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Clinical-Bladder cancer

Expression and prognostic utility of PD-L1 in patients with squamous cell carcinoma of the bladder

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Abstract

Objectives: Checkpoint inhibitors are approved for the treatment of urothelial bladder cancer. However, there have been no reports on the prognostic value of programmed-death receptor ligand 1 (PD-L1) expression in squamous cell carcinoma (SCC) of the bladder. We assessed the relationship between PD-L1 expression, clinicopathological features, and oncologic outcomes in bladder SCC.

Methods and materials: Immunohistochemistry of PD-L1 was performed on 151 radical cystectomy specimens with pure SCC treated in Mansoura, Egypt from 1997 to 2004.

Results: Median patient age was 52 years (range: 36–74 years) and median length of follow up was 63 months (range: 1–100 months). Schistosomiasis was present in 81% of the specimens and 93% had muscle-invasive disease on pathologic staging. PD-L1 expression was negative in 50 (33%) of the specimens. Negative PD-L1 expression was associated with higher pathologic tumor stage ($P = 0.04$), higher grade lesions ($P = 0.01$), and the presence of lymphovascular invasion ($P < 0.01$). Kaplan-Meier analyses showed that negative PD-L1 expression is associated with worse recurrence-free ($P = 0.01$) and worse cancer-specific survival ($P = 0.01$). Multivariable Cox regression analyses showed negative PD-L1 expression was an independent predictor of disease recurrence (hazards ratio 2.05, 95% confidence interval 1.06–3.96, $P = 0.03$) and cancer-specific mortality (hazards ratio 2.89, 95% confidence interval 1.22–6.82, $P = 0.02$).

Conclusions: Negative PD-L1 expression is associated with higher pathologic tumor stage, higher grade lesions, presence of lymphovascular invasion, and worse oncologic outcomes after radical cystectomy for SCC. These findings support the need for the inclusion of patients with bladder SCC into immunotherapy clinical trials.

Keywords: biomarker; bladder cancer; cystectomy; programmed cell death 1 ligand 1 protein; squamous cell carcinoma

1. Introduction

Bladder squamous cell carcinoma (SCC) is a relatively uncommon disease, accounting for approximately 2–5% of all new bladder cancer cases diagnosed in the United States. The incidence of SCC has historically been higher in Egypt, where schistosomiasis is more prevalent [1]. Compared to urothelial carcinoma, SCC often presents at a higher stage [2,3], and is less responsive to chemotherapy and radiation [4]. The standard of treatment for SCC of the bladder is radical cystectomy (RC) [5]. Contemporary series demonstrate that RC with pelvic lymphadenectomy can provide at best a 5-year cancer-specific survival (CSS) rate of only 58% [6].

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Thus, there is clearly an unmet clinical need for new treatment options to improve survival in patients with SCC of the bladder.

Recent advancements in cancer immunotherapy and our understanding of how cancer cells evade immunologic surveillance have led to the approval of novel checkpoint inhibitors for the treatment of metastatic urothelial carcinoma of the bladder [7–11]. Biomarkers for these immune checkpoints, such as programmed-death receptor ligand 1 (PD-L1), have been evaluated for prognostic value in patients with urothelial carcinoma of the bladder [12–14]. However, little remains known about the expression profile of PD-L1 in SCC of the bladder. To date, there has only been one small study of PD-L1 expression in 17 patients with bladder SCC in which the authors found no correlation of PD-L1 expression with clinicopathological parameters [15].

Given the success of checkpoint immunotherapy in metastatic bladder cancer and the known association between bladder SCC and chronic inflammation, we aimed to investigate the expression and prognostic value of PD-L1 in SCC of the bladder.

2. Material and methods

2.1. Study population

The study cohort included 151 patients with pure SCC who underwent RC with bilateral pelvic lymphadenectomy for bladder cancer in Mansoura, Egypt between 1997 and 2004. Patients who received either radiotherapy or chemotherapy in the neoadjuvant/adjuvant setting were excluded. Patients with inadequate formalin-fixed paraffin-embedded (FFPE) archival tissue or with insufficient clinical/pathological data or with insufficient follow-up and oncologic outcomes were also excluded. We constructed a database with clinicopathological characteristics and oncologic outcomes after institutional review board approval was obtained.

