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Original Article

PD-L1 instead of PD-1 status is associated with the clinical features in human primary prostate tumors

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Abstract: Immunotherapy targeting programmed cell death protein 1 (PD-1)/programmed cell death-ligand 1 (PD-L1) has shown efficacy in a variety of solid tumors. However, prostate cancer has often been a non-responder to anti-PD-1/PD-L1 therapies. The objective of this study was to determine PD-1 and PD-L1 expression status and its correlation with clinical features of the patients. A total of 279 patients who underwent radical prostatectomy for prostate cancer were included in this study. PD-1 and PD-L1 expression in primary prostate tumors was detected using immunohistochemical staining. Analyses were made between PD-1/PD-L1 status and patients' age, ethnicity, body mass index (BMI), diabetes mellitus, tumor stage, lymph node metastasis, prostate-specific antigen (PSA), Gleason score, grade group, and survival. We found that 6.5 (standard deviation 14.3; range 0-161.6) tumor-infiltrating lymphocytes per high power field were positive for PD-1 staining and 50/279 (17.9%) tumors were positive for PD-L1 staining. PD-L1-positive tumors had significantly more PD-1-positive lymphocytes than PD-L1-negative tumors. The number of PD-1-positive lymphocytes was not correlated with any clinical features except that patients with diabetes had significantly less PD-1-positive lymphocytes than patients without diabetes. In contrast, more PD-L1-positive tumors were found in older patients (≥ 65 years), obese patients (BMI ≥ 30), and patients with advanced tumor stage, lymph node metastasis, and high Gleason score. Neither PD-1 nor PD-L1 status was correlated with ethnicity, PSA, or survival. Our findings suggest that PD-L1 instead of PD-1 status is associated with the clinical features in human primary prostate tumors.

Keywords: PD-1, PD-L1, prostate tumor, immunohistochemical staining, correlation

Introduction

The American Cancer Society estimates that about 174,650 new cases of prostate cancer will be diagnosed and 31,620 deaths will be caused by prostate cancer in the United States in 2019, which shows that prostate cancer is the most common cancer and the second leading cause of cancer-related deaths in American men [1]. The lethality of prostate cancer is mainly due to locally advanced and particularly metastatic castration resistant diseases where no cure is available [2, 3]. The United States Food and Drug Administration has approved six therapies for patients with metastatic castration resistant prostate cancer, namely, docetaxel, cabazitaxel, sipuleucel-T, enzalutamide, abiraterone, and radium-223 [4]. However, these treatments only extend patients' overall survival by 2 to 4.8 months [5]. It has become obvious

that new therapeutic approaches are needed in dealing with prostate cancer. Recently, immune checkpoint proteins such as cytotoxic T lymphocyte-associated protein 4 (CTLA-4) and programmed cell death 1 (PD-1) have become the targets in cancer immunotherapy. Anti-PD-1 and anti-programmed cell death-ligand 1 (PD-L1) therapies have been approved for the treatment of Hodgkin's disease, desmoplastic melanoma, Merkel cell carcinoma, skin melanoma, non-small cell lung cancer, small cell lung cancer, head and neck cancer, gastroesophageal cancer, bladder and urinary tract cancer, renal cell carcinoma, hepatocellular carcinoma, and any solid cancer with high-level of microsatellite instability [6]. The objective response rate varies from 15% to 87% [6]. Nevertheless, prostate cancer has often been found as a non-responder to anti-PD-1 monotherapies [7].

Previous studies have shown that expression of PD-L1 in tumor tissues is associated with the response to anti-PD-1 therapies [8, 9]. It has been speculated that the likely reason for failure of anti-PD-1 monotherapies in prostate cancer is due to paucity of PD-L1 expression in prostate tumor microenvironment, as only 3 samples of 20 primary prostate tumors showed positive PD-L1 staining [10]. In another cohort of 44 patients with intermediate to high risk prostate cancers who received neoadjuvant abiraterone acetate plus leuprolide plus prednisone treatment prior to radical prostatectomy, 3/44 (7%) of primary prostate tumors were stained positive for PD-L1 (defined as ≥ 1% tumor cells stained positive). Meanwhile, 9/44 (20%) of otherwise matched control tumors without the neoadjuvant treatment were stained positive for PD-L1, though the difference (7% vs 20%) was not statistically significant (P = 0.062) [11]. In that study, expansion of the cohort without the neoadjuvant treatment to 130 cases revealed 18 cases (14%) that were stained positive for PD-L1. In contrast, a study of 209 cases showed that 52.2% of primary prostate tumors presented moderate to high levels of PD-L1 staining [12]. To the further extreme end, another study showed that 371/402 (92%) primary prostate tumors presented positive PD-L1 staining in tumor epithelial cells and 156/396 (39%) cases had intratumoral PD-1+ lymphocytes [13]. The discrepancy among these studies is hard to resolve because the immunohistochemical staining conditions are different.

