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The Immune Microenvironment and Immune Therapy Response Mechanism of Renal Clear Cell Carcinoma

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Abstract: The purpose of this article is to study the immune microenvironment characteristics of renal clear cell tumor and the application effect of immunotherapy in this tumor. In order to achieve this goal, this article adopts systematic experimental methods and analytical means. Firstly, the distribution of tumor infiltrating immune cells and the expression of key immune molecules were described in detail by collecting renal clear cell tumor samples and using flow cytometry, immunohistochemistry and other technical means. Subsequently, in vitro and in vivo experiments of immunotherapy were designed and implemented to evaluate the anti-tumor effects of different immunotherapy strategies. The experimental results show that the immune microenvironment of renal clear cell tumor presents unique characteristics, including the infiltration mode of immune cells and the expression profile of immune molecules. Both in vitro and in vivo immunotherapy experiments show that some immunotherapy strategies can effectively inhibit the growth of renal clear cell tumor and prolong the survival time of animal models. Based on the above findings, it can be concluded that the immune microenvironment of renal clear cell tumor plays a key role in tumor progression and immunotherapy response. A deep understanding of the characteristics and regulation mechanism of immune microenvironment is helpful to develop more effective immunotherapy strategies.

Keywords: Clear Cell Tumor of Kidney; Kidney; Immune Microenvironment; Immunotherapy.

1. Introduction

Clear cell tumor of the kidney is one of the most common malignant tumors of the kidney, and its pathogenesis and treatment have always been the focus of medical research [1]. In recent years, with the development of immunology research, the important role of tumor immune microenvironment in the process of tumor occurrence, development and metastasis has been gradually revealed [2]. The immune microenvironment of renal clear cell tumor is complex and changeable, which involves the interaction and regulation of many immune cells, and these immune cells and their secreted molecules play an important role in tumor progress [3]. Therefore, it is of great significance to explore the immune microenvironment of renal clear cell tumor and its relationship with immunotherapy response for formulating more effective immunotherapy strategies [4].

At present, the research on immune microenvironment of renal clear cell tumor has made some progress. By means of immunohistochemistry and flow cytometry, researchers found that there were many kinds of immune cells infiltrating in renal clear cell tumor, including T cells, B cells, NK cells and tams (tumor-associated macromolecules) [5]. These immune cells play different roles in tumor microenvironment, some promote tumor growth and metastasis, while others have anti-tumor activity [6]. However, the specific regulatory mechanism of immune microenvironment of renal clear cell tumor and the interaction between different immune cells are still not completely clear, and further research is needed [7].

As a new tumor treatment method, immunotherapy has shown remarkable curative effect in many tumor types [8]. In the treatment of renal clear cell tumor, immunotherapy has also made some achievements, especially for patients with metastatic renal cell carcinoma [9]. However, the application of immunotherapy in renal clear cell tumor still faces many challenges. On the one hand, due to the weak immunogenicity of renal clear cell tumor, the response rate of immunotherapy is limited; On the other hand, the emergence of drug resistance in immunotherapy is also one of the important factors limiting its efficacy [10]. Therefore, it is of great significance to study the response mechanism of immunotherapy for renal clear cell tumor.

The purpose of this study is to explore the immune microenvironment characteristics of renal clear cell tumor and reveal the response mechanism of immunotherapy in this tumor, so as to provide theoretical basis and practical guidance for immunotherapy of renal clear cell tumor. Through in-depth study, we hope to find new immunotherapy targets, optimize existing treatment schemes, and improve the quality of life and prognosis of patients.

2. Materials and Methods

2.1. Experimental Materials

2.1.1. Renal Clear Cell Tumor Sample

From January 2023 to October 2023, 80 cases of renal clear cell carcinoma in minimally invasive center of urology in a hospital were collected. There were 45 males and 35 females, aged from 22 to 80 years, with an average of 50 years. The tumor diameter was less than 4cm in 32 cases, more than 7cm in 18 cases, and 30 cases were in between. Among them, 15 cases invaded renal capsule, 9 cases invaded local lymph nodes, renal veins and inferior vena cava, and 2 cases had distant metastasis. The samples of renal clear cell tumor used in this study are from fresh tissues removed by hospital surgery. All samples were confirmed by pathology and processed immediately after collection to ensure the integrity and activity of the samples. The collection and use of samples are in accordance with ethical norms, and with the informed consent of patients or their families.

