Coexpression of LAG-3 and PD-L1 in tumor infiltrating immune cells predicts worse outcome in clear cell renal cell carcinoma

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Abstract

Lymphocyte-activation gene 3 (LAG-3) protein is a potential immune checkpoint target for cancer treatment. Thus, we investigated LAG-3 expression and its prognostic value in clear cell renal cell carcinoma (ccRCC) and correlated LAG-3 expression with programmed cell death ligand 1 (PD-L1) using immunohistochemistry on tissue microarrays incorporating 134 ccRCC primary excision specimens. The study patients were analyzed as two groups: the whole cohort and those with metastatic renal cell carcinoma (mRCC). LAG-3 expression was significantly correlated with PD-L1 expression in the whole cohort and in the mRCC group (all, $p < 0.05$). Both LAG-3⁺ RCC and PD-L1⁺ RCC in the whole cohort presented with a higher TNM stage and higher Fuhrman nuclear grade (all, $p < 0.05$). According to the International Metastatic Renal Cell Carcinoma Database Consortium risk classification, there were more intermediate- and poor-risk patients in both LAG-3⁺ mRCC and PD-L1⁺ mRCC than LAG-3⁻ mRCC and PD-L1⁻ mRCC (all, $p < 0.05$). PD-L1⁺/LAG-3⁺ RCC and PD-L1⁺/LAG-3⁻ RCC in the whole cohort showed poorer cancer-specific survival (CSS) than PD-L1⁻/LAG-3⁺ RCC (all, $p = 0.01$). Similarly, PD-L1⁺/LAG-3⁺ mRCC and PD-L1⁺/LAG-3⁻ mRCC showed poorer CSS than PD-L1⁻/LAG-3⁺ mRCC (all, $p < 0.05$). Multivariate analysis showed that PD-L1⁺/LAG-3⁺ mRCC (hazard ratio: 3.19; $p = 0.033$) was a predictor of poor CSS. Our findings provided a scientific rationale for LAG-3 blockade in combination with programmed cell death 1/PD-L1 blockade as a potential therapeutic approach for RCC.

Introduction

Renal cell carcinoma (RCC) comprises approximately 90% of kidney cancer cases, of which 70% are clear cell RCC (ccRCC) [1]. Although the surgical resection of early-stage RCC has a good prognosis, the 5-year survival rates for relapsed or metastatic RCC (mRCC) have traditionally been quite low (0%–20%) [2, 3]. In systemic treatment for mRCC, systemic cytokine therapy, followed by targeted therapies, including tyrosine kinase inhibitors (TKIs) and inhibitors of mammalian target of rapamycin (mTOR), have been reported to prolong survival. However, approximately 20%–25% of patients derive no benefit from first-line targeted therapy or become treatment-resistant [4, 5]. Fortunately, the introduction of immune checkpoint inhibitors (ICIs) has revolutionized the treatment paradigm for mRCC. ICIs targeting cytotoxic T-lymphocyte-associated protein 4, programmed cell death 1 (PD-1) receptor, or PD-1 ligand (PD-L1) have shown clinical efficacy in mRCC treatment [6, 7]. Despite improved survival and treatment efficacy using ICIs, the treatment efficacy of the checkpoint blockade in mRCC remains limited to a specific subpopulation of patients [8]. Thus, efforts are being made to find alternative pathways and auxiliary targets to overcome the limited efficacy of ICI treatment and treatment resistance.

Lymphocyte-activation gene 3 (LAG-3), also known as CD233, is an example of a new immune checkpoint target. This inhibitory receptor is mainly found on activated immune cells (ICs) and is involved in negative regulatory effects on T-cells and their biological functions related to immune and inflammatory responses [9, 10]. Based on the coexpression of LAG-3 with other inhibitory receptors, recent preclinical and clinical evidence has revealed PD-1 pathway blockade in combination with LAG-3 inhibition as a potentially effective immunotherapy strategy [11]. However, the expression of LAG-3 and its coexpression with PD-L1 in primary RCC tissue has not been fully investigated. Additionally, the prognostic role of LAG-3 expression

in RCC and the synergistic effect on prognosis upon coexpression with PD-L1 remains unclear. Therefore, we investigated the clinicopathological and prognostic significance of the coexpression of LAG-3 and PD-L1 in primary ccRCC tumors.

