages ($P=.954/P=.155$), or either or both of them ($P=.964/P=.767$) versus disease recurrence after radical prostatectomy.

PD-1/PD-L1 expression was detected in a subset of prostate cancers. In particular, PD-L1 expression was considerably up-regulated in nonorgan-confined tumors. However, PD-1/PD-L1 expression in our TMA was found to be not very helpful in predicting tumor recurrence in prostate cancer patients who underwent radical prostatectomy.

Abbreviations: PD-1 = programmed cell death protein 1, PD-L1 = programmed death-ligand 1, PSA = prostate-specific antigen, TMA = tissue microarray.

Keywords: immunohistochemistry, PD-1, PD-L1, prognosis, prostate cancer

1. Introduction

Prostate cancer is the most frequently diagnosed neoplasm, with an estimated 174,650 new cases and 31,620 deaths occurred in 2019, in United States.[1] Although radical prostatectomy represents the standard treatment for localized prostate cancer, disease recurrence after the surgery remains a major clinical challenge. Importantly, only few molecular markers, other than clinicopathological features including Gleason score of the tumor and preoperative level of prostate-specific antigen (PSA), are known to precisely predict disease progression.[2] Therefore, identification of key molecules involving prostate cancer outgrowth is urgently required, which may successively provide novel prognostic markers and/or novel targeted therapies in patients with prostate cancer.

Programmed cell death protein 1 (PD-1) and its ligand programmed death-ligand 1 (PD-L1) have been implicated in suppressing the adaptive immune system.[3] Remarkably, recent development of immune checkpoint inhibitors, including PD-1/PD-L1 inhibitors, has facilitated research assessing their efficacy in various types of malignant diseases.[4] Indeed, anti-PD-1/PD-L1 antibodies have been approved by the US Food and Drug Administration in the United States for the treatment of, for example, melanoma, lung nonsmall cell carcinoma, head and neck squamous cell carcinoma, urothelial carcinoma, and renal cell carcinoma. Meanwhile, responses to PD-1/PD-L1 blockade have been suggested to correlate with increased expression of PD-1/PD-L1 in tumor cells and/or tumor-infiltrating lymphocytes/macrophages.[5] Nonetheless, antitumor activity of immune

checkpoint inhibitors against prostate cancer remains to be established.

Recent studies have immunohistochemically assessed the expression levels of PD-L1 (and/or PD-1 only in a few studies) in prostate cancer specimens, which yields highly variable results.[6–12] The incidence and clinical implication of PD-1 and PD-L1 expression in benign and cancerous prostate thus remain far from being fully understood. The current study aimed to determine the status of PD-1/PD-L1 expression in prostate cancer tissue specimens and its prognostic significance.

2. Materials and methods

2.1. Prostate tissue microarray

We retrieved 220 prostate tissue specimens obtained by radical prostatectomy performed at the University of Rochester Medical Center. Appropriate approval from the Institutional Review Board was obtained before construction and use of the tissue microarray (TMA) consisting of representative lesions of non-neoplastic prostate and prostatic adenocarcinoma, as described previously.[13,14] The institutional review board also approved the request to waive the documentation of informed consent from the patients. Their mean age at presentation was 60.3 years (range 42–78 years) and the mean follow-up after the surgery was 48.2 months (range 3–116 months). None of the patients had received therapy with hormonal reagents, radiation, or other anticancer drugs pre or postoperatively before clinical or biochemical recurrence. Biochemical recurrence was defined as a single PSA level of ≥ 0.2 ng/mL.

2.2. Immunohistochemistry

Immunohistochemical staining for PD-1/PD-L1 was performed, using a primary antibody to PD-1 (clone 22C3; Agilent Technologies, Santa Clara, CA) or PD-L1 (IHC 22C3 pharmDx; Agilent Technologies), and an automated staining system, on the sections (5-μm thick) from the prostate TMA, as described previously.[13,14] All stains were quantified independently by 2 pathologists (M.S. and H.M.) who were blinded to sample identity. The expression of PD-1/PD-L1 was designated as positive when moderate to strong staining was seen in at least 1% of tumor cells/tumor-infiltrating lymphocytes or macrophages, or weak staining was seen in at least 10% of tumor cells/tumor-infiltrating lymphocytes or macrophages. Cases with discrepancies in the positivity were re-reviewed simultaneously by the 2 pathologists until a consensus was reached.

