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Impact of PD-L1 Protein Expression on Renal Cell Carcinoma Histo-differentiation

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Abstract. *Background/Aim: Renal cell carcinoma (RCC) comprises a variety of pathological entities. Many RCCs are aggressive, demanding efficient targeted and immune-therapeutic strategies. Programmed cell death ligand-1 (PD-L1) is expressed mainly in hematopoietic cells and also in epithelial cells. The aim of this study was to correlate PD-L1 protein expression in a series of RCC tissues with their histo-pathological features. Materials and Methods: One hundred (n=100) archival, formalin-fixed and paraffin-embedded RCC tissue specimens were analysed by immunohistochemistry. Conventional tumor proportion score (TPS) qualitative assay was applied for evaluating protein expression levels. Results: Based on the TPS evaluation, 8 (8%) cases were characterized as positive, and the rest of them (n=92; 92%) as negative. A progressive increase in PD-L1 positivity was significantly associated with the differentiation grade of the examined malignancies (p=0.001). Conclusion: Although PD-L1 over-expression is detected in low rates in RCCs, its correlation with differentiation grade should be considered as a factor for discriminating sub-groups of patients with specific histo-pathological features eligible for targeted anti-PD-L1 immunotherapy strategies.*

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Immune checkpoint inhibitors act as suppressors by targeting specific pathways that regulate immune-responses. They are considered very promising agents for the treatment of patients suffering from malignant tumors (1). Programmed cell death-1 (*PD-1*) gene – located on chromosome 2 (gene locus: 2q37.3) – encodes a cell surface membrane protein that belongs to the CD28 family of receptors and acts as an inhibitor of the immune system, involved in tumor immune escape (2). PD-1 is expressed in pro-B-cells and involved in their differentiation, whereas its role in apoptosis is under consideration. Concerning its downstream pathway, PD-1 interacts with two potential ligands, PD-L1 and PD-L2. These ligands are trans-membrane proteins implicated in the regulation of specific cell-to-cell interactions and demonstrate different levels of expression (3). Programmed cell death ligand-1 (PD-L1), also known as CD274 (cytogenetic band: 9p24.1), is expressed predominantly in hematopoietic cells and in epithelial cells, including pancreatic islet cells and vascular endothelial cells. Furthermore, PD-L1 is expressed in the thymic cortex, thymocytes, and thymic medulla. Additionally, dendritic cells express PD-L1, which reduces early activation and expansion of self-reactive T cells. PD-L2, also known as CD273, expression is restricted to macrophages and dendritic cells. The PD-1/PD-L1 pathway delivers inhibitory signals that regulate both peripheral and central tolerance (4). Its main role is the inhibition of T lymphocyte proliferation, survival and other functions (cytotoxicity, cytokine release).

Table I. *Total PD-L1 IHC results and statistics.*

Clinicopathological Parameters		PD-L1 expression pattern		*p*-Value
RCC (n=100)		N*	P*	
	n (%)	92/100 (92%)	8/100 (8%)	
Gender				0.374
Male	63 (63%)	57/100 (57%)	6/100 (6%)	
Female	37 (37%)	35/100 (35%)	2/100 (2%)	
RCC anatomic location				0.099
Left kidney	47 (47%)	41/100 (41%)	6/100 (6%)	
Right kidney	53 (53%)	51/100 (51%)	2/100 (2%)	
RCC histotype				0.542
Clear cell	75 (75%)	68/100 (68%)	7/100 (7%)	
Papillary	13 (13%)	12/100 (12%)	1/100 (1%)	
Chromophobe	12 (12%)	12/100 (12%)	0/100 (0%)	
Fuhrman's Grade				**0.001**
F1	12 (12%)	12/100 (12%)	0/100 (0%)	
F2	39 (39%)	39/39 (39%)	0/100 (0%)	
F3	31 (31%)	30/100 (30%)	1/100 (1%)	
F4	18 (18%)	11/100 (11%)	7/100 (7%)	

RCC: Renal cell carcinoma; IHC: immunohistochemistry; P: positive; N: negative. *PD-L1 score <1% is characterized as negative (N), whereas ≥1% is categorized as positive (P). Bold value indicates statistical significance.