Materials And Methods

Patients

Our study cohort comprised surgical specimens collected from 134 patients with ccRCC who had undergone radical or partial nephrectomy at the Inje University Busan Paik Hospital, South Korea, between January 2011 and January 2019. All pathological tissue specimens were provided by Inje Biobank. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki, as reflected in a prior approval by the Institutional Review Board of Inje University Pusan Paik Hospital (approval no. 20-0121). Data on the histopathological features, such as histological subtype, tumor size, lymphovascular invasion, sarcomatoid features, Fuhrman nuclear grade, and distant metastases at surgery, were collected. The pathological stage was determined according to the 2010 version of the American Joint Committee on Cancer TNM staging system and the Heidelberg classification of renal tumors. The follow-up duration was calculated from the date of surgery to the date of the last follow-up or death. The study patients were analyzed as two groups, namely, the whole cohort and those with mRCC (determined according to the metastatic disease and systemic treatment status during follow-up). Clinical information regarding demographic characteristics, International Metastatic Renal Cell Carcinoma Database Consortium (IMDC) risk classification, and follow-up data were extracted from the patients' electronic medical records.

Tissue microarrays (TMAs) and immunohistochemistry

Six TMA blocks comprising a total of 402 cores were constructed by selecting invasive margins containing viable and representative tumor cells (TCs) and stroma with tumor-infiltrating ICs, including macrophages and lymphocytes, from formalin-fixed paraffin-embedded (FFPE) primary RCC tissue blocks of 134 patients. Three 2-mm diameter cores were selected from each case. From each TMA block, 4- μ m-thick sections were cut and stained with LAG-3 (1:200; clone D2G40, Cell Signaling Technology, MA, USA) and PD-L1 (1:100; clone SP263, Ventana Medical Systems, Inc., AZ, USA) primary antibodies. Normal human FFPE tonsil sections treated with and without these primary antibodies were used as positive and negative controls, respectively. LAG-3 staining was performed using a Dako REAL EnVision Detection System (K5007; Agilent Technologies, CA, USA) according to the manufacturer's protocol, while PD-L1 staining was performed using an OptiView DAB IHC Detection Kit (Ventana) on a BenchMark ULTRA automated platform (Ventana), according to the manufacturer's protocol.

LAG-3 and PD-L1 scoring

TMAs and individual slides were visually scored by an experienced pathologist blinded to clinical information. LAG-3 and PD-L1 scoring of ICs is a controversial matter in the current literature; in our study, we used the method of Burugu et al. and Motzer et al. [12, 13]. LAG-3 scores were reported as absolute counts, and any positive expression on ICs (≥ 1 IC per TMA core) was used for dichotomization into positive

and negative cases. IC expression of PD-L1 was assessed as the percentage of ICs with membranous or cytoplasmic expression; any cores with $\geq 1\%$ of PD-L1⁺ ICs were considered positive. All IC types, including macrophages and lymphocytes, were counted together to calculate the LAG-3 and PD-L1 scores. PD-L1 expression in TCs was assessed as the percentage of carcinoma cells with membranous expression at any intensity. Any expression of $\geq 1\%$ in a TMA core that included at least 100 evaluable TCs was considered positive [7]. For the final statistical analysis, PD-L1⁺ cells were defined as any positive PD-L1 staining on ICs or TCs. The three tumor TMA cores for each case were independently scored, and if any of the three cores were positive, that case was considered positive in the statistical analysis.