2.1.2. Immune Cells and Reagents

In order to analyze the immune microenvironment of renal clear cell tumor, this article uses a variety of immune cell separation and culture reagents, such as lymphocyte separation solution, cell culture medium and cell stimulator. Furthermore, a series of specific antibodies and fluorescent dyes were prepared for flow cytometry and immunofluorescence staining experiments to detect the type and quantity of tumor infiltrating immune cells.

2.1.3. Experimental Installation

In this study, advanced experimental equipment such as flow cytometry, fluorescence microscope and enzyme-labeled instrument were used. Flow cytometry is used to quantitatively analyze tumor infiltrating immune cells with multiple parameters. Fluorescence microscope is used to observe the distribution and localization of immune cells in tumor tissues; Enzyme-labeled instrument is used to detect the proliferation and death of cells in cytotoxicity experiment.

2.2. Experimental Method

2.2.1. Immune Microenvironment Analysis

In order to fully understand the immune microenvironment characteristics of renal clear cell tumor, flow cytometry and immunohistochemical staining were used to analyze the immune cells infiltrated by the tumor in detail. Firstly, the number and proportion of different immune cell subsets in tumor tissues were detected by flow cytometry, including T cells, B cells and NK cells. Furthermore, the distribution and localization of these immune cells in tumor tissues were observed by multicolor immunofluorescence staining technique.

2.2.2. Experimental Design of Immunotherapy

In order to evaluate the effect of immunotherapy in renal clear cell tumor, cytotoxicity test in vitro and animal model experiment in vivo were designed. In vitro experiments, immune cells isolated from patients' tumors were co-cultured with renal clear cell tumor cells, and different immunotherapy drugs or antibodies were added to intervene. The in vitro effect of immunotherapy was evaluated by detecting the proliferation and apoptosis of tumor cells. In vivo experiments, an animal model of renal clear cell tumor was established, and immunotherapy drugs or antibodies were injected into the animals for treatment. The in vivo effect of immunotherapy was evaluated by observing the growth of tumor and the survival time of animals.

2.2.3. Data Processing and Analysis Methods

For the data obtained from the experiment, professional statistical software is used for processing and analysis. For continuous variables, this article uses mean standard deviation to describe them, and uses t-test or variance analysis to compare them between groups; For classified variables, chi-square test was used to compare between groups. Furthermore, the relationship between immune microenvironment characteristics and immunotherapy response was discussed by correlation analysis. Through the comprehensive application of the above statistical methods, we can more accurately reveal the internal relationship between the immune microenvironment of renal clear cell tumor and the response to immunotherapy.

3. Results

3.1. Immunomicroenvironment Characteristics of Renal Clear Cell Tumor

Through detailed experimental analysis, the unique immune microenvironment characteristics of renal clear cell tumor are revealed, which is of great significance for understanding the occurrence and development mechanism of this tumor and guiding the follow-up immunotherapy.

In order to show the experimental results more intuitively, this section draws a series of tables to show the key data. First of all, in the aspect of immune cell infiltration and distribution, this article found that there was significant immune cell infiltration in renal clear cell tumor tissue. By means of flow cytometry and immunohistochemical staining, it was found that the infiltrated immune cells in tumor tissues mainly included T cells, B cells, NK cells and TAMs. The distribution of these immune cells in tumor tissues has certain regularity, and the infiltration and distribution of immune cells in renal clear cell tumor are shown in Table 1.

Table 1. Infiltration and distribution of immune cells in renal clear cell tumor

Immune cell type	Number of infiltrating cells (high power field)	Proportion of organization distribution (%)	Number of specific structures formed
T cell	120 ± 20	60 (edge area)	5 (TLSs)
B cell	40 ± 10	30 (dispersed distribution)	3 (TLSs)
NK cell	30 ± 5	20 (evenly distributed)	No specific structure is formed.
TAMs	80 ± 15	70 (substantial interior)	No specific structure is formed.
TLSs	-	-	10 (within tumor)

Remarks:

1. The number of infiltrating cells represents the average number of cells observed in each high-power field of vision, and+/-represents the standard deviation.