Furthermore, it causes apoptosis of tumor-specific T cells and differentiation of $CD4^{+}T$, inducing resistance of tumor cells to cytotoxic T lymphocyte (CTL) lineage attack. Aberrant over-expression of PD-L1 enhances the inflammatory process and allows cancers to evade the host immune system by suppressing T cell activation and inducing peripheral tolerance (5).

Renal cell carcinoma (RCC) demonstrates an aggressive phenotype (increased metastatic potential) in sub-groups of patients with specific molecular signatures (6). In fact, localized malignancies represent about 70% of all RCCs, whereas a 30% is characterized by advanced stage, a progressive tumor dedifferentiation and poor response rates to chemo-targeted therapeutic regimens (7). In the current experimental study, we focused on detecting and evaluating aberrant PD-L1 protein expression in a series of RCC tissues and correlating it with the corresponding histo-pathological features.

Materials and Methods

Study group. For the purposes of our study, we used one hundred (n=100) archival, formalin-fixed and paraffin-embedded (FFPE) RCC tissue specimens, including 75 histologically confirmed clear cell RCCs, 13 papillary RCCs, and 12 chromophobe. The cohort included 63 males and 37 females. The hospital ethics committee consented to the use of these tissues in the Department of Pathology, "Elpis" General Hospital, Athens, Greece for research purposes, according to World Medical Association Declaration of Helsinki. The tissue samples were fixed in neutral-buffered formalin. Hematoxylin and eosin (H&E) - stained slides were reviewed for confirmation of histopathological diagnoses. All lesions were classified according to the histological typing criteria of World Health Organization (WHO) (8).

Antibodies and immunohistochemistry assay (IHC). Ready-to-use anti-PD-L1 (clone 223C3 Dako, North America Inc, Carpinteria CA, USA) monoclonal mouse antibody was applied in the corresponding RCC tissue sections. IHC for the selected antigen was carried out on 3 μm serial tissue microarray sections. The slides were deparaffinized and rehydrated. The EnVision FLEX Target Retrieval Solution, Low pH (50×) (Dako) combined with EnVision FLEX Wash Buffer (20×) was used. Blocking solution was applied to all slides for 10min, followed by incubation for 1 h with the corresponding monoclonal antibody at room temperature (25˚C). Following incubation with the secondary antibody for 10 min, diaminobenzidine-tetrahydrocloride-DAB (substrate-Chromogen Solution –0.03%, Dako) containing 0.1% hydrogen peroxide was applied as a chromogen and incubated for 5 min. Sections were counterstained for 5 min with Hematoxylin, dehydrated, and cover-slipped. For negative control slides, the primary antibody was omitted. The IHC protocol was performed with the use of an automated staining system. Membranous and cytoplasmic staining was considered positive staining, according to manufacturers' data sheets. Colon cancer tissue sections expressing the protein were used as the control group.

PD-L1 IHC-based expression scoring. A conventional (qualitative) assay for scoring the PD-L1 expression patterns was applied. According to the manufacturer's guidelines, Tumor Proportion Score (TPS) reflects the percentage of viable tumor cells showing

