Low Level of Macrophages M0 in Primary Tumors is a Favorable Factor for Metastasis in Renal Clear Cell Carcinoma

Yaohai Wu

Cheeloo College of Medicine, Shandong University; 2. Department of Urology, Qilu Hospital of Shandong University

Tong Yang

Cheeloo College of Medicine, Shandong University; 2. Department of Urology, Qilu Hospital of Shandong University.

You Zuo

1. Cheeloo College of Medicine, Shandong University; 2. Department of Urology, Qilu Hospital of Shandong University

Shuai Fu

Shandong Cancer Hospital and Institute, Shandong Academy and Medical Sciences.

Zhongwei Zhao

Department of Urology, Qilu Hospital of Shandong University

Dawei Li

Department of Urology, Qilu Hospital of Shandong University

Zhonghua Xu

Department of Urology, Qilu Hospital of Shandong University

Nengwang Yu (✉ qiluyunengwang@hotmail.com)

Qilu Hospital of Shandong University <https://orcid.org/0000-0002-3488-2270>

Research article

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Abstract

Background: The relationship between tumor-infiltrating immune cells (TIICs) and different stages or subtypes, especially the M-stage in primary tumors, remains unknown in renal clear cell carcinoma (KIRC). Therefore, the purpose of this study was to determine the relationship between TIICs and distant metastasis and provide new ideas for immunotherapy in KIRC.

Methods: Clinical outcomes and transcriptome data were obtained from The Cancer Genome Atlas (TCGA) kidney cohort to characterize the composition of 22 TIICs in KIRC. Propensity score matching (PSM) and CIBERSORT were used to proceed data.

Results: A bar plot, heat map, violin plot, bubble plot, box plots, and survival curves were generated. Bar plot and violin plot showed that T cells CD8 ($P = 0.032$), macrophages M0 ($P = 0.033$), macrophages M1 ($P = 0.035$), and mast cells resting ($P = 0.001$) were correlated to the M-stage. Survival curves showed that only the macrophages M0 group had a P value <0.05 (0.015) that a higher amount of macrophages M0 was associated with a better survival rate, whereas the remaining P values, including those for T cells CD8 (0.915), macrophages M1 (0.88), and mast cells resting (0.347) were >0.05 .

Conclusions: we got the conclusion that a low level of macrophages M0 in primary tumors was identified as a favorable factor for metastasis in renal clear cell carcinoma, which could provide us new ideas for immunotherapy in KIRC. In order to decrease the distant metastasis, more attention and focus should be paid to increase the level of macrophages M0 in primary tumors.

1 Background

Kidney cancer is one of the most common cancers of the urinary system among men and women. Its incidence has increased in recent years, accounting for 2.2% of cancer cases in 2018 worldwide (1). RCC is the most common form of kidney cancer, accounting for approximately 85% of kidney cancer cases (2). According to the pathological classification, RCC can be divided into a variety of tumors with different molecular, histologic, and genetic alterations. Renal clear cell carcinoma is the most common RCC (3). Despite advances in diagnosis, screening, surgery, and drug therapy, the prognosis of RCC remains unsatisfactory, particularly for patients who present with high stage disease (2).

Since William Coley first studied tumor immunotherapy in 1890, an increasing number of researchers have focused on immune-infiltrating cells in tumors and obtained a wide range of results, especially in recent years (4). For example, one finding is that macrophages/innate immunity can be used to fight cancer, thereby contributing to the treatment of cancer through immunotherapy (5). Analysis of the cellular immune response in tumors can improve clinical predictions and help identify patients who could benefit from immunotherapies (6). A breakthrough finding that B cell infiltration and tertiary lymphoid structure in soft tissue sarcoma are positively correlated with prognosis and response to immunotherapy underscores the importance of B cell infiltration (7).

Advances in immunotherapy for kidney cancer have led to the development of several immunotherapy drugs for kidney cancer (8). The CheckMate 214 study, which tests first-line treatments of metastatic RCC, found that the combination of the programmed cell death protein 1 (PD1) antibody nivolumab and the cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) antibody ipilimumab was significantly superior to the targeted drug sunitinib for improving the overall survival of patients with medium- and high-risk metastatic RCC in International Metastatic RCC Database Consortium (IMDC) (9). The KEYNOTE 426 study showed that the combination of parabolizumab and axitinib significantly outperformed sunitinib regarding progression-free survival of metastatic renal cancer (10). The results of these two studies changed the treatment of metastatic renal cancer, introducing a new era of immunotherapy using targeted drugs. The National Comprehensive Cancer Network (NCCN) and other major guidelines recommend nivolumab combined with ipilimumab, or parabolizumab combined with axitinib for the first-line treatment of IMDC with medium and high risk metastatic renal cancer (11). However, individualized immunotherapy options for kidney cancer, especially RCC, need to be further evaluated.