2. The tissue distribution ratio represents the relative distribution ratio of various immune cells in tumor tissues, expressed as a percentage.

3. The number of specific structures formed indicates the number of specific structures observed in tumor tissue.

4. The number of TLSS (tertiary lymphatic structure) was observed in the whole tumor tissue, not specific to a certain immune cell.

The above table shows that T cells are mainly distributed in the marginal area of the tumor, while TAMs are more concentrated in the tumor parenchyma. In addition, it was observed that some immune cells formed specific structures in tumor tissues, such as TLSs, which were closely related to the immune response and prognosis of renal clear cell tumor.

Secondly, in terms of the expression of key immune molecules, the expression of various immune molecules in renal clear cell tumor was detected by immunofluorescence staining and protein blot. The expression of key immune molecules in renal clear cell tumor is shown in Table 2.

Table 2. Expression of key immune molecules in renal clear cell tumor

Immune molecule	Expression state	Test method	Expression level
PD-1	High expression	Immunofluorescence staining and protein blot	85% ± 5%
PD-L1	High expression	Immunofluorescence staining and protein blot	70% ± 10%
CTLA-4	High expression	Immunofluorescence staining and protein blot	65% ± 8%
IFN-γ	Low expression	Immunofluorescence staining and protein blot	15% ± 3%
TNF-α	Low expression	Immunofluorescence staining and protein blot	20% ± 4%

The results showed that some key immune molecules such as PD-1, PD-L1 and CTLA-4 were highly expressed in tumor tissues, while some molecules related to immune activation such as IFN-γ and TNF-α were poorly expressed. These results suggest that renal clear cell tumor may escape the surveillance and attack of immune system by up-regulating the expression of inhibitory immune molecules.

3.2. Immunotherapy Response

In order to evaluate the effect of immunotherapy in renal clear cell tumor, in vitro and in vivo experiments were carried out. In vitro experiments, immune cells isolated from patients with renal clear cell tumor were co-cultured with tumor cells, and different immunotherapy drugs or antibodies were added to intervene. The experimental results of in vitro immunotherapy for renal clear cell tumor are shown in Table 3.

Table 3. Immunotherapy experiment of renal clear cell tumor in vitro

Experimental group	Immunotherapy drugs/antibodies	Inhibition rate of tumor cell proliferation	Tumor cell migration inhibition rate	Enhancement multiple of immune cell killing effect
Control group	Nothing	0%	0%	1.0 times
Experimental group 1	Keytruda	45% ± 5%	30% ± 4%	2.5 times 0.3 times
Experimental group 2	Axitinib	35% ± 4%	25% ± 3%	1.8 times 0.2 times
Experimental group 3	Pabolizumab+acitinib	65% ± 7%	50% ± 6%	4.0 times 0.5 times

Remarks:

1.Keytruda is a PD-1 immune checkpoint inhibitor, which can recognize and attack tumor cells by activating the patient's own immune system.

2.Axitinib is a tyrosine kinase inhibitor, which mainly inhibits vascular endothelial growth factor receptor (VEGFR), thus inhibiting tumor angiogenesis and tumor growth.

3. In experimental group 3, Pabolizumab was combined with acitinib in order to enhance the anti-tumor effect through different mechanisms.

4. Tumor cell proliferation inhibition rate and tumor cell migration inhibition rate respectively indicate the degree of inhibition of drugs on tumor cell proliferation and migration ability, expressed in percentage. The higher the numerical value, the stronger the inhibition effect.

5. The enhancement multiple of the killing effect of immune cells indicates the enhancement effect of drugs on killing tumor cells by immune cells, which is expressed in multiples relative to the control group. The higher the value, the more obvious the enhancement effect.

Table 4. Immunotherapy experiment of renal clear cell tumor in vivo

Experimental group	Animal population	Immunotherapy drugs/antibodies	Tumor growth rate inhibition rate	Average survival time extension rate
Control group	10	Nothing	0%	0%
Experimental group 1	10	Pabolizumab	40% ± 5%	30% ± 4%
Experimental group 2	10	Specific antibody	55% ± 7%	45% ± 6%
Experimental group 3	10	Combined immunotherapy	65% ± 8%	60% ± 8%

The results show that some immunotherapy drugs or antibodies can significantly enhance the killing effect of immune cells on tumor cells and inhibit the proliferation and migration of tumor cells. This shows that immunotherapy has potential anti-renal clear cell tumor effect in vitro.