2.2. Tissue microarray block construction

Histology, grade, and tumor stage were confirmed by blinded review of hematoxylin and eosin stained sections cut from duplicate FFPE archival blocks from each RC case. Three replicates of 1 mm core diameter samples were collected from a single block in each case and placed on separate, randomly arranged spaces to construct tissue microarray (TMA) blocks. Sections (4 μm) were obtained from the TMAs and stained with hematoxylin and eosin to confirm presence of the tumor and to review tumor histology and other pathological parameters before immunohistochemistry (IHC) staining. Staging was performed according to the 2010 American Joint Commission on Cancer TNM classification [16]. Extravesical extension was defined as a pathologic tumor stage of 3 or greater. Grading was based on the 2004 World Health Organization classification and done according to the amount of keratinization and the degree of nuclear pleomorphism. Lymphovascular invasion (LVI) was defined as the presence of tumor cells within an endothelial-lined space lacking muscular walls. Other pathological variables collected included lymph node involvement, deoxyribonucleic acid ploidy, and concomitant (squamous cell) carcinoma in situ.

2.3. IHC staining and scoring

Standard IHC staining procedures for PD-L1 were performed with ready to use Biocare Medical rabbit monoclonal antibody, clone CAL10 at a dilution of 1:300. Briefly, FFPE tissue sections were cut at 4 μm and dried at 65°C. Sections were then deparaffinized, rehydrated, and subjected to heat-induced epitope retrieval for 20 minutes with EDTA buffer solution at a pH of 9.0. Sections were then incubated with the appropriate primary antibody for 30 minutes at room temperature.

Slides were developed using the Autostainer (Dako, Carpinteria, CA). Tonsillar tissues served as positive controls and independent staining with isotype (negative) control antibodies was performed.

The percent of PD-L1 positive tumor and immune cells was assessed by a semi-quantitative score from 0% to 100%. PD-L1 positive tumor cells were discerned cytomorphologically from infiltrating macrophages and inflammatory cells. Misinterpretation of rarely occurring unspecific staining reactions at the edges of the punches was excluded by restricting analysis to the central parts of the cores. We determined the mean percent of PD-L1 positive tumor and immune cells in 10 random hot spots in each core specimen. The mean of the triplicate cores in each case was calculated for data analysis. A membrane/cytoplasm staining percentage of 1% or greater for PD-L1 on tumor and immune cells was considered positive. Separate analyses were also done using a threshold of 5% or greater for positive PD-L1 expression. Of note, immune cells were available in TMA punches and suitable for analyses of PD-L1 expression in only 120 patients. Scoring was done by 2 uropathologists blinded to clinical outcomes.

2.4. Surveillance regimen

All patients were followed for disease progression every 2 months for the first 6 months, and every 6 months thereafter. Generally, follow-up visits consisted of a history, physical examination, and laboratory tests, including serum chemistry evaluation, liver function tests, and alkaline phosphatase measurements, when clinically indicated. Diagnostic imaging of the upper tract, such as ultrasound and/or excretory urography, and chest x-ray, were done semiannually or when clinically indicated. Computerized tomography or magnetic resonance imaging was performed at the treating physician's discretion at least once annually

and/or when findings suggested disease progression. Bone scans were done if patients presented with bony aches or a high serum alkaline phosphatase level.

Based on imaging, tumor recurrence in the pelvis or retroperitoneal lymph nodes as well as distant recurrence was coded as disease recurrence. Cause of death was determined by the treating physician, chart review with confirmation by death certificate, or by death certificate alone. Clinicopathological data were maintained in an institutional review board-approved database with regular data controls, as well as quality assurance checks, to ensure data validity and completeness.

2.5. Statistical analysis

The chi-square and Mann-Whitney *U* tests were used to assess the association between PD-L1 expression and clinicopathological variables. The Kaplan-Meier product-limit method and the log-rank test were applied to estimate survival probabilities and compare survival, respectively. Censored survival values represent patients who were alive without clinical evidence of disease at the time of last follow-up. Univariable and multivariable Cox proportional hazard regression models were fitted. Variables significant on univariable analyses were included in the multivariable analysis. All statistical analyses were done with IBM SPSS, version 24. Reported *P* values are 2-sided. Significance was defined as $P \leq 0.05$.

