

Gene expression-based immune infiltration analyses of renal cancer and their associations with survival outcome

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Abstract

Renal cancer is a common malignant tumor with an increasing incidence rate. In this study, based on the gene expression profiles, we analyzed the compositions of tumor infiltrating immune cells (TIICs) in renal cancer and paracancerous samples by using CIBERSORT. The proportions of 22 TIICs subsets in 122 paired renal cancer and paracancerous samples, and 224 Wilms tumor (WT) samples varied between intragroup and intergroup. After analyzed the difference in TIICs composition between renal cancer and paired paracancerous samples, we found that compared with paracancerous samples, M0 macrophages and CD8 T cells were significantly elevated, while naive B cells were significantly decreased in renal cancer samples. Survival analysis showed that high overall TIICs proportion, low proportion of resting mast cells and high proportion of activated memory CD4 T cells were associated with poor prognosis of renal cancer patients. In addition, 3 clusters were identified by hierarchical clustering analysis and they presented distinct prognosis. Cluster 1 had superior survival outcome, while cluster 2 had inferior survival outcome. In summary, our study indicated that overall TIICs proportion, certain TIICs subset proportion, and distinct cluster patterns were associated with the prognosis of renal cancer, which was significant for the clinical surveillance and treatment of renal cancer.

1. Background

Renal cancer is one of the common malignant tumors with an increasing incidence rate over time (Corgna et al. 2007; Weir et al. 2016). Wilms tumor (WT), also known as nephroblastoma, is recognized as the second most common solid organ abdominal tumor in children and the most frequent primary malignant renal cancer (Ehrlich 2017). It accounts for 6% of pediatric cancers, and an estimated 95% of renal cancer is WT among children (Barber et al. 2007). As an embryonic tumor of kidney, WT arises from metanephric mesenchyme and presents high incidence rate among 2–3 years old children (Perotti et al. 2007). The treatment of WT is determined by several factors, such as genetic, pathologic, and demographic factors and current therapies for WT consist of surgery, chemotherapy, and radiation therapy (Ehrlich 2017; Barber et al. 2011). Although the survival of WT has improved with the development of treatment strategies, the prognosis-related factors, including hematogenous and pulmonary metastasis, relatively high recurrence rate, and late effects make the prospects of treatment noteworthy (Njuguna et al. 2017; Davidoff 2012). Therefore, further investigation on the biological processes and underlying molecular mechanism of WT may facilitate the prognosis improvement of patients.

Currently, an increasing evidence has proved that the occurrence and development of tumors are not only associated with tumor cells, but also related to tumor microenvironment (Liu et al. 2017). Tumor microenvironment is composed of complex components, such as mesenchymal stem cells, fibroblasts, and immune cells (Isaac and Sharma 2015). Among them, tumor infiltrating immune cells (TIICs) are the pivotal component and considered as the leading players of tumor microenvironment (Ino et al. 2013). Accumulating studies have shown that TIICs are closely associated with the development and survival outcome of cancers. For example, infiltrated macrophages were found to promote the progression of

prostate cancer, and NK cells showed anti-tumor effects against tumor development (Gannon et al. 2009). In colorectal cancer, TIICs were associated with the clinical outcome of patients, and considered as key signatures for prognosis (Caro et al. 2014). The immunohistologic features and immunotype of TIICs played a crucial role in metastatic melanoma and were related to the survival outcome (Erdag et al. 2012). In addition, dysfunctional infiltrating lymphocytes were observed in renal cell carcinoma (RCC), an immunogenic renal cancer (Thompson et al. 2007). It was indicated that tumors might exert an impairment effects on the immune system and more studies on the associations between TIICs and tumor occurrence and prognosis should be helpful for the application of immunotherapeutic strategies on renal cancer.