The role of TIICs has been investigated by comparing normal kidney tissue to tumor tissue using TCGA data (12). However, the relationship between TIICs and different stages or subtypes, especially the M-stage in primary tumors, remains unknown in KIRC. Further studies of the relationship between TIICs and M-stage cancer are necessary, particularly in RCC. Here, we investigated the relationship between TIICs and M-stage in primary tumors from patients with KIRC.

2 Methods

2.1 Data acquisition

Raw data, including gene expression and corresponding clinical information, were identified and downloaded from TCGA (<https://portal.gdc.cancer.gov/repository>) kidney cohort in February 2020. TCGA database doesn't need any administrative permissions to access the raw data and everyone could download raw data freely. Important information was extracted from the raw data using the Perl scripts. The dataset used included primary kidney tumors, with no particular exclusion criteria applied. Each tumor case corresponded to one KIRC patient. The relevant clinicopathological data collected included age, gender, grade, T-stage, N-stage, M-stage, clinical stage, survival status, and survival duration in days.

2.2 Assessment of immune infiltration

First, the unknown or MX data were deleted, and the clinical dataset was divided into two groups, the M0 group and the M1 group according to the M-stage. Second, propensity score matching (PSM) was performed according to three clinical variables, age, gender, and grade, using the R code (R MatchIt package, method = "nearest", ratio = 1, caliper = 0.02). Third, data were processed using CIBERSORT (CIBERSORT R script v. 1.03, perm = 100), which identified patients in the M0 group and M1 group (6, 13, 14). Finally, after deleting data with a P value > 0.05, a bar plot, heat map, violin plot, bubble plot, box plots, and survival curves were generated. The study framework is displayed in Fig. 1.

CIBERSORT can characterize the composition of TIICs of complex tissues based on normalized gene expression profiles. This method quantifies the abundance of a particular cell type and has been verified by fluorescence activated cell sorting. We used CIBERSORT (<http://cibersort.stanford.edu/>) to examine the relative fractions of 22 infiltrating immune cell types in each tumor tissue. These TIICs included B cells naive, B cells memory, plasma cells, seven T-cell types (T cells CD8, T cells CD4 naive, T cells CD4 memory resting, T cells CD4 memory activated, T cells follicular helper (Tfh), T cells regulatory (Tregs), and T cells gamma delta), NK cells resting, NK cells activated, monocytes, macrophages (macrophages M0, macrophages M1, and macrophages M2), dendritic cells resting, dendritic cells activated, mast cells resting, mast cells activated, eosinophils, and neutrophils.

2.3 Statistical analysis

Statistical analyses were performed using R v. 3.6.2 and Bioconductor (<https://www.bioconductor.org/>). The bar plot and heat map (R pheatmap package), violin plot (R vioplot package), and the bubble plot were plotted using R. Gene expression information and the corresponding M-stage were integrated to generate a boxplot with R. Survival information and the corresponding gene expression information were integrated to generate a survival curve with R (R survival package). The Wilcoxon test was used to calculate and compare the differences between the M0 and M1 groups. The survival curve was plotted using the Kaplan-Meier method, and the differences were analyzed with the log-rank test. All statistical analyses were performed using R 3.6.5. $P < 0.05$ was considered statistically significant.

3. Results

3.1 Summary of renal cell carcinoma clinicopathological information

After deleting the unknown or MX data, including 505 cases, the dataset included 122 cases after PSM of KIRC samples. The clinicopathological characteristics of these samples before and after PSM are shown in Supplementary Tables (Table S1 and Table S2).

3.2 Distribution of tumor-infiltrating immune cells

Figure S1 shows the percentages of 22 TIICs in each KIRC sample. Columns 1–28 represent the M0 group, and columns 29–60 represent the M1 group. T cells CD4 memory resting, macrophages M2, and T cells CD8 were abundant, whereas the fraction of B cells memory, T cells CD4 naive, eosinophils, T cells gamma delta, and mast cells activated was small in most KIRC samples. Similar results were obtained in the two groups. The heat map shows the same results (Figure S2). The M0 group had a higher fraction of macrophages M0 and mast cells resting than the M1 group, and the fraction of macrophages M1 and T cells CD8 showed the opposite pattern to that of macrophages M0. For each tumor sample, the sum of all evaluated immune cell type fractions was 1. The violin plot results were the same as those described above (Fig. 2). Except for the comparison of each type of TIICs, a P value was obtained for the remaining

differences. The P value of the comparisons including T cells CD8, macrophages M0, macrophages M1, and mast cells resting was < 0.05 , whereas the remaining comparisons had a P value > 0.05 .