Statistical analysis

Continuous variables were presented as means (standard deviation) or medians (interquartile ranges, IQRs). Categorical variables were presented as frequencies with percentages. Differences in the distribution of variables among groups were evaluated using the chi-square test, Fisher exact test, and linear-by-linear association for categorical variables. The Student t-test was used for continuous variables. CSS according to the expression status of immune markers was estimated using the Kaplan–Meier method and compared using the log-rank test. Univariate and multivariate Cox proportional-hazard models adjusted by LAG-3 and PD-L1 expression status were utilized to identify any clinicopathological factors that might have affected CSS. The risk was expressed as the hazard ratio (HR), and the 95% confidence interval (CI) was determined using the reference groups. Statistical analysis was performed with SPSS v25.0 (IBM Corp., Armonk, NY, USA) and MedCalc v20.0 (MedCalc Software, Ostend, Belgium). In all tests, a two-sided p-value < 0.05 was considered statistically significant.

Results

Patient characteristics

The study cohort comprised 134 patients who underwent surgical resection of primary ccRCC. The patients' demographic data and clinicopathological features are summarized in Table 1. The median age of the whole cohort at the time of surgery was 61 years (IQR, 34–82), and the 134 patients included 94 men (70.1%). Among the 134 cases, 25 (18.7%) presented with synchronous metastasis, and 26 (19.4%) experienced disease recurrence during the follow-up period. Of the 51 patients with metastatic disease, 45 were treated with targeted therapy, including TKIs and mTOR inhibitors. According to the IMDC risk classification, 20 (44.4%) of these patients were classed as favorable risk, 10 (22.2%) as intermediate risk, and 15 (33.3%) as poor risk. During a mean follow-up period of 89.6 months (95% CI: 80.0–99.3 months; median survival, not reached), 32 of the 134 (23.9%) patients died after surgery for RCC. In the case of mRCC, 28 patients (62.2%) died after diagnosis of metastatic disease, with a median follow-up of 18.0 months (95% CI: 11.0–43.0).

LAG-3 and PD-L1 expression

Immunohistochemistry detected the expression of LAG-3 protein in the nucleus and cytoplasm of ICs, but LAG-3 protein was not detected on TCs. In the whole cohort of 134 RCC patients, LAG-3 was positive in 68

(50.7%) cases. The expression of PD-L1 on ICs was positive in 45 (33.6%) cases, whereas 34 (25.4%) expressed PD-L1 on TCs. Taken together, PD-L1⁺ RCC was observed in 59 (44.0%) RCC patients. LAG-3 was positive in 31 (68.9%) mRCC patients. PD-L1⁺ ICs and TCs were observed in 20 (44.4%) and 17 (37.8%) mRCC patients, of which 25 (55.6%) were PD-L1⁺ mRCC. LAG-3 expression was significantly correlated with PD-L1 expression in the whole cohort and with mRCC. In summary, in the whole cohort, 64.7% of the cases with LAG-3⁺ RCC were PD-L1⁺, whereas 77.3% of the cases with LAG-3⁺ RCC were PD-L1⁺ ($p < 0.001$). Similarly, in mRCC, 67.7% of the cases with LAG-3⁺ mRCC were PD-L1⁺, whereas 71.4% of the cases with LAG-3⁺ mRCC were PD-L1⁺ ($p = 0.016$) (Table 2) (Fig.1).

Clinicopathological significance of LAG-3 and PD-L1 expression

Both LAG-3⁺ RCC and PD-L1⁺ RCC presented with higher TNM stage and higher Fuhrman nuclear grade (all, $p < 0.05$) (Fig.2). PD-L1⁺ RCC presented with more tumor necrosis ($p = 0.028$) (Table 3). Both LAG-3⁺ mRCC and PD-L1⁺ mRCC presented with more IMDC intermediate and poor risk patients than LAG-3⁺ mRCC and PD-L1⁺ mRCC (all, $p < 0.05$). Only PD-L1⁺ mRCC showed higher TNM stage, higher Fuhrman nuclear grade, more sarcomatoid features, and more tumor necrosis (all, $p < 0.05$).