We have previously studied PD-L1 expression in human cervical intraepithelial neoplasia [14], endometrial cancer [15], and non-small cell lung cancer [16]. We have demonstrated that 17β-estradiol increased PD-L1 protein expression via activation of phosphoinositide 3-kinase/Akt pathway in estrogen receptor α-positive endometrial and breast cancer cell lines [17]. We have also shown that interleukin-17 and tumor necrosis factor-α up-regulated PD-L1 expression in human prostate and colon cancer cell lines [18]. We have assessed expression of PD-1, PD-L1, and PD-L2 in mouse prostate tumors in phosphatase and tensin homolog (*Pten*)-conditional knockout models. We found that increased expression of PD-1, PD-L1, and PD-L2 was associated with increased number of invasive prostate tumors formed in the interleukin-17 receptor c (*Il-17rc*) wild-type and obese mice compared to the *Il-17rc*-knockout and lean mice [19]. Given the controversial findings of PD-L1 expression reported in the literature [10-13], we decided to assess PD-1 and PD-L1 expression in our cohort of 279 human primary prostate tumors, in order to obtain the first-hand data. We found that PD-L1 staining was positive in 50/279 (17.9%) cases and PD-L1 status, but not PD-1 status, was associated with the clinical features in our cohort of primary prostate tumors.

Materials and methods

Human primary prostate tumor specimens

This study was approved by the Institutional Review Board of the Ochsner Health System (IRB# 2015.122.A, approval date: January 17, 2018). The procedures to obtain the medical records and specimens of all patients were in accordance with the Ethical Principles for Medical Research Involving Human Subject as formulated in the World Medical Association Declaration of Helsinki (revised 2013). The medical records of all prostate cancer patients treated at the Ochsner Health System from January, 2001 to March, 2016 were retrieved through the Electronic Research Study Application system [20]. The inclusion criteria were: 1) patients underwent radical prostatectomy; and 2) with pathological reports containing the term "Gleason"; and 3) at least one paraffin block of primary prostate cancer could be retrieved. The exclusion criteria were: 1) patients

diagnosed as not having primary prostate cancer by the pathologists; or 2) patients had only biopsy reports; or 3) multiple clinical data were missing. Once included, the patient's electronic and scanned medical records were reviewed manually by two investigators (VW and AP). The patient's age was the age at the time of surgery. Ethnicity and diagnosis of diabetes mellitus were retrieved as shown in the medical records. The patient's body weight and height at the time of surgery were retrieved to calculate body mass index (BMI) using the formula: BMI = body weight (kilogram)/body height^2 (meter). The tumor stage was based on the American Joint Committee on Cancer Prostate Cancer Staging (7th edition, 2009) and was retrieved from the pathological reports: stage T1 represents clinically inapparent tumor neither palpable nor visible by imaging; stage T2 represents tumor confined within prostate; stage T3 represents tumor extends through the prostate capsule; and stage T4 represents that tumor is fixed or invades adjacent structures other than seminal vesicles, such as external sphincter, rectum, bladder, levator muscles, and/or pelvic wall. Lymph node metastasis (N stage) was retrieved from the pathological reports. Pre-surgical prostate-specific antigen (PSA) levels and Gleason grades/scores were retrieved. Grade groups were defined in a new grading system based on Gleason grades/scores [21, 22], which were accepted by the World Health Organization (WHO) in 2016. Patient follow-up and vital status were provided by Louisiana Tumor Registry. Patient survival time was calculated as the time from the date of diagnosis to the date of last contact as of January 31, 2018. Death was from any cause.