Figure 3 shows the composition of 22 tumor-infiltrating immune cells in KIRC. In this bubble plot, each dataset was processed using a weighted average method to compare the differences in the composition of TIICs between two groups of KIRC patients. The M0 group contained abundant fractions of T cells CD4 memory resting (24.7%), macrophages M2 (24.4%), and T cells CD8 (11.6%), whereas the fraction of T cells CD4 naive and B cells memory was 0, and the fraction of eosinophils (0.01%), T cells gamma delta (0.01%), and mast cells activated (0.06%) was small. The M1 group was similar to the M0 group. The M1 group contained abundant fractions of macrophages M2 (23.3%), T cells CD4 memory resting (23.1%), and T cells CD8 (18.4%), whereas the fraction of B cells memory and T cells CD4 naive was 0, and the fraction of eosinophils (0.03%), mast cells activated (0.11%), and T cells gamma delta (0.18%) was small. The composition of TIICs differed considerably between the M0 group and the M1 group. The M0 group had a higher fraction of macrophages M0 (7.6%) and mast cells resting (3.4%) than the M1 group (macrophages M0, 2.7%, and mast cells resting, 1.3%), whereas the fraction of macrophages M1 (5.6%) and T cells CD8 (11.6%) in the M0 group was opposite to that of macrophages M0 in the M1 group (macrophages M1, 8.2%, and T cells CD8, 18.4%). For each group, the sum of all evaluated immune cell type fractions equaled 1.

3.3 Violin plot of tumor-infiltrating immune cells

Based on the expression data, we chose the corresponding M-stages and integrated them into the expression data. Then, TIICs box plots were generated based on M-stage. The P value of these comparisons, including T cells CD8 ($P = 0.032$), macrophages M0 ($P = 0.033$), macrophages M1 ($P = 0.035$), and mast cells resting ($P = 0.001$) was correlated to the M-stage, whereas the rest was not (Figure S3). The results were the same as those of the violin plot.

3.4 Relationship between survival rate and tumor-infiltrating immune cells

Next, we determined whether the survival rate was related to the fractions of 22 TIICs, especially macrophages M0, mast cells resting, macrophages M1, and T cells CD8. According to the expression data, we chose the corresponding survival outcomes and integrated them into the expression data. Then, the survival curve for each TIIC was plotted after dividing the data into a high expression group and a low expression group according to the median. Only the macrophages M0 group had a P value < 0.05 (0.015), whereas the remaining P values, including those for T cells CD8 (0.915), macrophages M1 (0.88), and mast cells resting (0.347) were > 0.05 (Fig. 4).

4. Discussion

In the present study, PSM was used to decrease the bias and confounding variables between the M0 and M1 groups. Age, gender, and grade were the independent risk factors for survival, which were matched in

this study.

A comprehensive and detailed analysis of immune infiltration in RCC was performed based on the deconvolution of bulk gene expression data downloaded from TCGA. In addition, the independent prognostic value of TIICs was determined. The composition of TIICs differed greatly between the M0 and M1 stages of KIRC. This difference may be important for predicting prognosis and designing new treatment strategies for KIRC.

In recent years, several studies investigated the tumor-infiltrating immune system, and many significant results were reported (5–7). Mills et al. reported that macrophages M2 promote cancer growth, whereas macrophages M1 slow or stop cancer growth (5). Ali et al. found that a large difference in TIICs in breast cancer is an important determinant of both prognosis and response to treatment (6). Tawbi and Fridman unraveled the potential of B-cell-rich tertiary lymphoid structures to guide clinical decision-making and treatments (7). Several studies focused on renal cell carcinoma, such as a report by Bex et al., who indicated that combination therapies involving checkpoint inhibitors could be the next standard of care for metastatic clear cell renal cell carcinoma (8). Zeng studied the composition of TIICs in RCC and reported the independent prognostic value of TIICs (12). However, there are few studies analyzing the tumor-infiltrating immune response according to different stages and subtypes of kidney cancer, which was the focus of the present study. In this study, we analyzed the tumor-infiltrating immune system according to M-stage with data downloaded from TCGA.