Relationship of cancer specific survival with LAG-3 and PD-L1 expressions

Patients with LAG-3⁺ RCC had significantly poorer CSS than patients with LAG-3⁻ RCC. The mean CSS was 60.4 months (95% CI: 50.9–69.9) in LAG-3⁺ RCC and 101.4 months (95% CI: 89.1–113.6) in LAG-3⁻ RCC (HR: 3.11; 95% CI: 1.53–6.30; $p = 0.0016$) (Fig. 3a). Similarly, patients with PD-L1⁺ RCC showed significantly poorer CSS than patients with PD-L1⁻ RCC (mean, 63.7 months [95% CI: 53.6–73.7] vs. mean, 96.3 months [95% CI: 84.8–107.8]; HR: 2.31; 95% CI: 1.11–4.77; $p = 0.0236$) (Fig. 3b). The differences between the four subgroups classified according to LAG-3 and PD-L1 expression were not statistically significant, but PD-L1⁺/LAG-3⁺ RCC showed poorer CSS than PD-L1⁻/LAG-3⁺ RCC (mean, 62.7 months [95% CI: 50.9–74.5] vs. mean, 105.4 months [95% CI: 92.5–118.4]; HR: 4.62; 95% CI: 1.80–11.87; $p = 0.0014$). Additionally, PD-L1⁺/LAG-3⁻ RCC showed poorer CSS than PD-L1⁻/LAG-3⁻ RCC (mean, 60.5 months [95% CI: 46.7–74.3] vs. mean, 105.4 months [95% CI: 92.5–118.4]; HR: 6.26; 95% CI: 1.93–20.26; $p = 0.0022$) (Fig. 3c).

Similar results were also observed in mRCC. The median CSS was 13.0 months (95% CI: 6.0–36.0) in LAG-3⁺ mRCC and 43.0 months (95% CI: 9.0–43.0) in LAG-3⁻ mRCC (HR: 2.30; 95% CI: 1.05–5.06; $p = 0.0369$) (Fig. 3d). The median CSS was 22.5 months (95% CI: 13.0–31.9) in PD-L1⁺ mRCC and 11.0 months (95% CI: 5.0–45.0) in PD-L1⁻ mRCC (HR: 2.09; 95% CI: 0.95–4.59; $p = 0.0639$) (Fig. 3e). PD-L1⁺/LAG-3⁺ mRCC showed poorer CSS than PD-L1⁻/LAG-3⁺ mRCC (median, 11.0 months [95% CI: 5.00–45.0] vs. not reached; HR: 2.91; 95% CI: 1.05–8.04; $p = 0.0389$). PD-L1⁺/LAG-3⁻ mRCC also showed poorer CSS than PD-L1⁻/LAG-3⁻ mRCC (median, 30.0 months [95% CI: 4.00–37.0] vs. not reached; HR: 4.74; 95% CI: 1.35–16.61; $p = 0.0149$) (Fig. 3f).

In the univariate analysis, LAG-3⁺, PD-L1⁺, and PD-L1⁺/LAG-3⁺ (all, $p < 0.05$) were significant unfavorable prognostic factors in the whole cohort and in the mRCC group (Supplementary Table 1). Multivariate analysis revealed only PD-L1⁺/LAG-3⁺ mRCC (HR: 3.19; 95% CI: 0.77–13.67; $p = 0.033$) as a predictor of poor CSS (Supplementary Table 2).

Discussion

Recently, several immune checkpoints on tumor-infiltrating ICs, which are key regulators of the immune escape of cancer cells, were studied and clinically applied to the treatment of various solid tumors. In primary ccRCC, PD-1 or PD-L1 inhibitors, which are hallmark immunological treatments, have offered a survival benefit in this decade [14]. However, even after such immunotherapy, a significant number of patients still show refractory disease or acquire resistance [8]. Therefore, demands for new therapeutic targets have emerged, of which is LAG-3 is attracting attention.