Immunohistochemical staining

Paraffin blocks were cut into 4 μm-thick tissue sections, which were mounted on glass slides (Superfrost Plus; Fisher Scientific, Pittsburgh, PA, USA). Sections were baked for 150 minutes (min) in a 60°C incubator. After being deparaffinized in xylene and rehydrated through a series of decreasing concentrations of ethanol, the sections were put in 0.01 M ethylenediaminetetraacetic acid in Tris buffer and boiled at 98°C for 5 min for antigen retrieval. Then, the sections were cooled down at room temperature for 20 min, followed by treating with 0.3% H_2O_2 for 10 min to block endogenous peroxidase activity. Non-specific binding was blocked with 1.5% normal goat or horse serum for 60 min (Catalog# PK-6102/PK-6101, Vector Laboratories, Burlingame, CA, USA). The following primary antibodies were used: mouse monoclonal antibody against PD-1 (Catalog# ab52587, used at 1:150 dilution, Abcam, Cambridge, MA, USA) and rabbit monoclonal antibody against PD-L1 (Catalog# 13684, used at 1:240 dilution, Cell Signaling Technology, Danvers, MA, USA). The sections were incubated with primary antibodies overnight at 4°C. Human tonsillectomy specimens were used as positive controls and non-immune serum replacing primary antibodies was used as negative controls to determine the dilutions of anti-PD-1 and anti-PD-L1 antibodies. A dilution of 1:150 of anti-PD-1 antibody resulted in a strong membranous staining without any obvious background staining, so did a dilution of 1:240 of anti-PD-L1 antibody. After incubation with the primary antibodies, the sections were washed three times in phosphate-buffered saline and then incubated with biotinylated horse anti-mouse IgG for PD-1 staining and goat anti-rabbit IgG for PD-L1 staining for 75 min, followed by avidin peroxidase using the Vectastain ABC elite kit (Catalog# PK-6102/PK-6101, Vector Laboratories, Burlingame, CA, USA). The chromogenic reaction was carried out with 3'-diaminobenzidine substrate kit (Catalog# SK-4100, Vector Laboratories, Burlingame, CA, USA) following the manufacturer's protocol. Then, the sections were counterstained with a hematoxylin solution. Finally, the sections were dehydrated through a series of increasing concentrations of ethanol, cleared with xylene, and covered with cover glasses. The stained sections were evaluated under a light microscope by an investigator who was blinded to the clinicopathological data of the specimens. For PD-1 staining, areas of the tumors with lymphocyte infiltration were identified under low-power (×40 magnification) in each slide. Typically, there were a few lymphocytes scattered around the tumor areas in most specimens, while some specimens had a large number of lymphocytes clustered around the tumor areas. Five representative high-power fields (×400 magnification) per tissue section were randomly selected in the tumor areas and evaluated by counting the number of positively stained lymphocytes in each high-power field.

Then, the average number of PD-1 positive lymphocytes was calculated from the five high-power fields to represent each specimen. For PD-L1 staining, tumor cells with positive staining at the cell membrane and/or cytoplasm were counted under five random high-power fields (×400 magnification), so were the tumor cells with negative staining. The percentage of PD-L1 positive tumor cells was calculated. A grading plan was designed as the following: negative staining, < 1% positive; 1+, 1-5% positive; 2+, 5-25% positive; 3+, 25-50% positive; and 4+, 50-100% positive. After blinded evaluation, only 6/279 specimens presented 2+ or above. Therefore, in order to simplify statistical analysis, any specimen with ≥ 1% positive tumor cells was considered as PD-L1 positive and any specimen with < 1% positive tumor cells was considered as PD-L1 negative.

Statistical analysis

All statistical analyses were performed using GraphPad Prism, version 7.04 (GraphPad Software, San Diego, CA). Both Student's t test and Mann-Whitney U test were used to compare PD-1 expression levels between two groups, while one-way analysis of variance (ANOVA) was used to compare PD-1 expression levels among three or more groups. Chi-square test was used to compare PD-L1 expression levels between the groups. $P < 0.05$ was considered statistically significant.

Results

Clinical features of the patients

A total of 279 patients who had undergone radical prostatectomy at Ochsner Health System between 2001 and 2016 were included in this study. The average age was 61.1 years old (range 39-76 years). The mean follow-up time was 106.5 months (range 3-180 months) as of January 31, 2018. The clinical features of the cohort is listed in **Table 1**, including age, ethnicity, BMI, diabetes, tumor stage, N stage, PSA, Gleason score, grade group, and survival status.