Immunohistochemistry is a commonly used method to evaluate the TIICs in tumors, however, the results are often inaccurate due to the extensive expression of markers in non-immune cells (Liu et al. 2017). In our research, the CIBERSORT algorithm was adopted to assess the TIICs subsets in renal cancer based on gene expression profiles retrieved from The Cancer Genome Atlas (TCGA). Meanwhile, we also analyzed the relationship of TIICs fraction and immune patterns with survival outcome of renal cancer to explore the prognostic values of TIICs fraction and immune clusters in prostate cancer.

2. Methods

2.1 Data source

We downloaded the gene expression profiles of renal cancer from the Gene Expression Omnibus (GEO, <https://www.ncbi.nlm.nih.gov/geo/>, Affymetrix HG-U133A platform) and The Cancer Genome Atlas (TCGA, , Illumina HiSeq platform). The TCGA dataset was composed of 889 renal cancer samples and 128 paracancerous samples, including chromophobe renal cell carcinoma, kidney renal clear cell carcinoma, and kidney renal papillary cell carcinoma samples. The GEO dataset (access no. GSE31403) included 224 Wilms tumor (WT) samples.

2.2 Tumor infiltrating immune cells calculation

CIBERSORT, a deconvolution algorithm based on gene expression profiles (Xiong et al. 2018), was used to calculate the relative proportions of 22 tumor infiltrating immune cells (TIICs) subsets. CIBERSORT is able to evaluate the composition of TIICs with 547 barcode gene expression values, using P-value as a parameter for measurement of the confidence in results.

In addition, a previous research has proved that the mean expression levels of genes GZMA and PRF1 represent the immune cytolytic activity of immune cells, which also reflect the fraction of TIICs (Rooney et al. 2019). Therefore, the expression levels of GZMA and PRF1 were determined to assess the immune cytolytic activity and evaluate TIICs composition comprehensively.

2.3 Statistical analyses

We used R language to calculate the proportions of 22 TIICs subpopulations in the 1,241 samples from TCGA and GEO datasets. Correlation of the results from different datasets was analyzed by Pearson correlation analysis. The compositions of TIICs subpopulations in 122 paired cancer and paracancerous samples from TCGA were computed, and the difference in compositions was further analyzed. Besides, the fraction of TIICs subsets in WT samples from GEO was also calculated.

Then we stratified the 1,241 samples by P-value of 0.05, and calculated the compositions of TIICs subsets as well as mean expression values of GZMA and PRF1 in the groups with $P \geq 0.05$ and $P < 0.05$ respectively.

Survival analyses of the 1,010 samples from TCGA which were also stratified by P-value of 0.05 were performed using survival package. With the relative proportions of 22 TIICs subsets as continuous variable, we calculated the hazard ratios (HR) using Cox regression analysis. We performed survival analyses to explore the effects of significant factors on survival outcome.

Euclidean distance method was adopted for hierarchical clustering of TIICs subsets in the 1,010 samples. Compositions of all immune clusters were analyzed, and the survival curve was drawn.

3. Results

3.1 Performance of CIBERSORT for TIICs evaluation in renal cancer

The TIICs composition was analyzed by CIBERSORT. As shown in Fig. 1A, the samples from GEO and TCGA presented distinct proportions of 22 TIICs subsets. As the renal cancer samples in GEO were all WT patients, we speculated that the TIICs in WT samples were significantly different from those in other renal cancer samples. Then we analyzed the correlation of immune cell proportions in renal cancer samples from TCGA and GEO. It was revealed that the renal cancer samples from the two datasets had highly consistent immune cell proportions (Fig. 1B), illustrating that CIBERSORT could evaluate the fraction of TIICs independently of data sources and platforms.

3.2 Landscape of TIICs in renal cancer

The compositions of TIICs subsets in 122 paired paracancerous and renal cancer samples from TCGA, and 224 WT samples from GEO were calculated by CIBERSORT respectively. As shown in Fig. 2A, 2B and 2C, the intragroup and intergroup differences in TIICs fraction were manifest. Thus, we inferred that the proportion of TIICs subsets was an inherent characteristic which varied significantly among different individuals. In addition, we analyzed the difference in TIICs composition between renal cancer and paired paracancerous samples. As shown in Fig. 2D, M0 macrophages and CD8 T cells were significantly elevated, while naive B cells were significantly decreased in renal cancer samples, compared with those in paired paracancerous samples.