3. Results

A total of 151 patients underwent RC with pelvic lymphadenectomy for pure SCC of the bladder. The median (interquartile range, IQR) number of lymph nodes removed was 19 (14–26). Positive surgical soft tissue margins were found in 5 (3%) cases. Mean ± SD age was 51.8 ± 7.9 years. Schistosomiasis was present in 81% of the specimens. Fig. 1a and b show examples of negative and positive PD-L1 staining on tumor cells, respectively, using 1% as the cutoff value for positive expression. Table 1 describes the demographic, clinical, and pathological characteristics of this cohort of patients stratified by PD-L1 expression on tumor cells with 1% as the cutoff value for positive expression. Negative tumoral expression of PD-L1 was associated with extravesical disease ($P = 0.032$), high-grade lesions ($P = 0.009$), and the presence of LVI ($P = 0.004$). There was no significant association between tumoral PD-L1 expression and schistosomiasis ($P = 0.862$). Immune cell expression of PD-L1 was not significantly associated with any of our demographic, clinical, or pathological variables, regardless of whether we used a cutoff value of 1% or 5%.

Median follow-up after RC was 63 months (range: 1–100). At the time of analysis, 39 (25.8%) patients had experienced disease recurrence and 24 (15.9%) had died of SCC.

Of the patients who experienced disease recurrence, 22 (56.4%) had local pelvic recurrence, 11 (28.2%) had distant metastasis, and 6 (15.4%) had local pelvic recurrence as well as distant metastasis. Sites of metastatic involvement included the bones in 7 cases, the lungs in 4 cases, the liver in 2 cases, and the lymph nodes in 1 case. Three cases had multiple sites of metastatic involvement.

Fig. 2a and b show the Kaplan-Meier plots of recurrence-free and CSS, respectively, stratified by tumoral PD-L1 expression with 1% as the cutoff value for positive expression. The recurrence-free survival (RFS) rate 5 years postoperatively was 76% and 53% in those with positive and negative tumoral PD-L1 expression, respectively ($P = 0.01$). The CSS rate 5 years postoperatively was 83% and 64% in those with positive and negative tumoral PD-L1 expression, respectively ($P = 0.01$). There was no

