Expression and clinical significance of interleukin-9 in renal tumors

Zhang Zhiyu

First Affiliated Hospital of Soochow University

Ouyang Jun

First Affiliated Hospital of Soochow University

Pu Jinxian

First Affiliated Hospital of Soochow University

Hou Jianquan

First Affiliated Hospital of Soochow University

Jianglei Zhang (✉ yooseen@126.com)

First Affiliated Hospital of Soochow University

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Abstract

Background To measure expression levels of interleukin-9 (IL-9) in renal tumors and to determine the clinical significance of those levels.

Methods We enrolled 66 patients who underwent surgical resection of renal tumors between January and December 2018 at the First Affiliated Hospital of Soochow University. Their tumor tissues were paired with adjacent normal tissues and IL-9 expression levels were measured using immunohistochemistry. We determined the correlation of IL-9 expression with clinicopathological features and progression-free survival (PFS).

Results Expression of IL-9 in renal tumors was significantly lower than in adjacent normal tissues ($P < 0.0001$). There was a significant negative correlation between IL-9 expression levels and R.E.N.A.L. scores ($P = 0.0277$) as well as with differentiation ($P = 0.0041$). However, no significant correlation was found between IL-9 levels and clinicopathological features, including gender ($P = 0.0716$), age ($P = 0.02566$), BMI ($P = 0.7941$), tumor size ($P = 0.4193$) or TNM staging ($P = 0.5402$). PFS time in renal tumor patients with positive IL-9 expression was similar to that of patients with negative IL-9 expression.

Conclusions IL-9 expression was higher in adjacent normal tissues than in renal tumors. Low expression of IL-9 was detected when R.E.N.A.L. score was high or cell differentiation was poor, suggesting that IL-9 may positively participate in renal tumor microenvironments.

Background

Renal cell carcinoma (RCC) originates from the epithelial system of renal parenchyma and urinary tubules. Renal clear cell carcinoma (RCCC) is the most common type. Currently, treatment of renal tumors consists of surgical resection and targeted drug therapy. Nevertheless, there remains 30%–40% of patients who experience metastasis or recurrence [1]. Some studies have found that destruction of the immune microenvironment is associated with RCCC occurrence [2–3]. This suggests that immunotherapy can be used as a treatment method for RCCC.

Interleukin-9 (IL-9) is a cytokine secreted mainly by T cells; it has a directional regulatory effect on tumors [4]. IL-9 significantly inhibited the growth of melanoma and squamous cell carcinoma in mice [5–6]. IL-9 levels were low on pathological examination of colon cancer tissues; however, levels were high in adjacent normal tissues [7], suggesting that IL-9 had an anti-tumor effect. Nevertheless, high expression of IL-9 was measured in cells from liver cancer, pancreatic cancer and chronic lymphoblastic leukemia, suggesting that IL-9 may accelerate the pathogenesis of some tumors [4,8–9]. The relationship between IL-9 and RCCC has not yet been reported. Therefore, the purpose of this study was to measure expression of IL-9 in RCCC and adjacent normal tissue, and to determine correlation between IL-9 expression and clinicopathological characteristics and progression-free survival (PFS).

Methods

General information

Patients who underwent surgical resection of renal tumors between January and December 2018 at the First Affiliated Hospital of Suzhou University with a pathology diagnosis of RCC were enrolled. Exclusion criteria included a history of autoimmune disease or infectious disease. A total of 66 patients were enrolled, including 40 males and 26 females, age 35–82 years, with an average age of 61.62 ± 10.87 . There were 40 patients in which the tumor was less than 4 cm in diameter, 17 in which the diameters were 4 cm to 7 cm, and nine patients in which diameters were greater than 7 cm. There were 27 patients with R. E. N. A. L. scores of less than 6 and 37 patients with scores of more than 6. According to Fuhrman's histological classification, 24, 28, 11 and 3 tumors were classified as grade I, II, III and IV respectively. According to TNM staging of American Cancer Federation in 2010, there were 46 tumors in stage I and 20 in stage II.

Immunohistochemistry

Specimen collection

We collected specimens from renal tumors and corresponding adjacent normal tissues (more than two centimeters away from the edge of the tumor and no invasion of tumor cells confirmed on post-surgical pathology). The specimens were fixed in formalin (10% formaldehyde solution) and then embedded in paraffin and sectioned for archiving.

Immunohistochemistry (SP method)

Preserved paraffin slices were sequentially treated by dewaxing, hydration, phosphate buffer saline (PBS) washing and rinsing with citric acid buffer. To inactivate endogenous peroxidase, 3% hydrogen peroxide was dropped onto the slices, maintained at room temperature for 10 minutes and washed again in PBS. Subsequently, 5% bovine serum albumin (BSA) 50 μ l were added to the slides and incubated at room temperature for 10 minutes. After adding primary antibody against IL-9 (diluted at 1:200), the slices were incubated overnight at 4 °C, and washed with PBS thereafter. Next, second antibody was added, incubated at room temperature for 60 minutes and then washed with PBS again. Then sections were reacted with DAB color reagent for 5 minutes, re-stained with hematoxylin, dehydrated with a gradient concentration of ethanol, and finally sealed with neutral resin for microscopic observation.