PD-1 and PD-L1 expression in human primary prostate tumors

Like the previous study [13], we used human tonsil tissues as positive controls (**Figure 1A** and **1B**) and non-immune serum replacing primary antibodies as negative controls (**Figure 1C** and **1D**) for PD-1 and PD-L1 staining. Typically, PD-1 positive staining was found in the tumor infiltrating lymphocytes (**Figure 1E**) based on the morphologic features of the cells. PD-1 staining was rarely found in the tumor cells or stromal fibroblasts. PD-L1 positive staining was typically found in the tumor cells on the cell membrane and/or in the cytoplasm (**Figure 1F**), but rarely in the infiltrating lymphocytes or stromal fibroblasts. Majority of the specimens were negative for PD-1 and/or PD-L1 staining (**Figure 1G** and **1H**). In a series of consecutively cut tumor sections, both PD-1 and PD-L1 were stained negative (**Figure 2A** and **2B**) in many specimens. The specimens might be stained positive for PD-1 (**Figure 2C**), but negative for PD-L1 (**Figure 2D**). In contrast, the specimens might be stained negative for PD-1 (**Figure 2E**), but positive for PD-L1 (**Figure 2F**). Some specimens were stained positive for both PD-1 (**Figure 2G**) and PD-L1 (**Figure 2H**). In the present cohort of 279 primary prostate tumors, 6.5 (standard deviation 14.3; range 0-161.6) tumor-infiltrating lymphocytes per high power field were positive for PD-1 and 50/279 (17.9%) tumors were positive for PD-L1 staining (**Table 1**). PD-L1-positive tumors had significantly more PD-1-positive cells than PD-L1-negative tumors (**Table 1**, $P = 0.0325$).

Correlation between PD-1 status and clinical features

As shown in **Table 1**, there was no statistically significant difference between patients aged < 65 years and those aged ≥ 65 years, in regard to the mean numbers of PD-1 positive cells. Similarly, there was no statistically significant difference between the White and African Americans, among patients with different BMI or tumor stages, between patients with or without lymph node metastasis, among patients with different PSA levels or Gleason scores or grade groups, or between patients who were alive or dead. The only statistically significant difference was found between patients without diabetes and with diabetes, in which patients with diabetes had significantly less PD-1 positive cells than patients without diabetes ($P = 0.0398$ using Student's t test and $P = 0.0204$ using U test).

Table 1. Clinical features of patients and their associations with PD-1/PD-L1 status

	Total cases (N = 279)	Mean number of PD-1 Positive cells[#] Overall 6.5 ± 14.3 (range 0-161.6)	*P*-value	PD-L1 Status		*P*-value
				PD-L1 (-) (N = 229)	PD-L1 (+) (N = 50)	
Age (%)						
< 65	186 (66.7%)	5.7	0.2572[a]	159 (85.5%)	27 (14.5%)	0.0360[b]
≥ 65	93 (33.3%)	8.2	0.632[c]	70 (75.3%)	23 (24.7%)	
Ethnicity (%)						
White	188 (67.4%)	6.3	0.645[a]	157 (83.5%)	31 (16.5%)	0.4537[b]
African American	84 (30.1%)	7.1	0.114[c]	67 (79.8%)	17 (20.2%)	
Other	5 (1.8%)	5.8		3 (60.0%)	2 (40%)	
Unknown	2 (0.7%)	8.9		2 (100.0%)	0 (0.0%)	
BMI (%)						
< 25	54 (19.4%)	5.9	0.4620[d]	43 (79.6%)	11 (20.4%)	0.0026[b]
25-29.99	137 (49.1%)	7.7		123 (89.8%)	14 (10.2%)	
≥ 30	81 (29.0%)	5.3		58 (71.6%)	23 (28.4%)	
Unknown	7 (2.5%)	2.7		5 (71.4%)	2 (28.6%)	
Diabetes (%)						
Non-diabetes	204 (73.1%)	7.3	0.0398[a]	170 (83.3%)	34 (16.7%)	0.3676[b]
Diabetes	75 (26.9%)	4.4	0.0204[c]	59 (78.7%)	16 (21.3%)	
Tumor stage (%)						
T1 (%)	16 (5.7%)	5.2	0.9504[d]	14 (87.5%)	2 (12.5%)	0.0196[b]
T2 (%)	152 (54.5%)	6.6		133 (87.5)	19 (12.5%)	
T3 (%)	88 (31.5%)	6.3		67 (76.1%)	21 (23.9%)	
T4 (%)	23 (8.2%)	7.8		15 (65.2%)	8 (34.8%)	
T1+T2 (%)	168 (60.2%)	6.4	0.9362[a]	147 (87.5%)	21 (12.5%)	0.0037[b]
T3+T4 (%)	111 (39.8%)	6.6		82 (73.9%)	29 (26.1%)	
N Stage (%)						
N0 (%)	255 (91.4%)	6.7	0.6169[a]	213 (83.5%)	42 (16.5%)	0.0294[b]
N1 (%)	21 (7.5%)	8.2		13 (61.9%)	8 (38.1%)	
Unknown (%)	3 (1.0%)	1.6				
PSA (%)						
0-4 (%)	51 (18.3%)	8.3	0.5357[d]	44 (86.3%)	7 (13.7%)	0.2750[b]
4-10 (%)	169 (60.6%)	5.9		133 (79.2%)	35 (20.8%)	
> 10 (%)	54 (19.4%)	7.3		48 (87.3%)	7 (12.7%)	
Unknown (%)	5 (1.8%)	1.2		4 (80.0%)	1 (20.0%)	
Gleason score (%)						
≤ 6 (%)	109 (39.1%)	7.3	0.6851[d]	102 (93.5%)	7 (6.5%)	0.0001[b]
= 7 (%)	97 (34.8%)	6.4		76 (78.4%)	21 (21.6%)	
≥ 8 (%)	73 (26.2%)	5.4		51 (69.9%)	22 (30.1%)	
Grade group (WHO 2016) (%)						
1 (≤ 6) (%)	109 (39.1%)	7.3	0.8195[d]	102 (93.5%)	7 (6.5%)	0.0010[b]
2 (3+4) (%)	60 (21.5%)	5.4		48 (80.0%)	12 (20.0%)	
3 (4+3) (%)	37 (13.3%)	7.9		28 (75.7%)	9 (24.3%)	
4 (8) (%)	51 (18.3%)	5.3		36 (70.6%)	15 (29.4%)	
5 (≥ 9) (%)	22 (7.9%)	5.7		15 (68.2%)	7 (31.8%)	
Survival (%)						
Alive (%)	255 (91.4%)	6.7	0.0569[a]	210 (82.4%)	45 (17.6%)	0.9118[b]
Dead (%)	24 (8.6%)	4.3	0.9376[c]	19 (79.2%)	5 (20.8%)	
PD-L1 Status						
PD-L1 (+) (%)	50 (17.9%)	13.1	0.0325[a]			
PD-L1 (-) (%)	229 (82.1%)	5.1				

