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Comprehensive assessment of the association between tumor-infiltrating immune cells and the prognosis of renal cell carcinoma

Comprehensive assessment of TIICs and RCC

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Abstract

BACKGROUND

According to current statistics, renal cancer accounts for 3% of all cancers worldwide. Renal cell carcinoma (RCC) is the most common solid lesion in the kidney and accounts for approximately 90% of all renal malignancies. Increasing evidence has shown an association between immune infiltration in RCC and clinical outcomes. For the purpose to discover possible targets for the immune system, we aimed to investigate the link between tumor-infiltrating immune cells (TIICs) and the prognosis of RCCs.

AIM

To investigate the effects of 22 TIICs on the prognosis of RCC patients and identify potential therapeutic targets for RCC immunotherapy.

METHODS

The CIBERSORT algorithm partitioned the 22 TIICs from the TCGA cohort into proportions. Cox regression analysis was employed to evaluate the impact of 22 TIICs on the probability to develop RCC. A predictive model for immunological risk was developed by analyzing the statistical relationship between the subpopulations of TIICs and survival outcomes. Furthermore, multivariate Cox regression analysis was used to investigate independent factors for the prognostic prediction of RCC. A significance level of $P < 0.05$ was regarded as statistically significant.

RESULTS

Compared to normal tissues, RCC tissues exhibited a different infiltration of immune cells. An immune risk score model was established while univariate Cox regression analysis revealed a significant association between four immune cell types and the survival risk connected to RCC. High-risk individuals were correlated to poorer outcomes, according to the KM curve ($P = 1e{-}05$). The immunological risk score model was demonstrated to be a dependable predictor of survival risk (AUC = 0.747) *via* the

ROC curve. According to multivariate Cox regression analysis, the immune risk score model independently predicted RCC patients' prognosis (HR = 1.550, 95%CI = 1.342–1.791; $P < 0.001$). Finally, we established a nomogram that accurately forecast the survival of patients with RCC comprehensively.

CONCLUSION

TIICs play various roles in RCC prognosis. An independent predictor of poor survival in kidney cancer cases was the immunological risk score.

Key Words: RCC; TIICs; Prognosis; Immune risk score model; Nomogram.

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Core Tip: Renal cell carcinoma (RCC) is a prevalent form of renal cancer that typically arises subtly and is distinguished by its propensity for easy metastasis and poor prognosis. Patients with advanced RCC frequently exhibit low overall survival rates owing to the extensive spread of cancer. However, the advent of immunotherapy in recent years has resulted in an improvement in the median overall survival for all risk groups of patients, making it imperative to identify potential immune targets. Our research has led to the development of an immune risk assessment model that underscores the critical role of tumor-infiltrating immune cells (TIICs)-based immune models in the prognostic prediction and clinical management of RCC.

INTRODUCTION

Renal cancer is a highly prevalent cancer that causes malignant tumors on globally. Its prevalence has been on the growth over the past decade, accounting for up to 2–3% of all newly diagnosed tumor cases[1,2]. Histologically, renal cell carcinoma (RCC) is

a prominent subtype of renal cancer, comprising approximately 85% of the total renal cancer cases[3-5]. Despite great developments in conception, diagnosis, surgery, and various tumor drug treatments, the clinical results of RCC are still insufficient. Therefore, accurate assessment of patient survival risk and prognostic prediction is critical.

The human immune system effectively protects the body from both exogenous and endogenous diseases. When uncontrolled proliferation of mutated or abnormal cells occurs in an organism, invading and spreading throughout the body, the immune system effectively recognizes the differences between tumors and healthy tissues, thereby secreting relevant immune cells to inhibit tumor growth. However, this process may be mistaken. Research has shown that during tumor progression, cancer cells take advantage of mutations in oncogenes that appear in the immune system to help them escape immune system attack to promote growth[6,7]. These findings indicate that there are diverse immune escape mechanisms by which tumor cells reduce killing by the immune system during progression. In addition, during tumor progression, changes in immune phenotype and composition cause secondary changes in immune responses within and outside the tumor microenvironment (TME)[8]. Reconstruction of tumor immunity occurs in a wide range of tumor types. Among the many types of cancer, RCC has a high metastatic rate and poor prognosis, with statistics showing that 30% of patients at the first diagnosis have already developed metastases, and nearly 30% of the remaining patients will have metastatic foci successively detected during the course of follow-up[9]. This characteristic is closely related to the interactions among the stroma, immune cells, and tumor cells in the immune microenvironment of RCC, which leads to reconstruction of the microenvironment[10]. Research on the complexity of the immune heterogeneity of RCC with respect to this characteristic will help us assess tumor heterogeneity clinically and thus facilitate the development of more effective and personalized therapies.