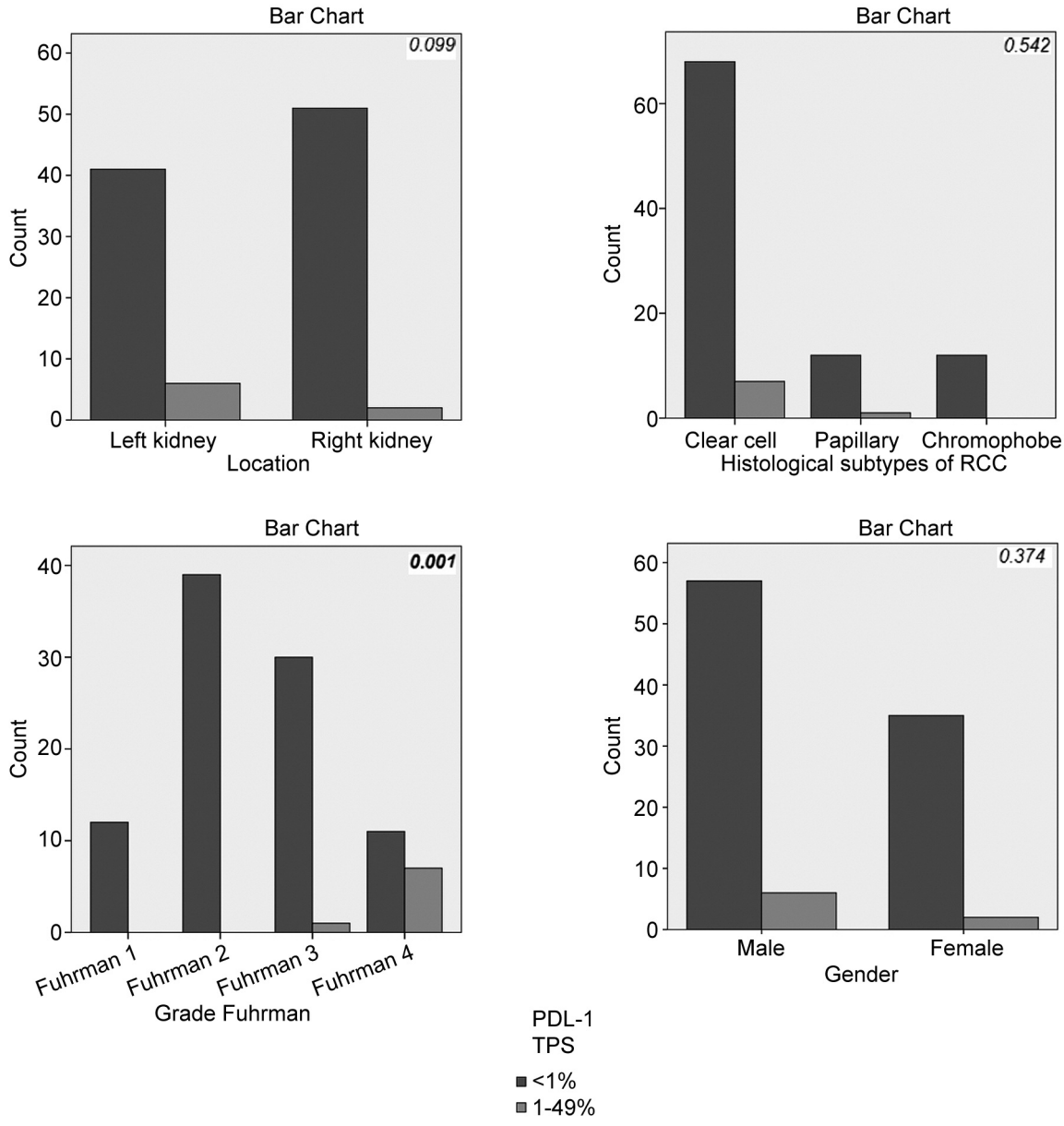

Figure 1. *Histograms and p-values of PD-L1 protein expression patterns (Negative: dark bars – Positive: grey bars) in the examined renal cell carcinomas.*

partial or complete membrane staining at any intensity. PD-L1 score <1% was characterized as negative, whereas ≥1% was categorized as positive (over-expression of the marker).