[#]Per high power (400×) field; [a]Student's t test; [b]χ^2 test; [c]U test; [d]one-way analysis of variance (ANOVA). BMI, body mass index; N stage, lymph node stage; PD-1, programmed cell death protein 1; PD-L1, PD-1 ligand 1; PSA, prostate-specific antigen; WHO, World Health Organization.

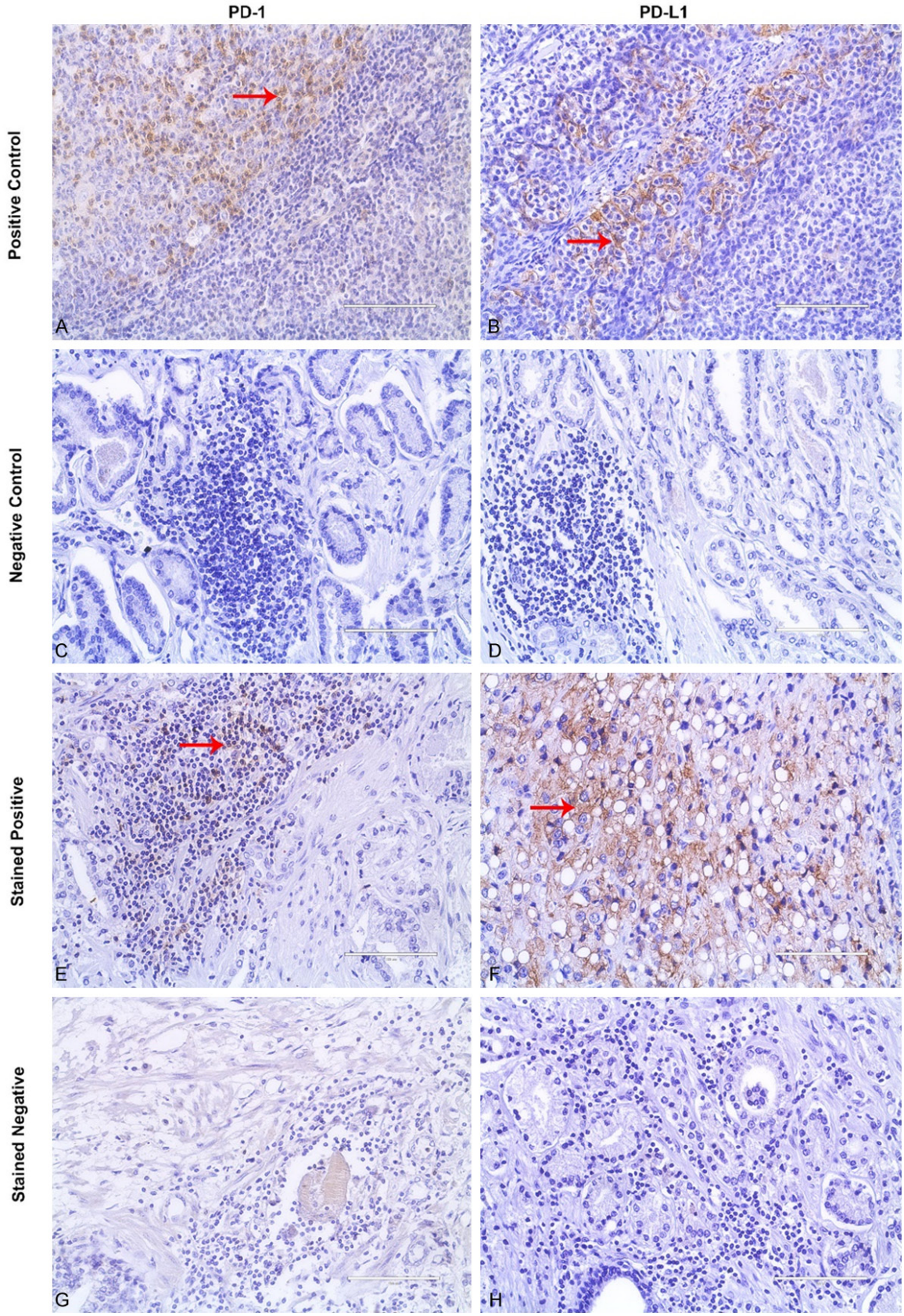

Figure 1. Representative photomicrographs of immunohistochemical staining of PD-1 and PD-L1. A, B. Human tonsil tissues used as positive controls; C, D. Non-immune serum replacing primary antibodies used as negative controls;

E, F. Representatives of positive staining; G, H. Representatives of negative staining. Arrows indicate the positively stained cells; magnification, ×400; scale bar, 100 μm. PD-1, programmed cell death protein 1; PD-L1, programmed cell death-ligand 1.

Correlation between PD-L1 status and clinical features

As shown in **Table 1**, patients aged 65 years or above had significantly more PD-L1-positive prostate tumors than patients younger than 65 years (P = 0.0360). Obese patients (BMI ≥ 30) had significantly more PD-L1-positive prostate tumors than overweight patients (with BMI = 25-29.99) (P = 0.0026 among obese, overweight, and BMI < 25 groups; not shown in **Table 1**, P = 0.0006 between obese and overweight groups). Patients with more advanced tumor stage had significantly more PD-L1-postive prostate tumors (P = 0.0196 among stage T1 to T4; P = 0.0037 between stage T1/2 and T3/4). Patients with lymph node metastasis had significantly more PD-L1-postive prostate tumors than patients without lymph node metastasis (P = 0.0294). Patients with more advanced Gleason scores had significantly more PD-L1-postive prostate tumors (P = 0.0001), which was still true when the patients were divided into 5 grade groups (P = 0.0010) (**Table 1**). As shown in **Figure 3**, Gleason score 6 tumors presented scattered positive PD-L1 staining in some tumor epithelial cells, and Gleason score 7 tumors presented weak positive PD-L1 staining, while Gleason score 9 tumors presented strong positive PD-L1 staining. However, no statistically significant differences were found among patients of different ethnicity, diabetes status, PSA levels, or survival status (P > 0.05, **Table 1**).