The TME is highly heterogeneous. Previous research has revealed that dynamic interactions between tumor cells and their microenvironment promote the formation, progression, metastasis, and development of drug resistance in solid tumors[11]. There is substantial evidence that the composition of multiple immune cells within the microenvironment affects antitumor immunity and immunotherapeutic responsiveness. An in-depth understanding of the TME, particularly the characteristics of TIICs, is essential for identifying critical regulatory molecules for tumor development and immunotherapy.

Most previous studies on RCC prognostic modeling have included only traditional clinical information, and the model has limitations such as poor sensitivity and lag, which makes it impossible to comprehensively assess the clinical application value of the model[12-14]. CIBERSORT is an algorithm which employs gene expression data to analyze barcode gene values of expression and estimate define the components of immune cells. The CIBERSORT algorithm is more efficient and precise than traditional immunohistochemical and flow cytometry approaches for calculating the relative proportions of 22 types of invasive tumor-infiltrating immune cells (TIICs)[15]. Based to the effectiveness of this methodology, numerous research have lately applied it to examine whether the prognosis of patients is affected by 22 TIIC subtypes[16-18].

Hence, our study aimed to explore the relationship between TIICs and RCC. For the purpose to investigate potential relationships between immunotherapy and RCC patients, we constructed an immune risk score methodology to prognostic prediction and thoroughly examined the impact of 22 TIIC subgroups on the prognosis of RCC patients by utilizing the CIBERSORT algorithm. The result provided a new avenue for the development of prognostic prediction models for RCC patients as well as the identification of therapeutic targets for immunotherapy.

MATERIALS AND METHODS

Data acquisition

The entire transcriptome data of RCC utilized in this investigation were obtained from the publicly available TCGA database. The research project encompassed a cohort of 957 patients diagnosed with RCC. The transcriptome data was obtained from the TCGA database using the R package "TCGA-Assembler". This dataset consisted of 128 cases of normal tissue and 829 cases of malignant tissue. Furthermore, we collected essential clinical information, including age, gender, TNM stage, grade, survival status, and survival duration, from a total of 829 patients diagnosed with RCC. The R software's "limma" package was executed to rectify the transcriptome data.

Assessment of immune infiltration

The deconvolution algorithm CIBERSORT divides 547 tag gene expression levels to describe the immune cell composition in tissues. In this research, the relative proportions of 22 TIICs in the transcriptome data of corrected RCC patients were estimated via this algorithm. We uploaded the transcriptome to the CIBERSORT website (http: //cibersort.stanford.edu/) and configured the algorithm to process 1000 rows. The criterion to calculate statistical significance was set at a P value < 0.05.

Statistical analysis

The analyses were conducted *via* SPSS 23.0 and R 3.5.3. Statistical tests were performed as two-sided tests. The criterion to calculate statistical significance was set at a P value < 0.05. The Kaplan–Meier curve was assessed *via* the log-rank test to examine the correlation between immune cell infiltration and overall survival. The sensitivity and specificity of the recurrence prediction model were analyzed employing time-dependent ROC curves. Independent impact factors linked to survival were examined using a multivariate Cox regression model, whereas the impacts of individual variables on survival were examined using a one-factor Cox regression model. A nomogram was generated to represent the regression coefficients in the Cox analysis.