Statistical analysis. Statistical analysis was performed with the use of IBM SPSS Statistics 21 (SPSS Inc, Chicago, IL, USA) software package for Windows. All he reported *p*-values were two-sided. Association between the two variables (conventional and digitized PD-L1 evaluation methods) was provided by kappa analysis. Total IHC results and correlations are described in Table I.

Results

According to the qualitative TPS–based scoring evaluation, PD-L1 protein expression demonstrated differences in the examined RCC tissue specimens. Among them, eight (n=8.8%) cases were characterized as positive, and the rest of them [ninety-two (n=92.92%)] as negative. Based on their histological subtypes, clear cell carcinomas demonstrated PD-L1 positive/over-expression in 7 out of 75 cases,

papillary carcinomas in 1 out of 13 cases, whereas all chromophobe carcinomas demonstrated no expression of the marker. Progressive increase in PD-L1 positivity was significantly associated with the differentiation grade (Fuhrman's categorization/Fuhrman's grade) of the examined malignancies (p=0.001). Statistically significant correlations with gender (p=0.374) tumor location (p=0.099), and histological type (p=0.542) were not confirmed (Figure 1).

Discussion

New treatment strategies in modern oncology –including RCC- are based on disrupting signal transduction pathways by targeting (blocking) specific molecules (9). Additionally, novel immunotherapeutic regimens –combined or not with other targeted agents- activate the immune system response for identifying and destroying cancerous cells in a variety of solid malignancies (10). Different PD-L1 over-expression patterns are associated with altered response rates to monoclonal antibodies (mAbs) and prognosis of the corresponding patients. Main examples of anti-PD-1 mAbs represent nivolumab and pembrolizumab (11). These mAbs has been administered as first line PD-1/PD-L1 checkpoint blockade therapy in esophageal cancer, gastric cancer, hepatocellular carcinoma, RCC, pancreatic cancer, ovarian cancer, and bladder cancer (12-14). Furthermore, eligible targets for specific anti-PD-1 immunotherapy include breast carcinoma, merkel cell carcinoma, lung cancer (non-small cell lung carcinoma), colorectal cancer, melanoma, and oral carcinoma (15-20).

In the current study, we analyzed PD-L1 protein expression in RCC by implementing an IHC assay. We observed that a small sub-group of the examined RCCs demonstrated positive PD-L1 expression rates according to the TPS scoring evaluation, whereas the majority of them were negative. Interestingly, a progressive increase in the expression of PD-L1 was significantly associated with the differentiation grade (Fuhrman's categorization) of the examined malignancies. Similar studies have shown a strong association of PD-L1 expression with advanced grade and stage (21). Furthermore, an increased incidence of disease recurrence was also observed. Additionally, it seems that subsets of positive PD-1/PD-L1 tumor-infiltrating leukocytes in the tumor microenvironment could be a useful prognostic and predictive biomarker for RCC patients with specific genetic signatures. Some studies have reported a strong correlation between cytotoxic T-cells/PD-L1 expression and poor survival rates (22, 23). There is strong evidence regarding the value of PD-L1 expression in RCC as a potential reliable prognostic biomarker, whereas in other carcinomas, such as colon adenocarcinoma, there are controversial data, although it seems to be an independent factor (24, 25).

In conclusion, although PD-L1 over-expression is detected in low rates in RCCs, its correlation with differentiation grade should be considered a factor for discriminating sub-groups of patients with specific histo-pathological features eligible for anti-PD-L1 targeted treatment. Furthermore, there is an increasing need for novel predictive/prognostic biomarkers in malignancies including RCCs. Understanding the molecular substrate and influence of PD-1/PD-L1 complex in these tumors may lead to the development of novel immunotherapy strategies.

Conflicts of Interest

The Authors declare no conflicts of interest regarding this study.

Authors' Contributions

Z-SA, MS: performed the research and statistics; TE, TP, TG: performed the research, and wrote the paper; PD, TG, TE, KN, LAC: acted as academic advisors, and reviewed the paper.

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