2.3. Statistical analysis

The Fisher exact test or chi-square test was used to evaluate the association between categorized variables. Nonparametric 2-group comparisons were carried out, using Mann-Whitney U test, to assess differences in variables with ordered distribution across dichotomous categories. The rates of recurrence-free survival were calculated by the Kaplan-Meier method, and comparisons were made by the log-rank test. P values less than .05 were considered to be statistically significant.

3. Results

We immunohistochemically stained for PD-1 and PD-L1 in 220 cases of prostatic carcinoma and corresponding non-neoplastic prostate present in radical prostatectomy specimens. Positive signals were detected in carcinoma cells (or benign epithelial cells), and also in tumor-infiltrating immune cells, such as lymphocytes and macrophages (Fig. 1).

Programmed cell death protein 1 and PD-L1 were positive in prostatic epithelial/carcinoma cells in 3 (1.5%) and 1 (0.5%) of 201 benign tissues, and also in 17 (7.7%) and 29 (13.2%) of cancer cases, respectively. Thus, the rate of PD-1 ($P = .003$) or PD-L1 ($P < .001$) positivity was significantly higher in cancer than in benign. All of the 4 benign cases showing PD-1 or PD-L1 expression were negative for each protein in tumor cells. PD-1 and PD-L1 were also expressed in tumor-infiltrating lymphocytes/macrophages in 172 (78.2%) and 33 (15.0%) cases, respectively. Tables 1–3 summarize the positivity of PD-1/PD-L1 in tumor cells, lymphocytes/macrophages, and either or both of them, respectively, and its correlations with clinicopathological characteristics of the tumors. PD-L1 expression in tumor cells was more often seen in high pT stage (pT2/2+: 10.8% vs pT3/4: 20.4%; $P = .072$; pT2/3a: 11.4% vs pT3b/4: 31.6%; $P = .013$) or lymph node-positive (pN0: 10.1% vs pN1: 27.3%; $P = .086$) cases, whereas PD-1 expression in tumor cells was not significantly associated with pT/pN stage. There were no significant associations between PD-1/PD-L1 expression in tumor cells and patient age, preoperative PSA level, or Gleason score. In addition, there were no significant associations of PD-1 or PD-L1 expression in tumor-infiltrating lymphocytes/macrophages or either tumor cells or tumor-infiltrating lymphocytes/macrophages or both with clinicopathological parameters examined. There was an inverse correlation between the positivity of PD-1 in tumor cells versus lymphocytes/macrophages ($P < .001$; Table 4), but not between that of PD-L1 in tumor cells versus lymphocytes/macrophages ($P = .190$; Table 5).

Kaplan-Meier analysis coupled with log-rank test was performed to assess the prognostic value of each stating (Fig. 2). Of the 220 patients, 39 (17.7%) had clinical or biochemical recurrence after radical prostatectomy. However, PD-1/PD-L1 expression in tumor cells ($P = .619/P = .315$), tumor-infiltrating lymphocytes/macrophages ($P = .954/P = .155$), or either or both of them ($P = .964/P = .767$) showed no strong association with disease recurrence.