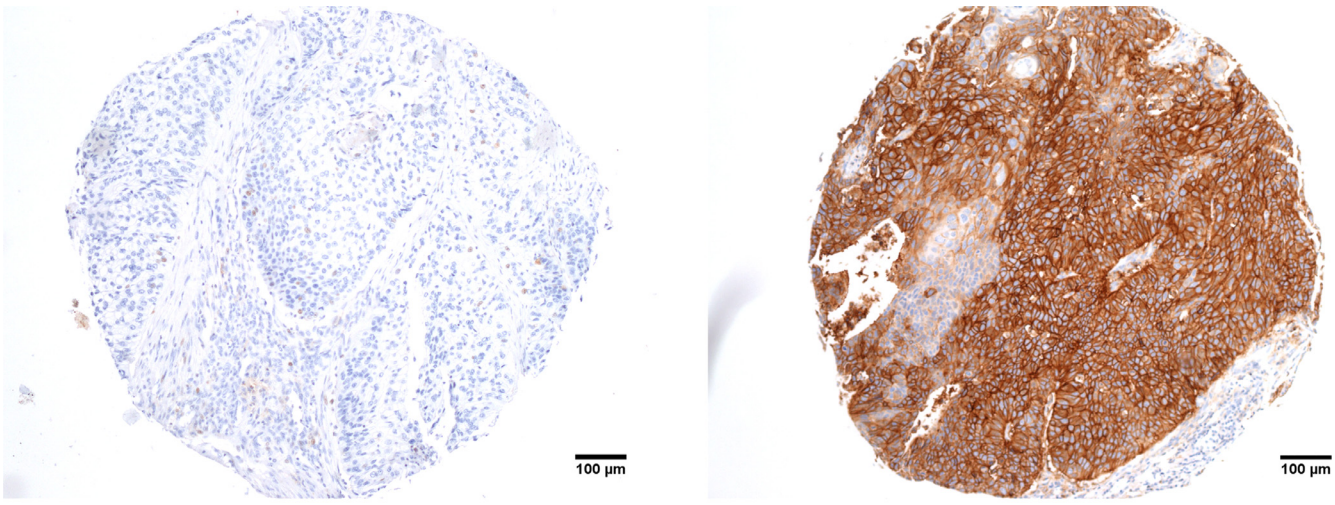

Fig. 1. Examples of negative (a) and positive (b) PD-L1 staining on tumor cells at 100× magnification.

Table 1
Demographic, clinical, and pathological characteristics of patients who underwent RC for SCC from 1997 to 2003

		PD-L1 expression		
	Total No. (%)	No. Positive (%)	No. Negative (%)	*P* value
Overall	151 (100.0)	101 (66.9)	50 (33.1)	
Age (in years)				
Mean (± SD)	51.8 (± 7.9)	51.7 (± 8.0)	52.1 (± 7.7)	0.800
Median (range)	52 (36–74)	51 (36–74)	52 (38–66)	
Sex				0.356
Male	98 (64.9)	63 (62.4)	35 (70.0)	
Female	53 (35.1)	38 (37.6)	15 (30.0)	
Pathologic tumor stage				0.039
pT1	10 (6.6)	5 (5.0)	5 (10.0)	
pT2	75 (49.7)	58 (57.4)	17 (34.0)	
pT3	57 (37.7)	34 (33.6)	23 (46.0)	
pT4	9 (6.0)	4 (4.0)	5 (10.0)	
Lymph node involvement				0.073
Present	46 (30.5)	26 (25.7)	20 (40.0)	
Absent	105 (69.5)	75 (74.3)	30 (60.0)	
Grade				0.009
Low	80 (53.0)	61 (60.4)	19 (38.0)	
High	71 (47.0)	40 (39.6)	31 (62.0)	
Associated carcinoma in situ				0.988
Present	9 (6.0)	6 (5.9)	3 (6.0)	
Absent	142 (94.0)	95 (94.1)	47 (94.0)	
Lymphovascular invasion				0.004
Present	24 (15.9)	10 (9.9)	14 (28.0)	
Absent	127 (84.1)	91 (90.1)	36 (72.0)	
Schistosomiasis				0.862
Present	122 (80.8)	82 (81.2)	40 (80.0)	
Absent	29 (19.2)	19 (18.8)	10 (20.0)	

significant difference in survival times when 5% was used as the cutoff value for positive expression.

Higher immune cell expression of PD-L1 was associated with better survival outcomes. The RFS rate 5 years postoperatively was 73%, 67%, and 64% in those with 5% or greater, 1% or greater but less than 5%, and less than 1% immune cell PD-L1 expression, respectively ($P > 0.05$). The CSS rate 5 years postoperatively was 77%, 74%, and 72% in those with 5% or greater, 1% or greater but less than 5%, and less than 1% immune cell PD-L1 expression, respectively ($P > 0.05$). Fig. 3a and b show the Kaplan-Meier plots of recurrence-free and CSS, respectively, stratified by immune cell PD-L1 expression with 5% as the cutoff value for positive expression.