Criteria for determining results

Because IL-9 is mainly expressed in the cytoplasm, the appearance of pale yellow, brown yellow or brown granules in the cytoplasm at 200-fold magnification was considered positive. For a section judged positive, ten microscopic views with 400-fold magnification were randomly observed and scored according to the proportion of positive cells and staining intensity. Specifically, when the proportion of positive staining cells was less than 5%, 5%–30%, 31%–60% and more than 60%, the scores were zero, one, two and three, respectively. The scoring criteria for staining intensity were as follows: colorless scored zero, light yellow scored one, brown yellow scored two and brown scored three. Finally, the score of positively stained cell proportion was multiplied by the staining intensity score, and the product of less than two points were classified as negative (-); otherwise it was considered positive (+) [10].

Follow-up

Patients were followed up for six to seven months with the deadline of June 1, 2019. Progression-free survival (PFS) was defined as the time from the day of surgery to the deadline or the time point when progression was observed. Patients with stage T1-T2 were followed up every six weeks after surgery, then every six months for up to three years, and annually thereafter. Patients with stage T3-T4 were followed up every six weeks after surgery, every three months up to two years, then every six months from the third year and annually thereafter. Follow-up was conducted by telephone and outpatient consultation.

Statistical analysis

The quantitative data were expressed as mean $\pm$ standard deviation (SD). Qualitative data were expressed as ratios; comparisons between two groups were determined using the χ^2 test. Statistical analyses were performed using SPSS 22.0. GraphPad Prism 8.0 was used to draw survival curves. Kaplan-Meier and Log-rank tests were used for survival analysis. All *p*-values were considered statistically significant if $P < 0.05$.

Results

Expression of IL-9 in RCCC and adjacent tissues

Scores of negative and positive expression of IL-9 in cancer tissues were 59 and 7, respectively, and the positive rate was 10.61% (Fig. 1 and Table 1). By contrast, the scores negative and positive expression of IL-9 in adjacent tissues were 5 and 61, respectively, and the positive rate was 92.42%. This suggests that expression of IL-9 was low in RCCC tissues but high in adjacent tissues. The difference was statistically significant ($P < 0.0001$).

Correlation between clinicopathological features and expression of IL-9 in RCCC

Gender, body mass index (BMI), age, tumor diameter and TNM stage did not correlate with IL-9 expression in renal tumors ($P > 0.05$). Patients with R. E. N. A. L. scores ≤ 6 showed significantly higher IL-9 expression than did patients with R. E. N. A. L. score > 6 ($P = 0.0277$). The expression of IL-9 in tumor cells with medium-to-low differentiation was significantly lower than that of those with high differentiation ($P = 0.0041$).

The correlation between expression of IL-9 and PFS in RCCC

Patients were followed up for 5 to 17 months and the follow-up rate was 100%. PFS was 87.88% (58/66) in 66 RCCC patients until the deadline. PFS of RCCC patients with negative expression of IL-9 was 88.14% (52/59), while that of patients with positive expression was 85.71% (6/7). Because of the limited number of patients, univariate survival analysis did not reveal a significant difference in survival time between patients with positive and negative expression in RCCC (Kaplan-Meier:² = 0.2301, $P = 0.6314$, Log-rank test:² = 0.06847, $P = 0.7936$, Fig. 2).

Discussion

Our study is the first study of IL-9 in RCCC. IL-9, a cytokine with several biological effects, is primarily synthesized by activated Th9 cells and is secreted by neutrophils and mast cells in small amounts. IL-9 promotes the transcription and expression of related genes in the nucleus and plays a corresponding biological role by activating signal transducers and activator of transcription 1 (STAT1), STAT3, and STAT5 [11–12]. Studies have found that the effector cells of IL-9, including cytotoxic T lymphocytes (CTL), dendritic cells (DC) and mast cells, play important roles in promoting cell inflammation, mediating immune diseases and tumor immunity.

In the present study, we found that the expression of IL-9 in RCCC was significantly lower than that of adjacent normal tissues. The relationship between IL-9 and tumors has not been fully clarified. On the one hand, IL-9 has been reported to inhibit the growth of melanoma and squamous cell carcinoma [5–7], possibly attributed to IL-9 in stimulating DC cells to enter sentinel lymph nodes, activating CTL cell antigens to initiate and intrude tumor tissues. On the other hand, high expression of IL-9 has been measured in the blood of patients with hematological malignancies such as lymphoma [16], and the use of antibodies to block IL-9 with antibodies slowed the progress of tumors [17]. These data suggest that IL-9 could also promote tumor progression [8–9]. In the present study, IL-9 was expressed at low levels in RCCC but was highly-expressed in adjacent normal tissues, suggesting that IL-9 may inhibit the growth of RCCC. This could be explained by the fact that the kidney, an important excretory organ with

abundant perfusion, is more conducive to infiltration and aggregation of immune cells, and it secretes a variety of cytokines including IL-9. Nevertheless, RCCC is mainly composed of renal parenchymal cells, and the immune effector cells in them are in a state of inhibition, resulting less secretion of IL-9.