LAG-3 is mainly expressed in activated T-cells and natural killer cells and was identified as a marker of CD4⁺ and CD8⁺ T-cell activation [15]. Enhanced LAG-3 expression on T-cells in combination with PD-1 has been reported under pathological conditions, such as inflammation or the tumor microenvironment [9]. In this study, we confirmed that LAG-3 expression in RCC tissue was expressed only in ICs, not TCs. Our study confirmed that LAG-3 was only expressed on ICs, not TCs, of RCC tissue. Similarly, tumor-infiltrating lymphocytes isolated from patients with hepatocellular carcinoma [16], ovarian cancer [17], breast cancer [12], and melanoma [18] showed significant upregulation of LAG-3. Additionally, these studies noted the function of LAG-3 as an immune checkpoint molecule, demonstrating its potential role as a target for cancer immunotherapy in various solid tumors. However, only a limited number of studies examining the role of LAG-3 in kidney cancer using tissues derived from kidney cancer patients have been reported. Giraldo et al. reported the prognostic role of PD-L2 and LAG-3 in the immunomodulation of ccRCC [19]. Zelba et al. used flow cytometry analysis to reveal that PD-1 and LAG-3 were the most frequently upregulated inhibitory receptors within RCC tumor-infiltrating lymphocytes [20]. Most recently, there was a report of poor survival in ccRCC with LAG-3 expression and LAG-3 DNA methylation [21].

The exact mechanism underlying LAG-3 function in cancer treatment, including RCC, has been poorly studied. Fortunately, based on the pan-cancer analysis of The Cancer Genome Atlas dataset, Panda et al. reported that the expression of cytotoxic T-cell marker CD8A was strongly correlated with LAG-3 expression in various cancers, including RCC [22]. This suggests that tumors with strong CD8⁺ T-cell infiltration tend to have a high level of LAG-3 expression. Because CD8⁺ T-cell infiltrates in cancer are correlated with local immune activation and the response to immune checkpoint therapy [23], LAG-3 blockade could be a reasonable strategy for cancer immunotherapy.

In this tissue-based cohort study on surgically resected ccRCC, we found that LAG-3 expression is a prognostic indicator for poor CSS in ccRCC. Unlike previous studies reporting a simple survival analysis according to the LAG-3 expression status [19, 21], our study analyzed the predictive and prognostic implication of LAG-3 in comparison with various clinicopathological features associated with aggressive tumor behavior, including pT stage, grade, lymphovascular invasion, tumor necrosis, and sarcomatoid features, in tissue samples from patients with RCC. In fact, LAG-3 immunopositivity on ICs in tumor tissues was associated with an advanced pT stage and higher Fuhrman nuclear grade. Systemic treatment using ICIs is the main therapeutic approach for mRCC rather than for localized RCC, which can be cured by surgical treatment. Thus, it is more relevant to evaluate the prognostic value of LAG-3 in mRCC. Accordingly,

the subgroup analysis consisted of mRCC patients who were treated with targeted therapy, and we demonstrated poor survival in patients with RCC who had LAG-3⁺ primary tumors.

The major strength of our study was the examination of the correlation between LAG-3 and PD-L1 expression and the synergistic effect on RCC prognosis when both immune checkpoint molecules were expressed simultaneously. The expression rate of PD-L1 on TCs was similar to that of previous studies, but the expression rate of PD-L1 on ICs was relatively lower than previous reports [13, 24]. Differences in patient cohorts, the types of antibodies used in immunohistochemistry experiments, and the selection of cutoffs can contribute to this discrepancy. The expression of PD-L1 on TCs or ICs was positively correlated with LAG-3 expression in our study. Our results demonstrated that PD-L1⁺/LAG-3⁺ RCC patients had a poorer CSS than PD-L1⁺/LAG-3⁻ RCC patients. Furthermore, in the multivariate analysis using a subgroup consisting of mRCC patients only, PD-L1 and LAG-3 coexpression was found to be a significant predictor of poor CSS. Because LAG-3 and PD-1 synergistically regulate T-cell function to promote tumoral immune escape [19], PD-L1⁺/LAG-3⁺ RCC patients could have poorer CSS than PD-L1⁺, LAG-3⁺, or PD-L1⁺/LAG-3⁻ RCC patients. A similar result was reported in a study comparing the relationship between LAG-3 and PD-L1 in non-small cell lung cancer [25].