Discussion

Prostate cancer has been shown to have little responses to immune checkpoint inhibitors such as anti-CTLA-4 antibodies [23] or anti-PD-1 antibodies [7]. In order to reveal the underlying mechanisms of resistance, many investigators have examined expression of PD-1 and PD-L1 in primary prostate tumors [10-13]. However, these studies found huge variations in terms of PD-L1 expression. PD-L1-positive rates varies from 15% [10], 7% and 14% [11], to 52.2% [12], and even 92% [13]. The present study found that 50/279 (17.9%) primary prostate tumors were positive for PD-L1 staining and on the average 6.5 tumor-infiltrating lymphocytes per high power field were positive for PD-1 expression. Our PD-L1-positive rate is consistent with two previous studies [10, 11], but much lower than other two studies [12, 13]. It is worth noting that we used the same anti-PD-1 and anti-PD-L1 antibodies used in the previous study that showed very high PD-1 and PD-L1-positive rates [13], however, we used different secondary antibodies and staining conditions. It is still puzzling that prostate cancer does not respond well to anti-PD-1 monotherapies, given that 17.9% tumors express PD-L1 in our cohort and 52.2% to 92% tumors express PD-L1 in other cohorts [12, 13]. One possibility may be that PD-L1 level is not high enough to reach the response threshold, as only 6/279 (2.2%) specimens showed 5-25% of the tumor cells that were positive for PD-L1, while the majority of PD-L1-positive tumors had 1-5% of the tumor cells stained positive for PD-L1. Another possibility may be that PD-L1 status is not able to predict anti-PD-1 responses in prostate cancer. If that is the case, other markers such as microsatellite instability-high (MSI-H) or mismatch repair-deficient (dMMR) should be checked as anti-PD-1 antibody pembrolizumab has been approved by the US Food and Drug Administration for the treatment of MSI-H/dMMR solid tumors. Recently, it has been reported that among 11 patients with MSI-H/dMMR castration-resistant prostate cancer who received anti-PD-1/PD-L1 therapy, 6 (54.5%) had a greater than 50% decline in PSA levels [24], which is promising.