RESULTS

Distribution of TIICs

With the CIBERSORT algorithm, we conducted immune cell infiltration analysis to facilitate the identification of relevant immune cell levels in all RCC patients in the TCGA database. The dataset included 829 patients with RCC and 128 healthy tissues. The clinicopathological characteristics of the samples are shown in Table 1. Figure 1A, 1B, and 1C display the arrangement of TIICs in RCC samples and the relationship with various immune cell subtypes. The violin diagram demonstrated the main advantages in composition between normal and RCC tissues in 22 TIICs (Figure 1D). The invasion of immune cells, such as native B cells, plasma cells, M2 macrophages, dendritic cells, and eosinophils, wasn't demonstrating any difference between normal tissues and RCC tissues. Further analysis revealed that M1 macrophages, M2 macrophages, monocytes, and T cells exhibited the strongest correlation with tumor advancement (Figure 2). On the basis of the above analysis, we explored the types and distributions of TIIC subpopulations that are closely related to RCC, which provides potential research directions for clinical immunotherapy.

Univariate and multivariate Cox regression analyses to constructe an immune risk score

We analyzed 22 immune cells using a univariate Cox regression technique in order to identify a subset that was significantly related with the possibility of RCC survival (Table 2). $P< 0.01$ was implemented as the screening threshold. Results suggested that the risk of RCC survival was related to macrophages M1 and M2, CD4+ memory activated T cells, and follicular helper T cells. Based on the assumption that these four immune cells significantly influence the survival risk of RCC patients, an immune risk scoring model for these four immune cells was generated *via* the multivariate Cox regression approach (Table 3). A risk score based on the model was assigned to each patient. The patients were separated into high- and low-risk groups based on their median risk score. Based on the KM curve analysis, individuals classified in the high-risk category exhibited a significantly unfavorable prognosis (Figure 3A). According to

the ROC curve, the risk model was useful in forecasting survival risk and had excellent both specificity and sensitivity (Figure 3B). Figures 3C, 3D, and 3E present the distributions of the immune risk score, patient survival status, and four immune cell types in RCC patients. The connections between the risk assessments and clinical indicators are presented in Figure 3F.

An impartial predictor of the prognosis for RCC patients was the immunological risk score model.

Age, sex, stage, TNM, and risk score were all analyzed using univariate and multivariate Cox regression algorithms (Figure 4A, Figure 4B) to observe whether the established immune risk scoring model was independent of patient age, sex, stage, and other clinicopathological statistics. Univariate analysis suggested that age, stage, T stage, and the risk score were related to prognosis ($P< 0.05$). Multifactor Cox proportional risk regression was used to construct predictive models. The results of multivariate regression analysis revealed that the risk score, age, stage, and T stage were all independent predictive factors for RCC. To predict patient survival, we constructed a nomogram utilizing the multivariate Cox regression analysis coefficients (Figure 4C).

DISCUSSION

Cancer tissues contain fibroblasts, immune cells, endothelial cells, and a variety of chemokines, growth factors, and cytokines in besides malignant tumor cells. The tumor microenvironment consists of these components and their interactions, which usually have an inhibitory effect on malignant cells[19,20]. Nevertheless, tumor cells have the ability to evade these inhibitory signals as they spread, utilizing immune cells and other favorable conditions encountered in their own surroundings to facilitate their expansion, infiltration, and metastasis. Research has shown that cancer prognosis is closely associated with the tumor microenvironment, especially TIICs. TIICs can promote tumor growth by providing signals, and this field of research has attracted

much attention in recent years[21,22]. Consequently, investigating immune-infiltrating cell subsets to evaluate risk and tumor prognosis is crucial.

By means of deconvolution of data derived from an extensive array of samples, we performed an exhaustive and thorough evaluation of immune infiltration in RCC. This study illustrated the diversity of immune cells in RCC patients as well as the identification of particular TIICs. It is well recognized that different immune cell subgroups penetrate RCC locally[23]. These distinct immune cells provide a unique "immune signature map" for each patient and provide novel proposals for specialized immunotherapy for RCC in the future. First, we explored immune cell infiltration in all RCC patients *via* the TCGA database. The difference analysis results indicated that the remaining immune cells failed to demonstrate distinct infiltration between normal and RCC tissues, with the exception of native B cells, plasma cells, M2 macrophages, dendritic cells, and eosinophils ($P< 0.05$). These outcomes correspond with conclusions from earlier research. The number of eosinophils and dendritic cells is greater in the blood of RCC patients than in that of normal individuals[24]. The proportions of tumor-infiltrating lymphocytes, native B cells, and plasma cells are increased[25]. In addition, the proportion of M2 macrophages is increased in RCC tissues, and the increased proportion of this subpopulation significantly promotes the production of inflammatory cytokines and facilitates tumor growth[26].