3.3 P-value of CIBERSORT represents the overall proportion of TIICs

It should be noted that instead of determining the actual values, CIBERSORT only calculates the relative ratios of TIICs subsets, which contributes to the dependency of results on each other. Therefore, we further analyzed the association between the P-value provided by CIBERSORT and TIICs composition. Figure 3A showed that the proportions of samples with P-value < 0.05 and P-value \geq 0.05 in TCGA and GEO were obviously different.

It is known that the mean expression values of GZMA and PRF1 represent the immune cytolytic activity and are positively associated with TIICs proportions. After analyzing their mean expression values, we found that the samples with P-value < 0.05 had higher immune cytolytic activity in both TCGA and GEO cohorts ($p < 2.22e-16$ and $p = 0.043$ respectively, Fig. 3B and 3C). These results indicated that the proportion of TIICs in samples with P-value < 0.05 was higher in comparison to samples with P-value \geq 0.05.

3.4 Overall TIICs proportion, resting mast cells and activated memory CD4 T cells are associated with prognosis of renal cancer

To investigate the effects of overall TIICs proportion or certain TIICs subset on renal cancer prognosis, the survival analyses of 1,010 renal cancer samples with survival information were performed. The survival curve of renal cancer samples stratified by P-value of 0.05 showed that samples with P-value < 0.05 presented inferior survival outcome, in comparison to samples with P-value \geq 0.05 ($p < 0.0001$, Fig. 3D). It was suggested that high TIICs proportions might be associated with poor prognosis of renal cancer patients.

Subsequently, in order to further explore the effects of certain TIICs subset on renal cancer prognosis, the univariate Cox regression analysis was conducted with 22 TIICs subsets as continuous variables. The relevant 95% confidence intervals and Hazard Ratios (HRs) were shown in Fig. 4A. It was found that activated memory CD4 T cells and resting mast cells were significantly associated with the prognosis of renal cancer patients (HR = $9.4e + 05$, $p = 0.004$ and HR = $3.7e - 03$, $p = 0.034$, respectively). The survival curves showed low proportion of resting mast cells and high proportion of activated memory CD4 T cells were related to poor prognosis of renal cancer patients (both $p < 0.0001$).

3.5 Different immune clusters are associated with the prognosis of renal cancer

The above results indicated TIICs alteration might affect the prognosis of renal cancer patients. Therefore, we speculated whether different immune clusters could be identified with the TIICs data.

Firstly, the optimal number of clusters was determined as 3 using the within cluster sum of square errors (WSS) method (Figure S1). Subsequently, hierarchical clustering of the samples was conducted by Euclidean distance model (Fig. 5A). After analyzed the association of different immune patterns with prognosis, cluster 1 exhibited superior survival outcome, while cluster 2 exhibited inferior survival outcome ($p = 0.0057$, Fig. 5B). Moreover, the compositions of 22 TIICs subsets varied significantly across different immune clusters (Fig. 5C).

4. Discussion

Tumor microenvironment is considered as a complex “society”, and exerts a regulatory effect on tumor progression with the participation of multiple cell types and extracellular matrix (Albini et al. 2010). The various cell types in tumor microenvironment include mesenchymal stem cells, fibroblasts, endothelial cells, and immune cells (Ungefroren et al. 2011). It is believed that the TIICs are indispensable members of tumor microenvironment, and reflect the host immune reaction to tumors (Ino et al. 2013).

Accumulating evidences have proved that TIICs are associated with tumor invasion, metastasis, prognosis and response to therapy (Jiang et al. 2011; Jochems et al. 2011). Given their important role, comprehensive researches on the TIICs in the tumor microenvironment may provide novel therapeutic approaches for tumors.