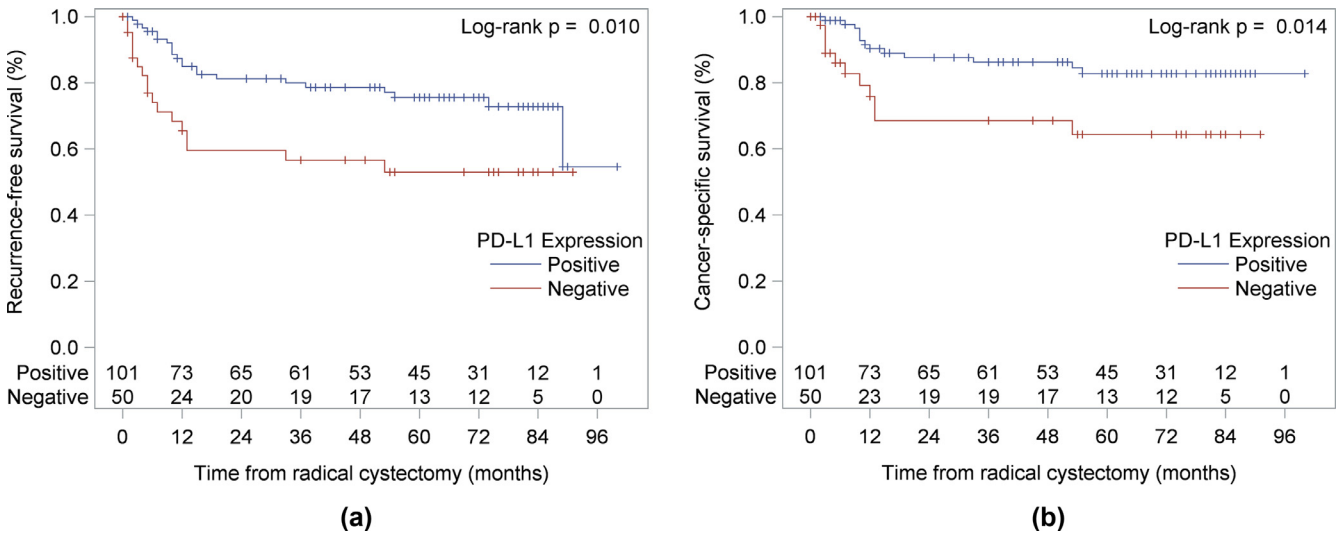

Fig. 2. Recurrence-free (a) and cancer-specific (b) survival probability stratified by tumoral PD-L1 expression in patients who underwent RC for SCC.

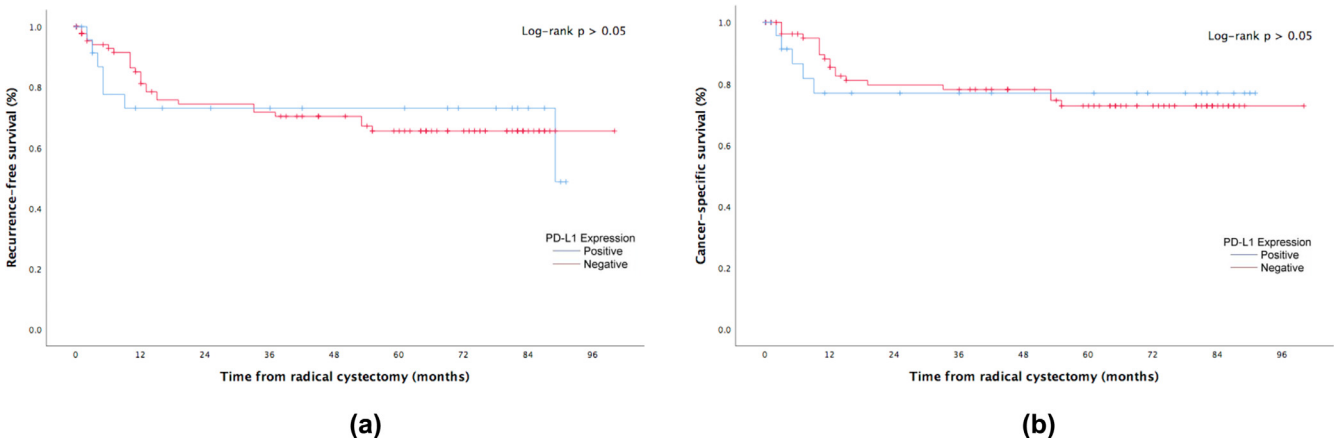

Fig. 3. Recurrence-free (a) and cancer-specific (b) survival probability stratified by immune cell PD-L1 expression in patients who underwent RC for SCC.