4. Discussion

Although the blockade of immune checkpoints via their inhibitors has become a promising immunotherapy for a variety of malignancies, the efficacy of anti-PD-1/PD-L1 therapy in patients with prostate cancer remains controversial. Meanwhile, the status of PD-1/PD-L1 expression has been assessed in prostate cancer specimens, primarily using immunohistochemistry.[6–12,15] An earlier study detected PD-1/PD-L1 in lymphocytes around lesions of prostate cancer (eg, 15 [88.2%]/14 [82.4%] cases showing ≥ 50 positive cells, respectively), but not in prostate cancer cells in any of these 17 cases, whereas none showed ≥ 50 PD-1-positive or PD-L1-positive lymphocytes in healthy prostate (n = 8) or benign prostatic hyperplasia (n = 4) tissues.[15] In 2 subsequent studies using prostate biopsy or radical prostatectomy specimens, PD-1 in cancer cells and tumor-infiltrating lymphocytes was shown to be positive in 8.0%[7] and 39.4%[9] of the samples, respectively. Other recent studies have demonstrated PD-L1 expression in prostate cancer cells in 3.7% to 92.3% of cases, and also in tumor-infiltrating lymphocytes in 9.9% to 14.6% of cancer cases.[6–11] There was a significant association of
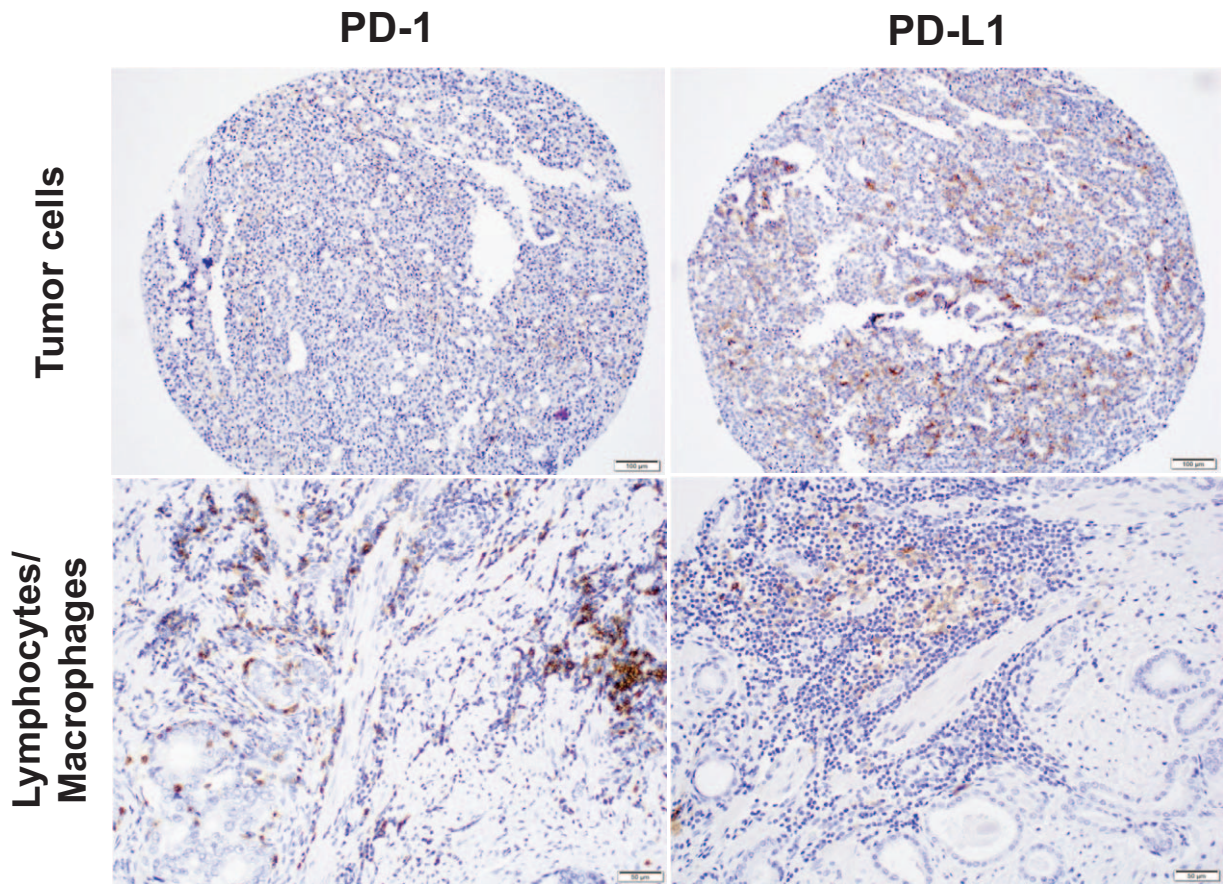
Figure 1. Immunohistochemistry of PD-1 and PD-L1 in prostate cancer tissues. Representative images (original magnification ×200) show PD-1/PD-L1 expression in tumor cells or tumor-infiltrating lymphocytes/macrophages. PD-1 = programmed cell death protein 1, PD-L1 = programmed death-ligand 1.