Numerous efforts have been devoted to explore the specific role of TIICs in renal cancer by multiple approaches. Jensen HK et al evaluated the TIICs in localized renal cell carcinoma by immunohistochemistry and found that neutrophils were independent prognostic signatures of survival outcome for localized renal cell carcinoma (Jensen et al. 2009). Donskov F et al identified the positive correlation of CD57 + NK cells and negative correlation of neutrophils with the prognosis of metastatic renal cell carcinoma by immunohistochemistry (Donskov 2006). Webster WS et al proved that the infiltrated mononuclear cells were able to predict the survival outcome of renal cell carcinoma patients independently through hematoxylin and eosin staining and flow cytometric analysis (Webster et al. 2006). Unlike above researches, the TIICs in renal cancer were analyzed using a distinct approach in our study. We assessed the 22 TIICs subpopulations composition of renal cancer and paracancerous samples from TCGA and GEO using CIBERSORT algorithm based on their gene expression profiles. The TIICs subsets proportions of renal cancer samples from TCGA and GEO showed consistent results after correlation analysis. It was suggested that CIBERSORT was able to assess the TIICs subsets composition, which was independently of data platforms and sources.

Then we investigated the association of certain TIICs subpopulation with survival outcome of renal cancer patients by Cox regression analysis and survival analysis. The results showed that elevated proportion of activated memory CD4 T cells and decreased proportion of resting mast cells were associated with poor prognosis of renal cancer. It was known that the activated memory CD4 T cells could secrete interleukin (IL) 17, a proinflammatory cytokine that promotes the proliferation and growth of cervical cancer (Tartour et al. 1999). IL-17 was found to promote the development of colorectal cancer and associate with poor prognosis (Liu et al. 2011). Thus, we speculated that the increased proportion of

activated memory CD4 T cells contributed to inferior survival outcome in renal cancer through enhanced secretion of IL-17. Mast cells, which reside in vascularized tissues, have two states including resting condition and activated condition (Carla et al. 2015). They are involved in multiple tumor-related processes. In thyroid cancer, thyroid cancer cells activated the mast cells, which could secrete extensive proinflammatory, angiogenic, and growth factors and exert protumorigenic effects [30]. We inferred that the decreased proportion of resting mast cells, which reflected the enhancement of activated mast cells activities, was associated with poor prognosis due to the protumorigenic effects of activated mast cells.

In addition, a total of 3 immune clusters was identified by hierarchical clustering analysis. After analyzing their fractions, we found that the proportions of TIICs subpopulations varied across different clusters. The survival analysis revealed that these clusters had different survival outcomes. These findings indicated that immune infiltrate exhibited variability across renal cancer, and distinct immune patterns could affect the prognosis of renal cancer patients.

5. Conclusions

In summary, the analysis of 22 TIICs proportions in renal cancer samples showed elevated activated memory CD4 T cells proportion, and decreased resting mast cells proportion predicted poor prognosis in renal cancer. Different immune clusters also presented distinct survival outcomes. The results might unveil novel prognosis prediction and immunotherapeutic strategies on renal cancer.

Declarations

Ethics approval and consent to participate: Not Applicable.

Consent for publication: Not Applicable.

Availability of data and material: All data generated or analyzed during this study are included in this published article.

Competing interest: The authors declare that there are no conflicts of interest.

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Authors' contributions: All authors read and approved the manuscript. Lei Chen and Liang Yin made substantial contributions to conception and design, acquisition of data, analysis and interpretation of data; Zilong Qi and Jinmin Li performed the experiments; Xinning Wang have been involved in drafting the manuscript or revising it critically for important intellectual content; Kun Ma given final approval of the version to be published. Xiangyang Liu agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Supplemental Figure

Figure S1. Selection of the optimal number of clusters.

