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Programmed death ligand 1 in non–clear cell renal cell carcinoma and its correlation with tumor microenvironment and prognostic factors

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SUMMARY

Non–clear cell renal cell carcinomas (non-ccRCC) are relatively uncommon tumors compared with clear cell RCC. Targeting immune checkpoints such as programmed death/programmed death ligand 1 (PD1/PD-L1) axis has been promising. In addition, intratumoral PD-L1 expression is a predictor of poor prognosis in some cancers. This study aimed to characterize the association between PD-L1 expression and clinicopathological prognostic factors and evaluate lymphocyte density displaying PD-1, CD4, and CD8 expression in the tumor microenvironment in non-ccRCC. Immunohistochemical analysis was performed on tissue microarray sections for the expression of PD-L1, PD-1, CD4, and CD8. PD-L1 positivity rate was found to be 24%. Dense lymphocytic infiltration staining with PD-1, CD4, and CD8 was significantly high in PD-L1-positive tumors. No correlation was found between PD-L1 expression and any of the tested negative prognostic factors. Only the rate of World Health Organization-International Society of Urologic Pathologists (WHO–ISUP) grade 3–4 tumors was considerably higher in PD-L1-positive cases than in negative ones. Further evaluation of PD-L1 as a potential predictive biomarker in larger series and standardization of staining procedures and scoring methods are warranted. The pathologists should be aware of the importance of lymphocytic infiltration in the tumor microenvironment, which potentially may be a predictive marker for targeted therapy.

Key words: Non–clear cell renal cell carcinoma, programmed death Ligand 1, tumor-infiltrating mononuclear cells

INTRODUCTION

Renal cell carcinoma is a heterogeneous disease with various histologic subtypes having different pathological and biological properties. Clear cell renal cell carcinoma (ccRCC) is the most common subtype of malignant kidney tumors. The remaining renal epithelial malignancies are categorized as non–clear cell RCC (non-ccRCC), including papillary (15%), chromophobe (5%), and other rare subtypes such as collecting duct carcinoma, Xp11.2 translocation carcinoma, medullary carcinoma, and unclassified RCC (1).

Because of the low prevalence of non-ccRCC, most clinical trials have focused on patients with clear cell histology. Various targeted therapies such as vascular endothelial growth factor monoclonal antibodies, tyrosine kinase inhibitors, and mammalian target of rapamycin inhibitors have an important role in ccRCC management. Retrospective analyses have pointed out the potential activity of targeted agents in non-ccRCCs (2). These drugs may have effects on non-ccRCCs, but advanced therapies for these patients are required (3, 4).

Immunotherapy strategies, such as interferon-alpha or high-dose interleukin 2 therapy, have been used in patients with advanced RCC, and improved survival has been achieved (5). PD-1 and PD-L1 have been investigated as novel targets in oncology (6). Programmed death 1 (PD-1) is a T-cell co-inhibitory receptor with two ligands: B7-H1 (PD-L1) and B7-DC (PD-L2) (7, 8). PD-1 is expressed on activated T cells, B cells, natural killer T cells, dendritic cells, and monocytes (8). T-cell activity is limited when PD-1 interacts with its ligand PD- L1 (9). The PD-1/PD-L1 signaling axis is an immunological escape mechanism triggered by the neoplasm (10). The inhibition of the PD-1/PD-L1 axis enhances effector T-cell proliferation and activity in the tumor microenvironment, leading to antitumor activity (10, 11). Some studies showed that intratumoral

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PD-L1 expression is a predictor of poor prognosis in patients with ccRCC (12-14). Furthermore, the presence of PD-1-positive tumor-infiltrating mononuclear cells (TIMCs) was correlated with poor clinical outcome and aggressive behavior in ccRCCs (15-17).

To date, only a few studies have addressed the expression of PD-L1 in non-ccRCC (18, 19); however, the results remain controversial. Therefore, the aim of this study was to hallmark the PD-L1 expression in patients with non-ccRCC and evaluate the impact of PD-L1 expression on clinicopathological prognostic factors. In addition, this study aimed to determine the correlation between the PD-L1 expression and PD-1-, CD4-, and CD8-expressing lymphocyte density in the tumor microenvironment in non-ccRCC series.