PD-L1 expression in prostate cancer cells with higher preoperative PSA levels identified in 2 of 4 studies.[6,8] Similarly, 2 studies showed an association between PD-L1 expression in prostate cancer cells and significantly higher tumor grade (eg, Gleason score, grade group),[6,8] whereas others failed to show such associations.[7,9,11] Interestingly, even in a single study,[6] the significant difference in the PSA or tumor grade was seen only in the test cohort (n = 611) or the training cohort (n = 209), but not

Table 1

PD-1/PD-L1 expression in tumor cells.

	PD-1			PD-L1		
	Negative	**Positive**	**P**	**Negative**	**Positive**	**P**
All cases (n = 220)	203 (92%)	17 (8%)		191 (87%)	29 (13%)	
Age (mean ± SD, y)	60.2 ± 7.0	59.8 ± 6.9	.826	60.4 ± 6.8	58.8 ± 7.7	.308
PSA (mean ± SD, ng/mL)	6.6 ± 5.4	6.1 ± 3.1	.559	6.4 ± 4.5	7.3 ± 9.0	.619
Gleason score 6 (n = 86)	80 (93%)	6 (7%)	.738*	72 (84%)	14 (16%)	.277*
Gleason score 7 (n = 110)	102 (93%)	8 (7%)	.354†	98 (89%)	12 (11%)	.917†
Gleason score ≥8 (n = 24)	21 (87.5%)	3 (12.5%)		21 (87.5%)	3 (12.5%)	
pT2 (n = 166)	153 (92%)	13 (8%)	.919‡	148 (89%)	18 (11%)	.072‡
pT3a (n = 35)	32 (91%)	3 (9%)	.670§	30 (86%)	5 (14%)	.013§
pT3b/4 (n = 19)	18 (95%)	1 (5%)		13 (68%)	6 (32%)	
pN0 (n = 138)	129 (93%)	9 (7%)	.743‖	124 (90%)	14 (10%)	.086‖
pN1 (n = 11)	10 (91%)	1 (9%)		8 (73%)	3 (27%)	
pNX (n = 71)	64 (90%)	7 (10%)		59 (83%)	12 (17%)	

PD-1 = programmed cell death protein 1, PD-L1 = programmed death-ligand 1.
* Gleason scores 6 versus ≥7.
† Gleason scores ≤7 versus ≥8.
‡ pT2 versus pT3/4.
§ pT2/3a versus pT3b/4.
‖ pN0 versus pN1.

Table 2

PD-1/PD-L1 expression in tumor associated lymphocytes/macrophages.

	PD-1			PD-L1		
	Negative	Positive	P	Negative	Positive	P
All cases (n=220)	48 (22%)	172 (78%)		187 (85%)	33 (15%)	
Age (mean ± SD, years)	61.0±6.6	60.0±7.1	.327	60.0±7.0	61.3±6.7	.306
PSA (mean ± SD, ng/mL)	6.6±4.0	6.5±5.6	.960	6.6±5.5	6.0±3.6	.453
Gleason score 6 (n=86)	21 (24%)	65 (76%)	.454[*]	72 (84%)	14 (16%)	.728[*]
Gleason score 7 (n=110)	23 (21%)	87 (79%)	.517[†]	98 (89%)	12 (11%)	.146[†]
Gleason score ≥8 (n=24)	4 (17%)	20 (83%)		21 (87.5%)	3 (12.5%)	
pT2 (n=166)	35 (21%)	131 (79%)	.644[‡]	139 (84%)	27 (16%)	.357[‡]
pT3a (n=35)	10 (29%)	25 (71%)	.506[§]	30 (86%)	5 (14%)	.214[§]
pT3b/4 (n=19)	3 (16%)	16 (84%)		18 (95%)	1 (5%)	
pN0 (n=138)	30 (22%)	108 (78%)	.782[‖]	116 (84%)	22 (16%)	.846[‖]
pN1 (n=11)	2 (18%)	9 (82%)		9 (82%)	2 (18%)	
pNX (n=71)	16 (23%)	55 (77%)		62 (87%)	9 (13%)	