Figures

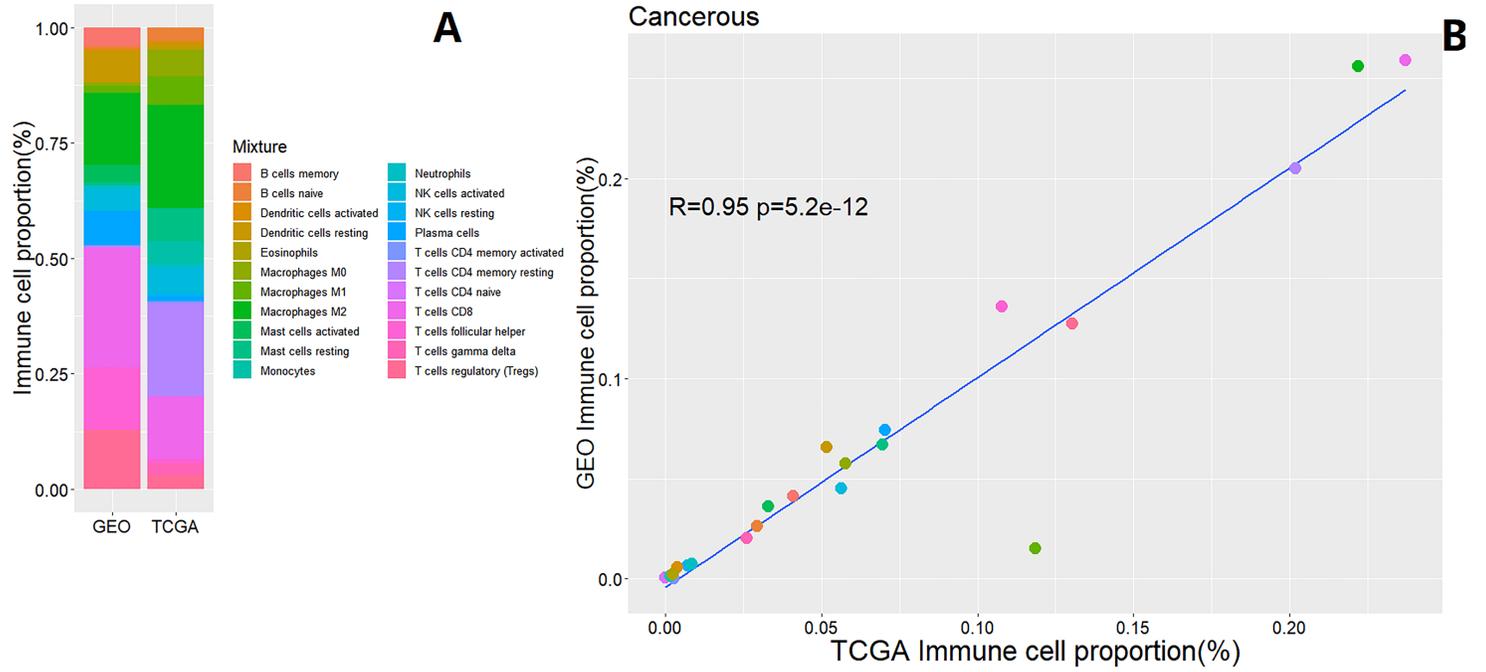


Figure 1

Renal cancer samples from GEO and TCGA had highly consistent immune cell proportion. (A) TIICs compositions of renal cancer and paracancerous samples from TCGA and GEO analyzed by CIBERSORT. (B) Proportions of TIICs subsets in renal cancer samples from TCGA and GEO.