A thorough investigation was conducted into the relationship between the prognosis of RCC patients and the subpopulations of TIICs. Additional analysis revealed that CD4⁺ memory-activated T cells, follicular helper T cells, M1 macrophages, and M2 macrophages were associated with RCC survival risk. Tumor-associated immune cells are considered important factors for predicting the prognosis of patients with tumors[27]. Previous studies revealed that both *ex vivo* and in vivo, infiltrating CD4⁺ T cells could promote RCC cell proliferation through the TGFβ1/YBX1/HIF2α axis[28]. Thompson *et al.*[29] observed that the interaction between PD-1 and B7-H1 expressed by activated T-cell PD-1 receptor immune cells may promote tumor progression by promoting immune dysfunction in patients with RCC, which is associated with the

survival risk of the disease. In addition, Menard *et al.*[30] demonstrated that an increased proportion of $CD4^+$ T cells in RCC tissues was significantly associated with RCC death according to flow analysis (P= 0.004). The results of our analysis further confirmed that an increased proportion of $CD4^+$ T cells was negatively associated with the prognosis of patients with RCC. Furthermore, researches have suggested that helper T cells, which are highly immunosuppressive, play important roles in organismal immunomodulation and the promotion of RCC immune escape[31]. This finding is consistent with our conclusion of a low overall survival rate in RCC patients with high infiltration of helper T cells.

Multiple studies have demonstrated that macrophages have significant functions in the inflammatory response throughout the process of tumor formation and growth[32,33]. In cancer and inflammation, the primary macrophage subsets are categorized into two distinct types: The normal activated subset (M1) and the alternating-activated subset (M2). M1 macrophages have anticancer properties by facilitating the host's immune response against microbes and cancer cells. On the opposite hand, M2 macrophages possess anti-inflammatory characteristics and promote tumor growth, which might contribute to the growth and migration of tumors. There is plenty of evidence that TAMs are related to tumor progression and metastasis. Tumors are able to utilize the remodeling ability of the macrophage matrix, allowing them to enter the surrounding matrix and migrate through it. On the other hand, TAMs are more highly expressed in highly malignant tumors. These findings reveal that high numbers of TAMs in the TME are more likely to predict adverse outcomes of the disease. Recent research has demonstrated that macrophages have a vital role in the progression of RCC, and specifically, M1 macrophages are strongly linked to the stage of RCC and the grade of tumor histology. Geissler *et al.*[27] reported the phenotypic characteristics of macrophages in RCC. They reported that the number of tumor-associated macrophages (TAMs) increased slightly with the degree of dedifferentiation. The ratio of macrophages to T cells was highest in G1-stage tumors. In addition, Yang *et al.*[34] found that macrophages were more easily absorbed into RCC tissues than into

nontumor tissues. The results revealed a new mechanism by which macrophages in the RCC TME increase RCC metastasis by activating AKT/mTOR signaling. These results further suggest that immune monitoring of RCC patients could be an effective tool for predicting the prognosis of RCC patients.

Additionally, a multivariate Cox regression method was employed to develop an immunological risk score model for the four categories of immune cells. The KM curve demonstrated a significant association between high-risk individuals and unfavorable outcomes (P= 1e−05). With an AUC value of 0.747, the ROC curve demonstrated the validity of the immunological risk evaluation model in forecasting the risk of survival. A multivariate Cox regression analysis was performed on the risk score, age, sex, stage, and TNM stage. Research results demonstrated that the immunological risk score model was a significant and separate factor in predicting the prognosis of RCC patients (HR = 1.550, 95%CI = 1.342–1.791; P< 0.001). A nomogram was developed to accurately forecast the overall survival of patients with RCC by utilizing the outcomes of a multivariate Cox regression assessment. Currently, the prognostic judgment of patients after RCC is mainly based on TNM staging[35]. As a visually and clinically valuable medical prediction model, a nomogram can objectively evaluate new predictive variables and provide a more accurate and personalized prognostic assessment.