PD-1 = programmed cell death protein 1, PD-L1 = programmed death-ligand 1.

[*] Gleason scores 6 versus ≥7.

[†] Gleason scores ≤7 versus ≥8.

[‡] pT2 versus pT3/4.

[§] pT2/3a versus pT3b/4.

[‖] pN0 versus pN1.

Table 3

PD-1/PD-L1 expression in tumor cells and/or lymphocytes/macrophages.

	PD-1			PD-L1		
	Negative	Positive	P	Negative	Positive	P
All cases (n=220)	35 (16%)	185 (84%)		159 (72%)	61 (28%)	
Age (mean ± SD, y)	61.6±6.3	59.9±7.1	.162	60.1±7.0	60.5±7.0	.716
PSA (mean ± SD, ng/mL)	6.5±4.2	6.5±5.5	.993	6.4±4.7	6.8±6.6	.734
Gleason score 6 (n=86)	15 (17%)	71 (83%)	.619[*]	60 (70%)	26 (30%)	.506[*]
Gleason score 7 (n=110)	18 (16%)	92 (84%)	.282[†]	84 (76%)	26 (24%)	.257[†]
Gleason score ≥8 (n=24)	2 (8%)	22 (92%)		15 (62.5%)	9 (37.5%)	
pT2 (n=166)	26 (16%)	140 (84%)	.861[‡]	122 (73%)	44 (27%)	.478[‡]
pT3a (n=35)	6 (17%)	29 (83%)	.988[§]	25 (71%)	10 (29%)	.353[§]
pT3b/4 (n=19)	3 (16%)	16 (84%)		12 (63%)	7 (27%)	
pN0 (n=138)	23 (17%)	115 (83%)	.897[‖]	102 (74%)	36 (26%)	.166[‖]
pN1 (n=11)	2 (18%)	9 (82%)		6 (55%)	5 (45%)	
pNX (n=71)	10 (14%)	61 (86%)		51 (72%)	20 (28%)	

PD-1 = programmed cell death protein 1, PD-L1 = programmed death-ligand 1.

[*] Gleason scores 6 versus ≥7.

[†] Gleason scores ≤7 versus ≥8.

[‡] pT2 versus pT3/4.

[§] pT2/3a versus pT3b/4.

[‖] pN0 versus pN1.

the other, respectively. No significant associations of PD-L1 expression in prostate cancer cells with tumor stage (eg, pT, pN) were also noted in all studies,[6,9,11] but 1,[8] showing that with higher pT stage (P=.011). The impact of PD-L1 expression in prostate cancer cells on the prognosis has additionally been investigated in a few studies. For instance, PD-L1 positivity was associated with a significantly higher risk of biochemical recurrence after radical prostatectomy (hazard ratio 1.49–2.37, P=.004–.011)[6] or metastasis in patients with lymph node-positive disease (P=.00774).[12] Another study showed a tendency to correlate between PD-1 expression in tumor-infiltrating lymphocytes (hazard ratio 1.96, P=.084) or PD-L1

Table 4

PD-1 in tumor cells versus lymphocytes/macrophages.

	PD-1 in lymphocytes/macrophages		
	Negative	Positive	P
PD-1-negative in tumor cells	35 (17%)	168 (83%)	<.001
PD-1-positive in tumor cells	13 (76%)	4 (24%)	

PD-1 = programmed cell death protein 1, PD-L1 = programmed death-ligand 1.

Table 5

PD-L1 in tumor cells versus lymphocytes/macrophages.