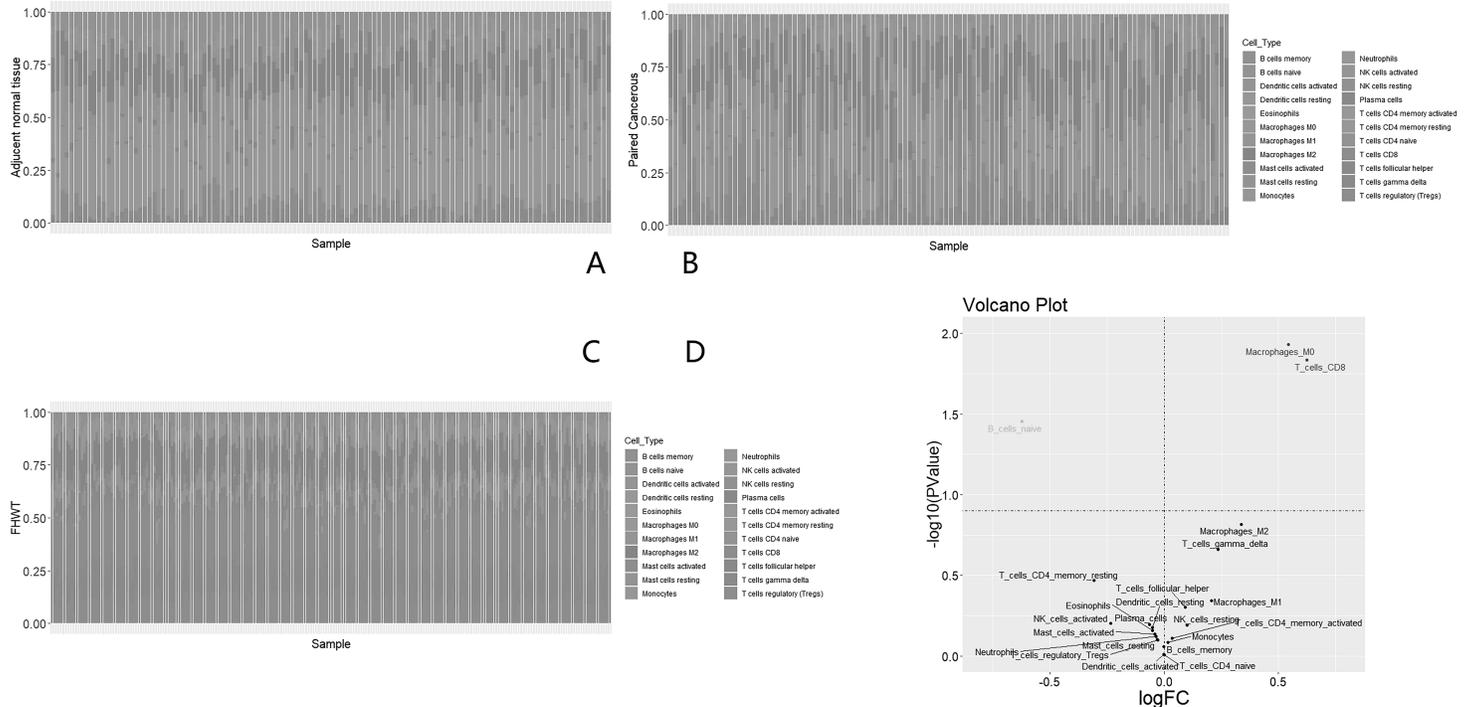


Figure 2

Obvious intragroup and intergroup differences in TIICs fraction were observed among paired renal cancer and paracancerous samples from TCGA, and WT samples from GEO. (A) Immune infiltration in paired paracancerous samples. (B) Immune infiltration in paired renal cancer samples. (C) Immune infiltration in WT samples. (D) Volcano plot of TIICs subsets proportions between paired renal cancer and paracancerous samples. Compared with paired paracancerous samples, M0 macrophages and CD8 T cells were significantly elevated, while naive B cells were significantly decreased in renal cancer samples.