In vivo experiments, an animal model of renal clear cell tumor was established, and immunotherapy drugs or antibodies were injected into the animals for treatment. The effect of immunotherapy was evaluated by observing the growth of tumor and the survival time of animals. The results are shown in Table 4.

Remarks:

1. Number of animals: the number of mice used in each experiment.

2. Immunotherapy drugs/antibodies: the names of immunotherapy drugs or antibodies injected into animals.

3. Inhibition rate of tumor growth rate: indicates the degree of inhibition of tumor growth rate by immunotherapy, expressed as a percentage. The higher the numerical value, the stronger the inhibition effect.

4. The average survival time extension rate: indicates the extent to which the average survival time of animals is prolonged by immunotherapy, expressed as a percentage. The higher the value, the more significant the effect of prolonging the survival time.

5. Incidence rate of toxic and side effects: indicates the proportion of toxic and side effects in animals during immunotherapy. In this experiment, all experimental groups did not have obvious toxic and side effects, so the incidence rate was 0%.

The results showed that the growth rate of tumor was obviously slowed down and the survival time was prolonged in animals after immunotherapy, and there were no obvious toxic and side effects. This shows that immunotherapy also has a good anti-renal clear cell tumor effect in vivo.

4. Discussion

4.1. Effect of Immune Microenvironment on Renal Clear Cell Tumor

In this study, the immune microenvironment characteristics

of renal clear cell tumor were deeply discussed, and its complexity and diversity were revealed. The expression of tumor infiltrating immune cells and their related molecules together construct this special microenvironment, which has an important influence on tumor progress and metastasis. On the one hand, the infiltration and activation of immune cells can play an anti-tumor role and inhibit the growth and spread of tumors; On the other hand, tumor cells can also escape the attack of the immune system by up-regulating the expression of inhibitory immune molecules, thus promoting the immune escape of tumors. Therefore, an in-depth understanding of the immune microenvironment characteristics of renal clear cell tumor will help us better understand the occurrence and development mechanism of this tumor and provide accurate targets for immunotherapy.

4.2. Effectiveness and Limitations of Immunotherapy Strategies

This study evaluated the effect of immunotherapy in renal clear cell tumor through in vitro and in vivo experiments, and the results showed that some immunotherapy strategies had significant anti-tumor effects. However, there are still individual differences in the effectiveness of immunotherapy, and some patients have limited or even ineffective response to immunotherapy. This may be related to the heterogeneity of immune microenvironment, the emergence of drug resistance in immunotherapy and the immune state of patients themselves. Therefore, when formulating immunotherapy strategies, it is needed to fully consider the individual differences and tumor characteristics of patients and formulate personalized treatment plans. Furthermore, it is needed to further study the drug resistance mechanism of immunotherapy and find new ways and means to overcome drug resistance.

4.3. Research Significance and Future Direction

This study systematically studied and analyzed the immune microenvironment and immunotherapy of renal clear cell tumor, which provided theoretical basis and practical guidance for immunotherapy of renal clear cell tumor. Through in-depth study on the characteristics of immune microenvironment and the mechanism of immunotherapy, it is expected to find new immunotherapy targets, optimize existing treatment schemes and improve the quality of life and prognosis of patients. Future research directions include further exploring the immune escape mechanism of renal clear cell tumor, developing new immunotherapy drugs or antibodies and establishing more accurate and effective immunotherapy strategies.

5. Conclusion

This study revealed the complex immune background of renal clear cell tumor and the effectiveness and limitations of immunotherapy through in-depth study on the immune microenvironment characteristics and immunotherapy response. In this article, it is found that there are obvious immune cell infiltration and specific immune molecule expression patterns in renal clear cell tumor, which are closely related to the progress and prognosis of the tumor. Furthermore, it is also found that some immunotherapy strategies have shown remarkable anti-tumor effects in vitro and in vivo experiments, but the degree of response of patients varies from individual to individual. The contribution of this study is to provide a new theoretical basis and practical guidance for immunotherapy of renal clear cell tumor, which is expected to promote the research progress in this field and improve the clinical treatment level.

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