MATERIALS AND METHODS

A total of 87 patients diagnosed with non-ccRCC after excision at our institution (Ankara Yıldırım Beyazıt University/Atatürk Education and Research Hospital) between 2007 and 2014 were analyzed retrospectively. Tissue microarrays (TMAs) were constructed from the most representative paraffin block. Two cores, representing the center and the advancing edge of the tumor (diameter, 0.3 cm each) were selected from each primary tumor. Data on clinicopathological characteristics, including age, gender, WHO–ISUP grade, coagulative tumor necrosis, lymphovascular invasion, renal sinus involvement, metastasis, and overall survival (OS) were retrospectively collected for these patients. TMA blocks were cut into 4-μm histological sections and used for immunohistochemistry.

Immunohistochemical analysis was performed for the expression of PD-L1 (SP263; 1:100; Ventana, US), PD-1 (NAT 105; 1:250; Cell Marque, US), CD4 (SP351; 1:100; Ventana), and CD8 (SP57; 1:100 dilution; Ventana). All sections were stained with primary antibodies on Ventana GX benchmark equipment with standard antigen retrieval (CC1 buffer; pH 8.0; Ventana). UltraView Universal 3,3'-Diaminobenzidine (DAB) Detection Kit (Ventana) was used according to the manufacturer's protocols. Counterstaining was performed using hematoxylin. The slides were washed, dehydrated in alcohol and xylene, mounted, and coverslipped. PD-L1 expression was assessed semi-quantitatively. PD-L1 positivity in tumor cells was defined as ≥5% membranous staining with or without cytoplasmic staining. The extent of TIMCs (i.e., lymphocytes and macrophages) was evaluated depending on the density of CD4-, CD8-, and PD-1-expressing TIMCs in the tumor microenvironment. The tumor microenvironment was considered as the stroma of the tumor cells, but not the stroma around the renal mass. This study aimed to evaluate the expression in immunological cells, which have immediate interaction with individual tumor cells. The expression of CD4, CD8, and PD-1 in lymphocytes was scored as 1 point (0–4 cells per high-power field, x400), 2 points (5–8 cells), 3 points (9–12 cells), and 4 points (≥13 cells). The evaluation was performed using a Nikon Eclipse 80i microscope (Nikon, Japan).

Statistical analysis

Patient and tumor characteristics were summarized descriptively. Crosstabs were created and χ^2 values were calculated to determine the differences between categorical variables in accordance with the PD-L1 condition. The Mann–Whitney *U* test was used to evaluate the differences between continuous variables in accordance with the PD-L1 condition. The time to death was calculated using the Kaplan–Meier method, and the differences between the curves were assessed using the log-rank test. MS-Excel 2010 and the IBM SPSS Statistics 21.0 (released in 2012; IBM SPSS Statistics for Windows, Version 21.0; IBM Corp., NY, USA) were used for statistical analyses and calculations. A *P* value <0.05 was regarded as an indicator of a significant difference in statistical decisions.

RESULTS

Patients and tumor characteristics

The characteristics of patients are summarized in Table 1. This retrospective study included a total of 87 patients with non-ccRCC. The histological subtypes were chromophobe RCC ($n=27$), papillary RCC ($n=48$), mucinous tubular and spindle cell ($n=3$), multilocular cystic RCC ($n=2$), collecting duct carcinoma ($n=1$), and unclassified RCC ($n=6$). Further, 49 patients had a high WHO–ISUP grade (III or IV) and 38 had a low WHO–ISUP grade (I or II). The median tumor size was 5 cm (range 1.5–17.5). The median follow-up time was 44 months, and the median age was 61years (range 31–87).

PD-L1 expression in tumor cells and TIMCs

PD-L1 was positive in 21 (24%) of 87 assessed patients. Tumor samples with scores of 0–2 for CD4, CD8, and PD-1were encoded

Table 1: Non-ccRCC patient characteristics.