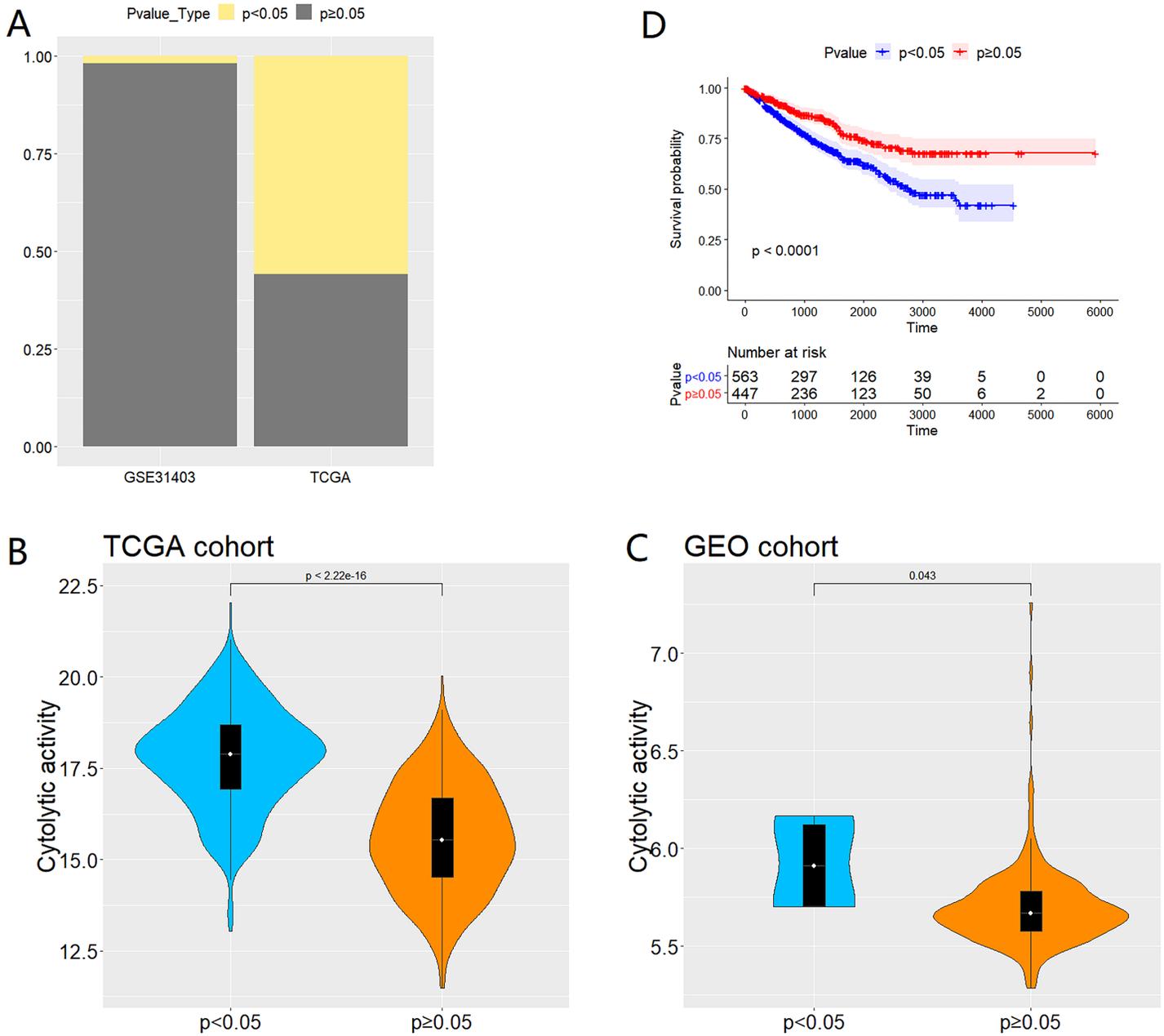


Figure 3

P-value of CIBERSORT represented the overall proportion of TIICs. (A) Proportions of samples with P-value < 0.05 and P-value \geq 0.05 in GEO and TCGA datasets. (B) Immune cytolytic activity of samples

with P-value < 0.05 and P-value \geq 0.05 in TCGA cohort. (C) Immune cytolytic activity of samples with P-value < 0.05 and P-value \geq 0.05 in GEO cohort. (D) Survival curves of samples stratified by P-value of 0.05. Compared with renal cancer patients with P-value \geq 0.05, patients with P-value < 0.05 had poor survival outcome ($p < 0.0001$).