	PD-1 in lymphocytes/macrophages		
	Negative	Positive	P
PD-1-negative in tumor cells	160 (84%)	31 (16%)	.190
PD-1-positive in tumor cells	27 (93%)	2 (7%)	

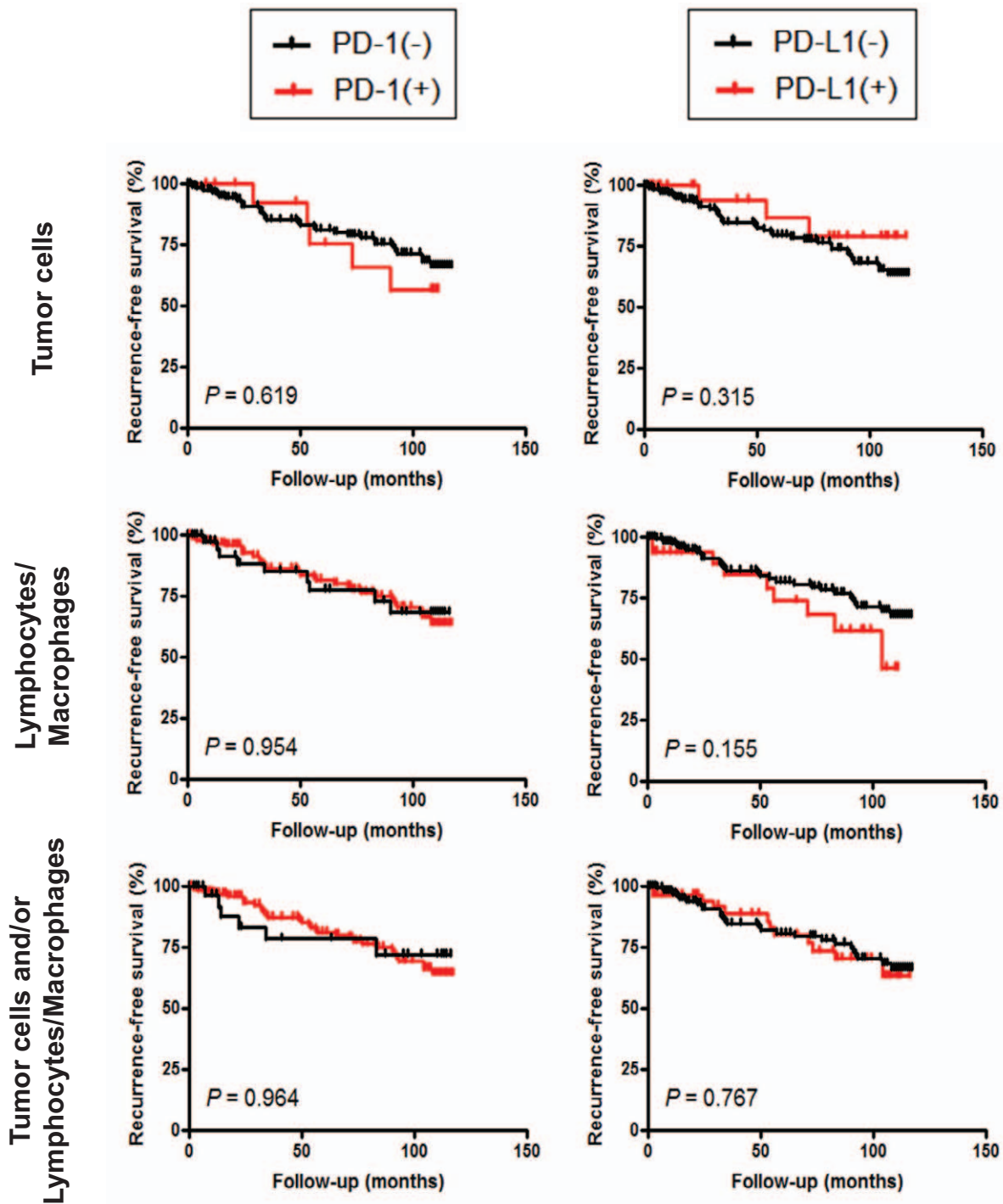PD-1 = programmed cell death protein 1, PD-L1 = programmed death-ligand 1.