Characteristic		Total (n = 87)	
		No. of patients	%
Gender	Male	66	75.9
	Female	21	24.1
Histology	Chromophobe	27	31
	Papillary	48	55.2
	Mucinous tubular and spindle cell	3	3.4
	Multilocular cystic	2	2.3
	Collecting duct	1	1.1
	Unclassified	6	6.9
		Median	Min, Max
Age at diagnosis		61	31–87
Tumor size (cm)		5	1.5–17.5

as "none or mild," whereas scores of 3–4 were encoded as "dense" lymphocytic infiltration in the tumor microenvironment. The rate of dense lymphocytic infiltration staining with PD-1, CD4, and CD8 was significantly high in PD-L1-positive tumors ($P < 0.05$) (Table 2; Fig. 1).

PD-L1 expression in tumor cells, clinicopathological features, and clinical outcome

No correlation was found between PD-L1 expression and any of the tested negative prognostic factors ($P > 0.05$). The rate of WHO–ISUP grade 3–4 tumors was considerably higher in PD-L1-positive cases than in negative ones (Table 3). The mean tumor size was 7.2 cm in PD-L1-positive cases and 6 cm in PD-L1-negative ones; however, the difference was not statistically significant.

The mean survival was 91.9 months for PD-L1-negative cases and 93.2 months for PD-L1-positive cases; this difference was not statistically significant ($P = 0.63$).

DISCUSSION

The prognostic significance of PD-L1 and PD-1 in clear cell renal cell carcinoma (RCC) has been investigated in previous studies (14, 16, 17, 20). Most of them demonstrated that intratumoral PD- L1 expression appeared to worsen clinical outcome.

In a meta-analysis including 1863 patients, PD-L1 expression was found to be associated with poor OS and advanced clinicopathological features in clear and non-clear cell RCC (21). Furthermore, it was found to be significantly associated with nuclear grade, tumor necrosis, metastases, regional lymph node involvement, and primary tumor stage in patients with RCC.

Table 2: PD-1, CD4, and CD8 expression in TIMCs in tumor microenvironment according to the PD-L1 status.

		PD-L1 staining		P value
		Negative, n (%)	Positive, n (%)	
CD4	Mild	44 (66.7)	7 (33.3)	0.007
	Dense	22 (33.3)	14 (66.7)	
CD8	Mild	43 (65.2)	7 (33.3)	0.010
	Dense	23 (34.8)	14 (66.7)	
PD-1	Mild	57 (86.4)	10 (47.6)	0.000
	Dense	9 (13.6)	11 (52.4)	

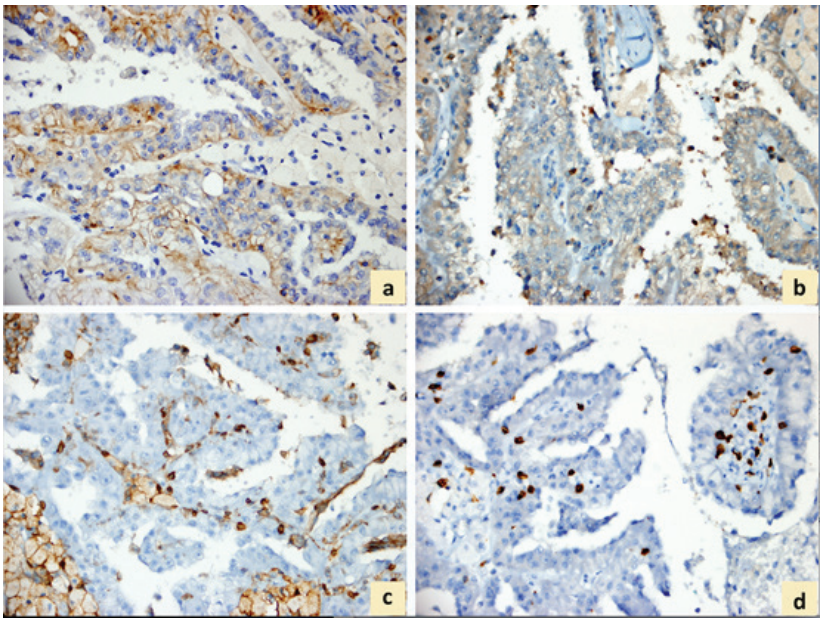

Figure 1: a) Membranous PD-L1 expression in tumor cells. b) Dense PD-1-positive TIMCs. c) Dense CD4-positive TIMCs. d) Dense CD8-positive TIMCs.