We found that the number of PD-1-positive lymphocytes was not correlated with age, ethnicity, BMI, tumor stage, lymph node metastasis, Gleason score or grade group, or survival status, except that patients with diabetes had significantly less PD-1-positive lymphocytes than patients without diabetes. This finding is consistent with the previous study where largely negative correlations were reported [13]. How PD-1 status is correlated with diabetes is not clear. It may reflect an overall low PD-1 expression in patients with diabetes who tend to have autoimmunity. It has been reported that anti-PD-1 therapy may cause immune-related side

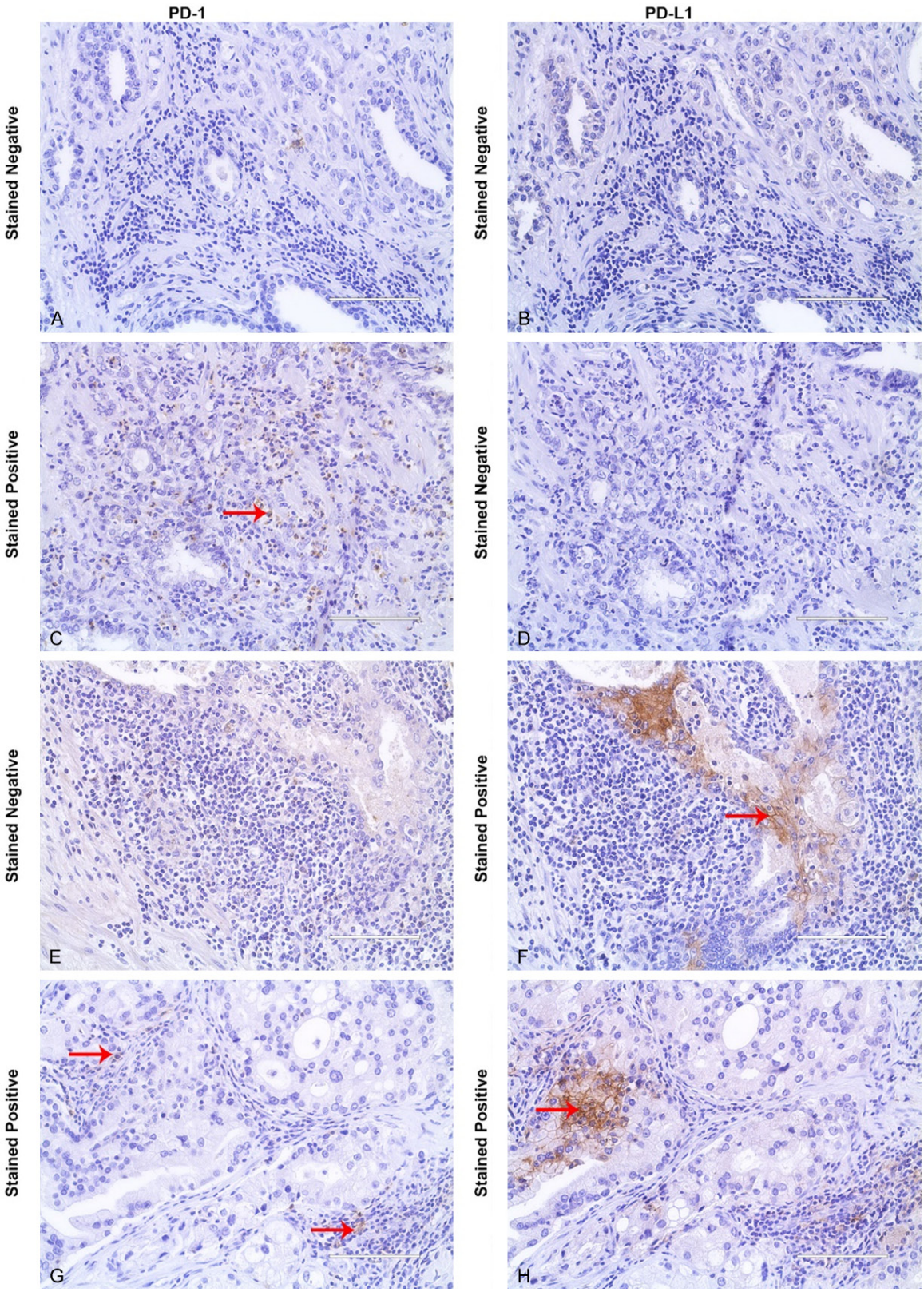

Figure 2. Representative photomicrographs of immunohistochemical staining on consecutively cut prostate tumor tissue sections to demonstrate variable staining patterns of PD-1 and PD-L1 staining. A, B. Negative staining for both PD-1 and PD-L1; C, D. Positive PD-1 staining and negative PD-L1 staining; E, F. Negative PD-1 staining and positive PD-L1 staining; G, H. Positive staining for both PD-1 and PD-L1. Arrows indicate the positively stained cells; magnification, ×400; scale bar, 100 μm. PD-1, programmed cell death protein 1; PD-L1, programmed cell death-ligand 1.

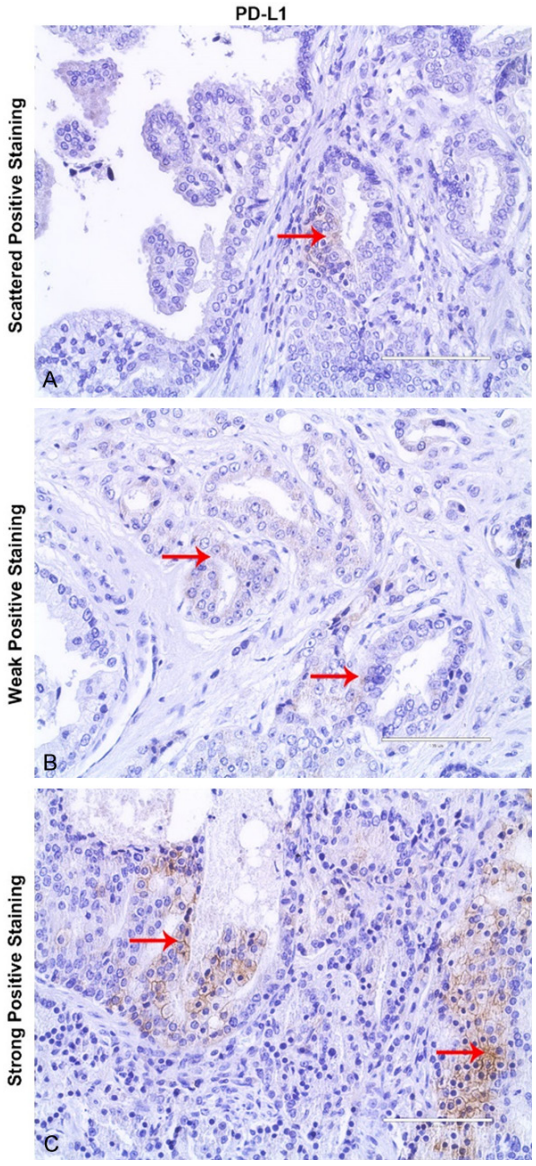

Figure 3. Representative photomicrographs of immunohistochemical staining of PD-L1 on prostate tumors with different Gleason scores. A. Scattered positive staining for PD-L1 in prostate tumors with a Gleason score of 6; B. Weak positive staining for PD-L1 in prostate tumors with a Gleason score of 7; C. Strong positive staining for PD-L1 in prostate tumors with a Gleason score of 9. Arrows indicate the positively stained cells; magnification, ×400; scale bar, 100 μm. PD-L1, programmed cell death-ligand 1.

effects such as autoimmune type 1 diabetes [25]. In contrast, we found that PD-L1 status was correlated with age, BMI, tumor stage, lymph node metastasis, Gleason score, and grade group. Older patients (≥ 65 years), obese patients, and patients with advanced tumor stage and lymph node metastasis as well as high Gleason score had significantly more PD-L1-positive prostate tumors. Our findings suggest that PD-L1 instead of PD-1 status is associated with the clinical features in human primary prostate tumors. These findings are unlike the previous study that showed negative correlations [13]. However, although PD-L1 status is correlated with the high risk factors such as obesity, advanced tumor stage, lymph node metastasis, and high Gleason score, it is surprising to see a negative correlation with patient's survival status. We speculate that this is due to the short period of follow up (average 8.9 years) and high survival rate within this short time for the patients who received radical prostatectomy. To overcome this limitation, Louisiana Tumor Registry is continuing to follow up this cohort of patients, and the data will be re-analyzed in the future.

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Disclosure of conflict of interest

None.

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