Recent clinical trials have shown that high levels of PD-L1 expression are associated with a worse prognosis, but when treated with PD-1/PD-L1 inhibitors, patients with higher levels of PD-L1 expression tend to respond better to therapy [26]. However, since a significant number of patients do not respond to PD-1/PD-L1-targeting therapy regardless of their PD-1/PD-L1 expression status, alternative pathways need to be identified to overcome refractory disease or resistance to anti-PD-1/PD-L1 treatment. After blocking PD-1/PD-L1, TCs can still counteract the activity of immune checkpoints and activate additional inhibitory pathways by expressing other immune checkpoints and their ligand within the tumor immune microenvironment [27]. Indeed, LAG-3 cell-surface expression was upregulated *in vitro* upon PD-1 blockade using patient-derived RCC tissue [20]. Fortunately, recent clinical research on melanoma demonstrated that LAG-3 might have a synergistic effect with PD-1/PD-L1, and the combination of anti-LAG-3 and anti-PD-1 treatment revealed exciting effects in tumors that were resistant to previous PD-L1 blockers [28]. In line with these findings, our results that PD-L1⁺/LAG-3⁻ or PD-L1⁺/LAG-3⁺ RCC and mRCC had significantly poorer CSS than PD-L1⁺/LAG-3⁺ RCC and mRCC suggest that anti-LAG-3 monotherapy or a combination of anti-LAG-3 and anti-PD-1/PD-L1 treatment could be beneficial for LAG-3⁺ RCC patients who are refractory or resistant to anti-PD-1/PD-L1 treatment.

Our study had several limitations. First, although we identified the association of LAG-3 expression in ccRCC with known risk factors for survival, some clinicopathological factors were not statistically significant because of our study's retrospective design and relatively small number sample size. Therefore, additional prospective studies with a large patient cohort are necessary to confirm our results. Second, the TMA of the primary tumor tissue cannot completely reflect the immune markers analyzed in the tumor microenvironment. Additionally, although we showed LAG-3 and PD-L1 expression on ICs, we could not evaluate the IC type-specific expression of either checkpoint molecule. Third, despite recent advances in ICIs in the treatment of mRCC, our study only includes a survival analysis of mRCC patients who were treated with targeted therapy. However, because previous studies show the poor prognostic role of PD-L1

expression in mRCC patients who were treated with targeted therapy, our results of the analysis of the prognostic role of LAG-3 in ccRCC and mRCC could be clinically relevant [29, 30]. In the near future, the results of ongoing clinical trials using an anti-LAG-3 mAb, such as relatlimab, will be used to determine the prognostic role of LAG-3 for various solid tumors, including RCC [11].

In conclusion, our study demonstrated that LAG-3 and PD-L1 expression in ccRCC is positively correlated with adverse clinicopathological features. Additionally, the coexpression of LAG-3 and PD-L1 predicts poor clinical outcomes in ccRCC. These findings provide a scientific rationale for LAG-3 blockade in combination with PD-1/PD-L1 blockade as a potential therapeutic approach for ccRCC. Data from ongoing clinical trials are required to validate these hypotheses.

Abbreviations

ccRCC Clear cell renal cell carcinoma

CSS Cancer specific survival

ICI Immune check point inhibitor

ICs Immune cells

IMDC International Mestastatic Renal cell carcinoma databe consortium

LAG-3 Lymphocyte-activation gene 3

mRCC Metastatic renal cell carcinoma

mTOR Mammalian target of rapamycin

PD-L1 Programmed death ligand-1

TCs Tumor cells

TKI Tyrosin kinase inhibitor

TMA Tissue microarray

Declarations

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Data availability: The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval: This study was approved by the Busan Paik Hospital Institutional Review Board (IRB No. 20-0121) and was in compliance with ethical guidelines according to the Declaration of Helsinki.

Consent to participate: Informed consent was waived by the Busan Paik Hospital Institutional Review Board on the grounds of being a retrospective study using tumor tissue already archived by the Inje Biobank

Consent for publish: This study did not include any form of personally identifiable data.