Choueiri et al. evaluated the role of PD-L1 in non-ccRCC. Eleven of the 101 (10.9%) non-ccRCC cases were PD-L1 positive. PD-L1 positivity in tumor cells was significantly associated with higher stage, nuclear grade, and shorter OS. PD-L1 positivity in both tumor cell membrane and TIMC cells were associated with shorter time to recurrence (18) but PD-L1 was not found to be an independent parameter for adverse prognosis. Consistent with the aforementioned study, this study also demonstrated that membranous PD-L1 expression was correlated with higher a WHO–ISUP grade in patients with non-ccRCC. However, no correlation was found with PD-L1 and other negative prognostic factors such as necrosis, metastases, or OS. Abbas et al. showed that neither PD-1-positive TIMC nor intratumoral PD-L1 expression was associated with tumor aggressiveness or advanced disease in non-ccRCCs (19).

A phase I study showed that some patients with PD-L1-positive RCC had an objective response to the anti-PD-1 monoclonal antibody, but none of the patients with negative PD-L1 expression

Table 3: Clinicopathological prognostic factors according to the PD-L1 status.

		PD-L1 staining		Test statistics (χ^2; *P*)
		Negative, *n* (%)	Positive, *n* (%)	
Renal sinus Involvement	No	56 (84.8))	15 (71.4)	1.912; 0.167
	Yes	10 (15.2)	6 (28.6)	
Necrosis	No	37 (56.1)	12 (57.1)	0.008; 0.931
	Yes	29 (43.9)	9 (42.9)	
Lymphovascular invasion	No	56 (84.8)	18 (85.7)	0.009; 0.923
	Yes	10 (15.2)	3 (14.3)	
Distant metastasis	No	47 (71.2)	17(81)	0.777; 0.378
	Yes	19 (8.8)	4 (19)	
WHO–ISUP grade	1–2	34 (51.5)	4 (19)	6.827; 0.009
	3–4	32 (48.5)	17 (81)	

exhibited objective response. According to these results, PD-L1 may be a promising predictive biomarker of response to agents targeted at the PD1/PD-L1 axis (6). However, the relation between the prediction of anti-PD-1 response and the PD-1/PD-L1 axis in tumors is still not completely understood. This is believed to result from complex interactions within the tumor microenvironment (22, 23). This study investigated the effect of the PD-1/PD-L1 axis in non-ccRCC and its correlation with the tumor microenvironment. This study demonstrated that PD-1-, CD4-, and CD8-positive dense lymphocytic microenvironment existed in most of the PD-L1-positive cases.

This study had some limitations. The rarity of collecting duct, multilocular cystic, mucinous tubular, and spindle cell carcinomas, and small number of patients with these subtypes, might have influenced the results. Also, the relatively short follow-up period might have affected the correlation between PD-L1 expression and OS. Since tissue microarrays were evaluated, the results might not represent the PD-L1 expression in the whole section of the tumor because of intratumoral heterogeneity. However, in practice, it is impossible to evaluate the entire tumor. Although the WHO–ISUP nuclear grading is not recommended in chromophobe non-ccRCCs, the grade of this group was not excluded from this study (24).

In conclusion, this study demonstrated that intratumoral PD-L1 expression was associated only with a high grade, but not with aggressive disease in non-ccRCC patients. The results suggested that patients with non-ccRCC, especially with higher PD-L1 expression and PD-1-, CD4-, and CD8-positive dense lymphocytic microenvironment should not be excluded from clinical trials of agents that target the PD-1/PD-L1 pathway. Even high-grade non-ccRCCs may receive these therapies without the evaluation of these immunohistochemical markers, but the pathology report may have the information on the presence of lymphocytic infiltrate if the results are replicated in larger cohorts. Despite the rare incidence of non-ccRCC, further evaluation of PD-L1 as a potential predictive biomarker in larger series and standardization of staining procedures and scoring methods are warranted. The pathologists should be aware of the importance of lymphocytic infiltration in the tumor microenvironment, which potentially may be a predictive marker for targeted therapy.

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