Due to the rapid evolution of high-throughput sequencing technologies, many tumor markers associated with tumor prognosis have been identified, but the number of these markers is still limited, and their value for clinical application still needs to be considered. Hence, it is imperative to investigate additional therapeutic targets to accurately forecast the prognosis of patients with RCC and to guide the development of clinically individualized treatment regimens. Compared with other solid tumors, RCC is a highly heterogeneous tumor with wide variations in therapeutic efficacy, and relevant specific therapeutic targets are still lacking. In the context of precision tumor therapy and multiline therapy, rational individualized selection of diverse treatments has become an important clinical issue for maximizing the efficacy of comprehensive

treatment for advanced RCC. Traditional cancer treatments have many drawbacks, such as pathological tissues that cannot be completely resected by surgery, leading to recurrence, drug resistance, or related serious adverse reactions in patients during radiotherapy and chemotherapy. In addition, the poor prognosis and easy metastasis of RCC make it difficult to control the disease effectively. Currently, immunotherapy is recognized as one of the most promising therapeutic tools for RCC treatment. Since 2005, with the introduction of a variety of antitumor neovascularization tyrosine kinase inhibitors (TKIs), targeted therapy has become the main systemic treatment for RCC[36]. Immune combinations (pabrolizumab + axitinib, avelumab + axitinib, nabulizumab + cabozantinib, and pabrolizumab + lenvatinib) and dual immune combinations (nabulizumab + ipilizumab) have been approved for the treatment of RCC. Macrophages, $CD4^+$ T cells and helper T cells play crucial roles in cancer immunotherapy. Therefore, we explored the role of four critical TIIC immune subpopulations, M1 macrophages, M2 macrophages, $CD4^+$ T cells and helper T cells in a model for RCC immune risk assessment. This model was confirmed as an independent predictor of RCC. In other words, the immune risk assessment model is a reliable indicator for determining RCC prognosis before immunotherapy. These findings suggest that patients with RCC with high levels of M1 macrophages, M2 macrophages, $CD4^+$ T cells and helper T cells may benefit from immunotherapy. At present, only a few studies have modeled and predicted the prognosis of RCC patients via immune risk scores. However, in other fields, an increasing number of researchers have realized the importance of establishing immune risk score models. Research has shown that the prognostic immune risk scoring model established by researchers on the basis of systematic assessment of the immune status of cancer patients is not only an independent prognostic factor for the recurrence-free survival of cancer patients but also has better prognostic value than TNM staging does. This conclusion was also verified in our research, and the established immune risk assessment model has greater risk stratification ability, accuracy of prognostic judgment, and value in guiding clinical decision-making than the current traditional single prognostic model for RCC. The

model can be used to assess the prognosis of patients with RCC and the therapeutic benefits of related drugs, which can help clinicians develop personalized treatment plans and improve the survival rate of patients with RCC.

There were specific constraints in our investigation. Initially, it is crucial to point out that the public dataset contains a restricted amount of data. Consequently, the clinical pathology characteristics that were specifically chosen for examination in this study are not exhaustive and may result in possible errors or biases. In subsequent studies, the sample size should be further expanded to reduce bias and confirm the results. Furthermore, we neglected to take into consideration the variability of the immunological microenvironment in relation to the specific site of immune infiltration. Lastly, the outcomes of the study aren't applicable to patients in Asian countries because all the data sets that were downloaded for developing an immunological risk scoring model were from Western countries. More investigation is needed to verify this.

CONCLUSION

In this study, we explored all immune cells that are closely associated with RCC. We identified and validated the types and distributions of key TIIC subpopulations closely associated with patients with RCC. Additionally, our research incorporated the major factors that influence the prognosis of patients and constructed a prognostic prediction-related model. The predictive model for immune risk assessment on the basis of key subgroups has good predictive value for patients in the RCC cohort. Considering the rapid growth of high-throughput technology, we are optimistic that our immunological risk score model provides significant promise for application in clinical practice. These results would potentially be extremely valuable for investigating novel immunodiagnosis and therapeutic approaches for cancer.

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