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Tables

Table 1 Clinicopathologic characteristics for renal cell carcinoma patients

Characteristic	Whole cohort	Metastatic RCC
	N = 134	N = 45
Age at surgery, years (median, IQR)	61 (34 - 82)	63 (40 - 81)
Gender, n (%)		
Male	94 (70.1)	32 (71.1)
Female	40 (29.9)	13 (28.9)
Tumor size, cm (median, IQR)	5.5 (1.2 - 13)	6.5 (1.2 - 9.2)
T stage, n (%)		
pT1	78 (58.2)	12 (26.7)
pT2	19 (14.2)	9 (20.0)
pT3	33 (24.6)	21 (46.7)
pT4	4 (3.0)	3 (6.7)
N stage, n (%)		
cN0	122 (91.0)	35 (77.8)
cN1	12 (9.0)	10 (22.2)
M stage, n (%)		
cM0	109 (81.3)	22 (48.9)
cM1	25 (18.7)	23 (51.2)
Stage, n (%)		
Stage I	72 (53.7)	6 (13.3)
Stage II	15 (11.2)	5 (11.1)
Stage III	22 (16.4)	11 (24.4)

Stage IV	25 (18.7)	23 (51.1)
Fuhrman nuclear grade, n (%)		
G1 - G2	65 (48.5)	10 (22.2)
G3	51 (38.1)	20 (44.4)
G4	18 (13.4)	15 (33.3)
Lymphovascular invasion, n (%)		
No	118 (88.1)	34 (75.6)
Yes	16 (11.9)	11 (24.4)
Sarcomatoid features, n (%)		
No	126 (94.0)	39 (86.7)
Yes	8 (6.0)	6 (13.3)
Tumor necrosis, n (%)		
No	103 (76.9)	24 (53.3)
Yes	31 (23.1)	21 (46.7)
IMDC risk classification		
Favorable	-	20 (44.4)
Intermediate	-	10 (22.2)
Poor	-	15 (33.3)

Table 2 Expression of LAG-3 and PD-L1 in clear cell renal cell carcinoma

Characteristics	LAG-3 in Whole cohort				LAG-3 in Metastatic RCC			
	Total	Positive	Negative	<i>p</i> value	Total	Positive	Negative	<i>p</i> value
PD-L1 in ICs, n (%)								
Positive	45 (33.6)	34 (50.0)	11 (16.7)	<0.001	20 (44.4)	16 (51.6)	4 (28.6)	0.154
Negative	89 (66.4)	34 (50.0)	55 (83.3)		25 (55.6)	15 (48.4)	10 (71.4)	
PD-L1 in TCs, n (%)								
Positive	34 (25.4)	25 (36.8)	9 (13.6)	0.002	17 (37.8)	15 (48.4)	2 (14.3)	0.030
Negative	100 (74.6)	43 (63.2)	57 (86.4)		28 (62.2)	16 (51.6)	12 (85.7)	
PD-L1 in overall, n (%)								
Positive	59 (44.0)	44 (64.7)	15 (22.7)	<0.001	25 (55.6)	21 (67.7)	4 (28.6)	0.016
Negative	75 (56.0)	24 (35.3)	51 (77.3)		20 (44.4)	10 (32.3)	10 (71.4)	

Table 3 Relationship of LAG-3 expression and clinicopathologic features in clear cell renal cell carcinoma

Characteristic	Whole cohort				Metastatic RCC			
	LAG-3 positive	<i>p</i> value	PD-L1 positive	<i>p</i> value	LAG-3 positive	<i>p</i> value	PD-L1 positive	<i>p</i> value
	n = 68		n = 59		n = 31		n = 25	
Pathologic T stage, n (%)								
pT1	31 (45.6)	0.006	28 (47.4)	0.008	7 (22.6)	0.210	3 (12.0)	0.002
pT2	12 (17.7)		8 (13.6)		5 (16.1)		4 (16.0)	
pT3	23 (33.8)		20 (33.9)		17 (54.8)		15 (60.0)	
pT4	2 (2.9)		3 (5.1)		2 (6.5)		3 (12.0)	
Clinical N stage, n (%)								
cN0	60 (88.2)	0.249	50 (84.7)	0.024	23 (74.2)	0.394	16 (64.0)	0.014
cN1	8 (11.8)		9 (15.3)		8 (25.8)		9 (36.0)	
Clinical M stage, n (%)								
cM0	50 (73.5)	0.018	41 (69.5)	0.002	13 (41.9)	0.169	7 (28.0)	0.002
cM1	18 (26.5)		18 (30.5)		18 (58.1)		18 (72.0)	
Stage, n (%)								
Stage I	27 (39.7)	0.001	26 (44.1)	0.002	3 (9.7)	0.076	1 (4.0)	<0.001
Stage II	9 (13.2)		4 (6.8)		2 (6.5)		0 (0.0)	
Stage III	14 (20.6)		11 (18.6)		8 (25.8)		6 (24.0)	
Stage IV	18 (26.5)		18 (30.5)		18 (58.1)		18 (72.0)	
Fuhrman nuclear grade, n (%)								
G1 - G2	25 (36.8)	0.004	19 (32.2)	<0.001	6 (19.4)	0.264	2 (8.0)	<0.001
G3	30 (44.1)		27 (45.8)		13 (41.9)		10 (40.0)	