Table 2 shows the multivariable Cox proportional hazards regression analysis with 1% as the cutoff value for positive expression. Negative tumoral expression of PD-L1 was significantly associated with both disease recurrence (HR 2.05, 95% CI 1.06−3.96, $P = 0.03$) and cancer-specific mortality (HR 2.89, 95% CI 1.23−6.82, $P = 0.02$), after adjusting for pathologic tumor stage, grade, lymph node involvement, and LVI. There were no significant associations with disease recurrence and cancer-specific mortality when 5% was used as the cutoff value for positive tumoral PD-L1 expression. Similarly, there were no significant associations between immune cell PD-L1 expression with disease recurrence and cancer-specific mortality, regardless of whether we used a cutoff value of 1% or 5%.

4. Discussion

To our knowledge, this is the first report of the prognostic value of PD-L1 expression on IHC in patients with SCC of the bladder. We found that negative PD-L1 expression is associated with adverse pathological characteristics. In addition, negative PD-L1 expression was an independent prognosticator for disease recurrence and cancer-specific mortality on multivariable analysis. This shows the importance of PD-L1 expression beyond simply adverse pathological criteria.

The ability of PD-L1 expressing tumor cells to maintain an immunosuppressive environment may explain the association between PD-L1 expression and oncologic outcomes in bladder SCC. In this study, we found that negative expression of PD-L1 was independently associated with worse oncologic outcomes, including disease recurrence (HR 2.05, 95% CI 1.06−3.96, $P = 0.03$) and cancer-specific mortality (HR 2.89, 95% CI 1.23−6.82, $P = 0.02$). Our findings are in contrast to previously published reports on the prognostic significance of PD-L1 in bladder urothelial carcinoma. In these studies, positive tumor PD-L1 expression not only doubled the risk of stage progression [12], but also was associated with worse outcomes, including RFS and CSS [13], as well as all-cause mortality in organ-confined disease [14]. Although positive PD-L1 expression is regarded to be a poor prognostic biomarker among most urologic malignancies [17−19], a recent report on upper tract urothelial carcinoma found PD-L1 positivity of tumor cells to be favorable with respect to disease recurrence and all-cause mortality [20]. Other studies on the prognostic value of PD-L1 expression in squamous nonsmall cell lung carcinoma and in head and neck SCC have yielded variable

Table 2
Adjusted HRs of oncological outcomes by pathological characteristics of patients who underwent RC for SCC from 1997 to 2003

	Disease recurrence		Cancer-specific mortality	
Feature (Referent)	HR (95% CI)	*P* value	HR (95% CI)	*P* value
Pathologic tumor stage (Organ-confined)				
Extravesical extension	1.80 (0.90−3.58)	.09	1.65 (0.70−3.89)	.25
Lymph node involvement (Absent)				
Present	2.09 (0.96−4.54)	.06	2.28 (0.84−6.16)	.10
Grade (Low)				
High	1.41 (0.65−3.03)	.39	1.04 (0.40−2.69)	.93
Lymphovascular invasion (Absent)				
Present	1.88 (0.83−4.25)	.13	3.42 (1.29−9.07)	.01
PD-L1 expression (Positive)				
Negative	2.05 (1.06−3.96)	.03	2.89 (1.23−6.82)	.02

results [21,22]. In addition, the percentage of our SCC specimens with positive tumoral PD-L1 expression was higher in comparison to prior reports evaluating PD-L1 in bladder urothelial carcinoma [12,14,23–27]. Our findings in this study indicate a possible beneficial role of PD-L1-mediated suppression of the immune response within the tumor microenvironment of bladder SCC. Furthermore, these findings support the need for the inclusion of patients with bladder SCC into clinical trials to assess whether these cancers have a response to immunotherapy with checkpoint inhibitors and if positive tumoral PD-L1 expression is predictive of that response.

Contemporary series have reported 5-year CSS rates around 57% and 58% after RC with pelvic lymphadenectomy for SCC [6,28]. In the present study, we report 5-year CSS rates of 83% and 64% in those with positive and negative PD-L1 expression, respectively. Our reported CSS rates compare favorably with those published in prior contemporary studies. However, this may be attributable to the differences in pathological characteristics of the study cohorts. Of their patients with SCC, Rogers et al.[28] and Ehdaie et al.[6] reported that 84% and 77%, respectively, had extravesical tumor extension on pathologic examination. In comparison, only 44% of our patients had pathologic evidence of extravesical extension.