We found that expression of IL-9 in RCCC was not significantly correlated with clinicopathological features such as gender, age, BMI, tumor diameter or TNM stage. Nevertheless, several related studies have found that the expression of IL-9 in peripheral blood of patients with laryngeal squamous cell carcinoma and small cell lung cancer were higher than those of normal controls, and the expression levels of IL-9 in patients with stage III and IV were higher than those of patients with stage I and II, suggesting that IL-9 may correlate with tumor staging [18–19]. In the present study, we found that expression levels of IL-9 negatively correlated with the R. E. N. A. L. scores and tumor differentiation, suggesting that the R. E. N. A. L. scoring system can be used not only as an auxiliary reference for the evaluation of surgical difficulty of renal tumors, but also for expression of IL-9 in tumors. Considering the limitation of a single center and small sample size in this study, the relationship between the R. E. N. A. L. scoring system and IL-9 expression in renal tumors remains to be confirmed in multi-center and large-volume studies. Consistent with previous findings, we found that poor tissue differentiation correlated with lower expression levels of IL-9, suggesting that IL-9 may inhibit occurrence and progression of renal tumors. Future well-designed studies of its potential mechanisms will be also needed to confirm this. Huang et al. found that PFS of colon cancer patients with positive expression of IL-9 was not superior to that of patients with negative expression; nevertheless, the authors found that survival time of patients with high expression of IL-9 was significantly longer than that of patients with low expression [10], suggesting that expression of IL-9 may correlate with survival after surgery. In future studies, we will expand the sample size and extend the follow-up time to further investigate the relationship between IL-9 and PFS.

Conclusion

In summary, IL-9 may have anti-tumor effects on RCCC; IL-9 levels correlated with R. E. N. A. L. scores and tumor differentiation. The immune mechanism of IL-9 against RCCC still needs to be investigated. There were some limitations in our study, including the small size and short follow-up period, both of which need to be remedied in the future.

Abbreviations

IL-9 interleukin-9; renal cell carcinoma RCC; renal clear cell carcinoma RCCC; progression-free survival PFS; phosphate buffer saline PBS; bovine serum albumin BSA; standard deviation SD; body mass index BMI; signal transducers and activator of transcription 1 STAT1; cytotoxic T lymphocytes CTL; dendritic cells DC

Declarations

Ethics approval and consent to participate

Our study was approved by the ethics committee of the First Affiliated Hospital of Soochow University. All the patients signed the consent form before surgery.

Availability of data and materials

All the data can be found by contacting corresponding author's e-mail.

Competing interests

Not applicable.

Funding

Not applicable.

Authors' Contributions

All the authors contributed equally to the study.

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Tables

Table 1. IL-9 expression in RCCC and corresponding adjacent normal tissues

Tissue	IL-9 expression [case (%)]		χ^2	P
	Positive	Negative		
Tumor tissues	7 (10.61)	59 (89.39)	88.44	<0.0001
Adjacent tissue	61 (92.42)	5 (7.58)		

Table 2. Correlation between expression of IL-9 and clinicopathological features of RCCC

Clinicopathological features	Cases	Expression of IL-9 [case (%)]		χ^2	P
		Positive	(negative		
Gender				3.247	0.0716
Male	40	3 (7.50)	27 (92.50)		
Female	26	6 (23.08)	20 (76.92)		
BMI (kg/m ²)				0.06811	0.7941
≤24	34	5 (14.71)	29 (85.29)		
>24	32	4 (12.50)	28 (87.50)		
Age				1.287	0.2566
≤65	40	7 (17.50)	33 (82.50)		
>65	26	2 (7.69)	24 (92.31)		
Diameters of tumors (cm)				0.6523	0.4193
≤7	57	7 (12.28)	50 (87.72)		
>7	9	2 (22.22)	7 (77.78)		
R.E.N.A.L. score				4.844	0.0277
≤6	29	7 (24.14)	22 (75.86)		
>6	37	2 (5.41)	35 (94.59)		
Degree of differentiation				8.234	0.0041
High	24	6 (25.00)	18 (75.00)		
medium to low	42	1 (2.38)	41 (97.62)		
TNM stage				0.01112	0.9160
I	46	5(10.87)	41(89.13)		
II	20	2 (10.00)	18 (90.00)		

Figures

Figure 1

Expression of IL-9 in RCC and adjacent normal tissues (SP*100). A: Negative expression of IL-9 in RCC; B: Positive expression of IL-9 in RCC; C: Negative expression of IL-9 in adjacent tissues; D: Positive expression of IL-9 in adjacent tissues.

Figure 2

Survival curve of patients with negative and positive IL-9 expression in RCCC.