Tumor-associated macrophages (TAMs) in the tumor microenvironment are related to metastasis, angiogenesis, and immunosuppression in different cancers (15, 16). TAMs are mainly composed of the M0 or inactivated state of TAMs, M1, and M2 cells; M0 can be converted to M1/M2 when M0 is polarized. Because there is little research on the M0 stage, its effect on tumors is not clear. The different macrophage subtypes, such as M1/M2, can serve as biomarkers for treatment and diagnosis (17), and macrophage polarization can affect cancer growth.

Macrophages M1, which are activated macrophages, can prevent tumor development by suppressing angiogenesis and inducing apoptosis and senescence (18). Inhibiting macrophage M1 polarization can reduce the antitumor immune response (19). Macrophages M2, which are activated macrophages, promote tumor angiogenesis or the release of proangiogenic factors (20, 21).

The present study had several limitations. First, additional data obtained under similar conditions are necessary to validate the conclusion. Second, the sample size was small, which could increase bias to some extent because of the use of PSM to process data. Third, using CIBERSORT, which has 100 times random computing, to analyze data may lead to some uncertainty. Third, we failed to explain the underlying mechanism, so more researches are still needed to figure it out.

PSM was used to analyze the data and increase the validity of the results. CIBERSORT was then used to process the data, and a bar plot, heat map, violin plot, and bubble plot were generated to analyze the outcomes. The results showed that T cells CD8, macrophages M0, macrophages M1, and mast cells

resting were correlated to the M-stage. Finally, the clinical outcomes were integrated with the corresponding expression data, and box plots and survival curves were generated, which led to the conclusion that macrophage M0 is a favorable factor for tumor metastasis in renal clear cell carcinoma. This is the first study analyzing the relationship between TIICs and M-stage in KIRC, and the first study using PSM to decrease bias.

5. Conclusions

In this study, macrophage M0 may be a favorable factor for tumor metastasis in renal clear cell carcinoma, which could provide us new ideas for immunotherapy in KIRC. Macrophage M0 may prevent tumor development by promoting M0 to M1 polarization in metastasized KIRC.

Abbreviations

TIICs, tumor-infiltrating immune cells; KIRC, kidney renal clear cell carcinoma; TCGA, The Cancer Genome Atlas; PSM, propensity score matching; TAMs, tumor-associated macrophages; RCC, renal cell carcinoma; PD1, programmed cell death protein 1; CTLA-4, cytotoxic T-lymphocyte-associated antigen 4; IMDC, International Metastatic RCC Database Consortium; NCCN, The National Comprehensive Cancer Network; EAU, European Association of Urology.

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

All authors approved of the version to be published and participated sufficiently in the work to take public responsibility for appropriate portions of the content.

Availability of data and materials

The data underlying this study are freely available from TCGA data portal (<https://portal.gdc.cancer.gov/repository>). Public access to the TCGA database is open and the authors did not have special access privileges.

Competing interests

The authors declare that there is no conflict of interests.

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Authors' contributions

YW and NY contribute to conception and design. YW, TY, YZ, SF acquired, analyzed and interpreted of data. YW draft the manuscript. NY, ZZ, DL, ZX and SF revised it critically for important intellectual content. all authors have read and approved the manuscript

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Figures

Figure 1

Overall flow chart of this study.

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Overall flow chart of this study.

Figure 2

The violin plot between M0 and M1 group of 22 TILCs. The blue columns represent the M0 group and red columns represent the M1 group. TILCs, tumor-infiltrating immune cells.

Figure 2

The violin plot between M0 and M1group of 22 TIICs. The blue columns represent the M0 group and red columns represent the M1 group. TIICs, tumor-infiltrating immune cells.

Figure 3

The bubble plot shows the percentages of 22 tumor-infiltrating immune cells in each KIRC sample between M0 and M1 group. The size of bubble is proportional to the percentage of immune cells. KIRC, kidney renal clear cell carcinoma.

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Figure 4

Survival analysis of immune cells in the TCGA cohort, including T cells CD8 (A), macrophages M0 (B), macrophages M1 (C), and mast cells resting (D). Red lines represent high expression of the immune cells and blue lines represent low expression. Only the macrophages M0 group was significantly positively with survival with a P value <0.05 (0.015). TCGA, The Cancer Genome Atlas.

Figure 4

Survival analysis of immune cells in the TCGA cohort, including T cells CD8 (A), macrophages M0 (B), macrophages M1 (C), and mast cells resting (D). Red lines represent high expression of the immune cells and blue lines represent low expression. Only the macrophages M0 group was significantly positively with survival with a P value <0.05 (0.015). TCGA, The Cancer Genome Atlas.

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