Figure 2. Kaplan-Meier curves for recurrence-free survival according to the expression of PD-1 or PD-L1 in tumor cells and/or tumor-infiltrating lymphocytes/macropahges. PD-1 = programmed cell death protein 1, PD-L1 = programmed death-ligand 1.

expression in tumor cells (hazard ratio 1.34, $P = .078$) and the risk of biochemical recurrence.[9] Thus, previous immunohistochemical studies, using prostate cancer specimens, have yielded variable results in the positivity of PD-1/PD-L1 expression, with little data in benign prostatic tissues. Moreover, only limited number of studies has assessed clinicopathological and/or prognostic significance of PD-1/PD-L1 expression in prostate cancer.

In the present study, we immunohistochemically assessed the status of PD-1/PD-L1 expression in a set of TMA consisting of 220 cases of radical prostatectomy specimens. We found that, for the first time using a large number of cases (n = 201), PD-1 (1.5%), and PD-L1 (0.5%) were expressed in the corresponding benign portion of the specimens in only a small subset of cases, and that, compared with these, the positive rates of PD-L1 (7.7%) and PD-L1 (13.2%) in prostate cancer cells were significantly

increased. We also demonstrated that PD-1 and PD-L1 signals in tumor-infiltrating lymphocytes/macrophages were detected in 78.2% and 15.0% cases, respectively. Thus, the positive rates of PD-1 expression in tumor cells (7.7% vs 8.0%[7]) or tumor-infiltrating lymphocytes (78.2% vs 39.4%–88.2%[9,15]) and PD-L1 expression in tumor cells (13.2% vs 3.7%–92.3%[6–11]) or tumor-infiltrating lymphocytes (15.0% vs 9.9%–82.4%[10,15]) were similar to that or within the ranges previously reported. Of note, PD-1 expression in tumor cells versus lymphocytes/macrophages was found to be inversely correlated. We further identified the elevated expression of PD-L1 in tumor cells in ≥pT3 disease ($P = .072$), especially pT3b/4 ($P = .013$), which was in accordance with the findings in some of the previous studies,[6,8] and also in lymph node-positive disease ($P = .086$). However, there was no significant difference or tendency in PD-1 expression in tumor cells between different pT/pN stages, and also in PD-1/PD-L1 expression in tumor cells between different tumor grades. In addition, PD-1/PD-L1 expression in tumor-infiltrating lymphocytes/macrophages was not significantly associated with tumor grade or stage, or other parameters examined. Furthermore, consistent[7,9] or inconsistent[6,8] with previous observations, no statistically significant associations between PD-1/PD-L1 expression in tumor cells or lymphocytes/macrophages versus preoperative PSA level were seen. Meanwhile, our survival analysis revealed no significant associations between PD-1/PD-L1 expression in tumor cells and/or tumor-infiltrating lymphocytes versus disease recurrence after radical prostatectomy, although a few studies suggested the role of PD-L1 expression as a prognosticator in patients with prostate cancer.[6,9,12]

There are several limitations in our investigation, including its retrospective design which is subject to potential selection bias, although consecutive prostatectomy cases were included in our prostate TMA. A potentially more problematic issue is the stains in the TMA consisting of 1-mm tissue cores that may not be representative of the entire lesion of interest in each case. This may have thus produced false-negative results.

5. Conclusions

PD-1 and PD-L1 expression in tumor cells was detected in a subset of hormone-naïve prostate cancers in the current study. In particular, PD-L1 expression was considerably up-regulated in nonorgan-confined tumors. However, immunohistochemical detection of PD-1/PD-L1 is found to be not very useful for predicting tumor recurrence in prostate cancer patients who underwent radical prostatectomy. Further validation studies with larger cohorts are thus warranted. In addition, the precise functional role of the PD-1/PD-L1 signaling pathway in the development and progression of prostate cancer needs to be further investigated.

Author contributions

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