The prognostic significance of pathologic tumor stage, nodal status, and grade in bladder cancer has previously been well described in the literature. Ghoneim et al. reported a significantly increased risk of disease recurrence after RC for lesions with advanced pathologic stage, lymph node involvement, and/or higher grade, after adjusting for tumor histology [2,3]. Our study found that negative expression of PD-L1 was significantly associated with both extravesical disease ($P = 0.032$) and high grade lesions ($P = 0.009$), with a trend toward significance for nodal involvement ($P = 0.073$).

Our study also found a significant association between negative PD-L1 expression and the presence of LVI ($P = 0.004$). It is generally accepted that LVI is an important prognostic marker of oncologic outcomes after RCC for urothelial carcinoma. Lotan et al. found a significantly increased risk of both disease recurrence and cancer-specific mortality in patients with LVI without any evidence of nodal disease [29]. Quek et al. reported the presence of LVI to be significantly associated with a higher risk of both cancer-specific and all-cause mortality [30]. We have previously reported similar findings with respect to the prognostic value of LVI in SCC of the bladder [31].

It has long been known that SCC of the bladder is associated with chronic inflammation secondary usually to persistent irritation of the urinary tract [1,32,33]. In this context, PD-L1 expression on tumor cells may be a marker of response to pressure from host immunity rather than an indication of immune evasion. Studies using murine tumor cell lines have shown that interferon-γ up-regulates the expression of PD-L1 and effectively inhibits tumor rejection by $CD8^+$ T cells.[34] Thus, the presence of inflammatory cytokines in the tumor microenvironment of SCC could up-regulate tumoral PD-L1 expression. In our study, we found that 66.9% of the specimens showed positive tumoral PD-L1 expression. Prior studies evaluating PD-L1 in urothelial carcinoma of the bladder through IHC have reported lower rates of positive staining, ranging from 12.4% to 50.9% [12,14,23–27]. This discrepancy could easily be due to differences in the tumor microenvironment of SCC and urothelial carcinoma. It could also be explained by the use of different cutoffs for positivity, antibodies for staining, and/or materials for analysis (i.e., whole tissue blocks vs. TMAs). However, it is likely that PD-L1 has an important role in maintaining a balance between immune activation and inhibition within the tumor microenvironment of SCC. Brenner et al. previously reported a case of invasive SCC after intravesical bacillus Calmette-Guerin immunotherapy in a patient with pre-existing squamous dysplasia [35]. It could be possible that the robust bacillus Calmette-Guerin-induced immune response overrides local mechanisms of immunosuppression and creates a microenvironment permissive for the progression of dysplasia to carcinoma. Further studies are definitely needed to assess other potential biomarkers, such as interferon-γ and CTLA-4, within the immunological milieu of bladder SCC.

There are several limitations to our study. First and foremost is its retrospective design. Another is the fact that staining for PD-L1 on TMAs is not necessarily homogenous and can be associated with significant variability. However, we used 3 punches of tumor for each patient and the interpretation of IHC findings was done by experienced uropathologists who were blinded to clinical outcomes. In addition, given the multiple antibodies on the market with which to perform IHC for PD-L1, we chose an antibody that was previously established at our processing institution for quality assurance purposes due to previous expertise and use of the antibody. We were further limited by the lack of a consensus in the literature on the definition of PD-L1 alteration with variability in antibodies and cutoff values being used [36]. Our study was also based predominantly on a cohort of patients with schistosomiasis-associated SCC, which may limit the extrapolation of our results to western European and American cohorts. Lastly, our findings will require external validation in the future.

5. Conclusions

Negative expression of PD-L1 is associated with higher tumor stage, higher grade, LVI, and worse oncologic outcomes after RC for SCC. Our study suggests that positive expression of PD-L1 may be part of the immune response associated with better outcomes in bladder SCC. These findings support the need for inclusion of SCC in immunotherapy clinical trials to evaluate whether positive tumoral expression of PD-L1 is associated with better response to therapy.

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