G4	13 (19.1)		13 (22.0)		12 (38.7)		13 (52.0)	
Lymphovascular invasion, n (%)								
No	57 (83.8)	0.125	50 (84.7)	0.296	22 (71.0)	0.292	17 (68.0)	0.192
Yes	11 (16.2)		9 (15.3)		9 (29.0)		8 (32.0)	
Sarcomatoid features, n (%)								
No	63 (92.6)	0.495	53 (89.8)	0.069	27 (87.1)	0.900	19 (76.0)	0.020
Yes	5 (7.4)		6 (10.2)		4 (12.9)		6 (24.0)	
Tumor necrosis, n (%)								
No	48 (70.6)	0.081	40 (67.8)	0.028	16 (51.6)	0.733	10 (40.0)	0.048
Yes	20 (29.4)		19 (32.2)		15 (48.4)		15 (60.0)	
IMDC risk classification								
Favorable	-	-	-	-	10 (32.3)	0.045	6 (24.0)	<0.001
Intermediate	-		-		9 (29.0)		6 (24.0)	
Poor	-		-		12 (38.7)		13 (52.0)	

Figures

Figure 1

Expression of lymphocyte-activation gen 3 (LAG-3) by immunohistochemistry according to Fuhrman nuclear grade of clear cell renal cell carcinoma. Fuhrman grade 2 (a), grade 3 (b), grade 4 (c), sarcomatous differentiation (d). Original magnification, x200

Figure 2

Expression of lymphocyte-activation gen 3 (LAG-3) and programmed cell death ligand-1 (PD-L1) by immunohistochemistry on serial section of same tissue. (a) LAG-3 and PD-L1 in immune cells (ICs) (b) LAG-3 in ICs and PD-L1 in tumor cells (TCs)

Figure 3

Kaplan-Meier curves of cancer specific survival (CSS) in the whole cohort and metastatic renal cell carcinoma according to expression of lymphocyte-activation gen 3 (LAG-3) and programmed cell death ligand-1 (PD-L1). CSS in patients with LAG-3+ (a) and PD-L1+ (b) were significantly lower than with LAG-3- and PD-L1- ($p=0.0016$, $p=0.0236$). PD-L1+/LAG-3+ group was showed significantly lower CSS than PD-L1-/LAG-3-, PD-L1-/LAG-3+ and PD-L1+/LAG-3- groups ($p=0.0014$, $p=0.0022$, $p=0.0474$) (c) in whole cohort. In metastatic RCC group, LAG-3+ patients were significantly lower than LAG-3- patients ($p=0.0369$) (d) and PD-L1+ patients were showed lower CSS than PD-L1- ($p=0.0639$) (e). PD-L1+/LAG-3+ group was showed significantly lower CSS than PD-L1-/LAG-3-, PD-L1-/LAG-3+ and PD-L1+/LAG-3- groups ($p=0.0389$, $p=0.0149$, $p=0.0401$) (f)

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementaryTable1.UnivariateandmultivariateCoxproportionalanalysisallpatients.docx](#)
- [SupplementaryTable2.UnivariateandmultivariateCoxproportionalanalysisinRCCpatients.docx](#)