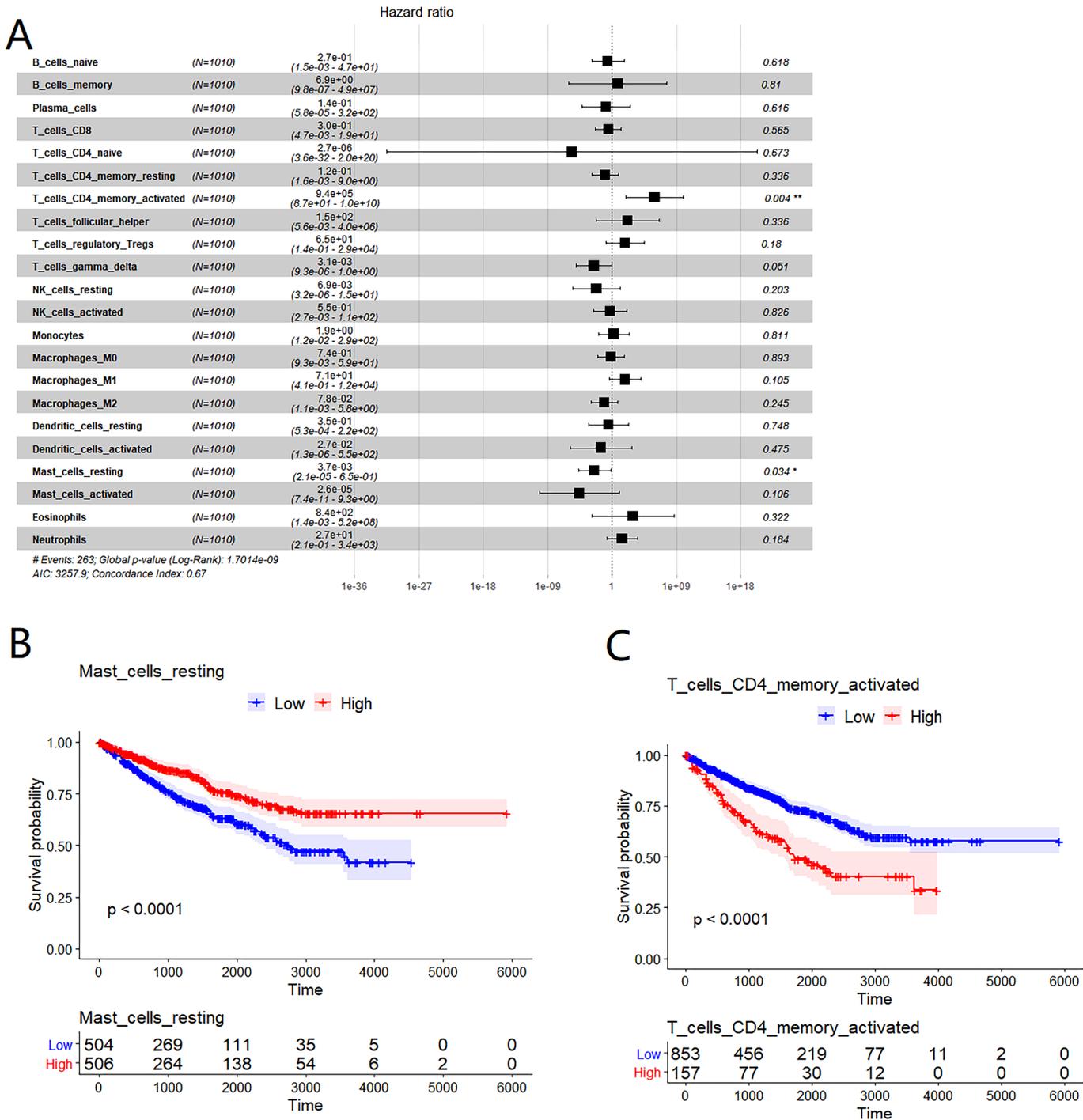


Figure 4

Decreased resting mast cells and elevated activated memory CD4 T cells were associated with poor prognosis of renal cancer patients. (A) Association of 22 TIICs subsets with survival outcome of renal

cancer patients. * and ** indicated statistical significance. (B) Survival curve of resting mast cells stratified by median proportion. (C) Survival curve of activated memory CD4 T cells stratified by median proportion.

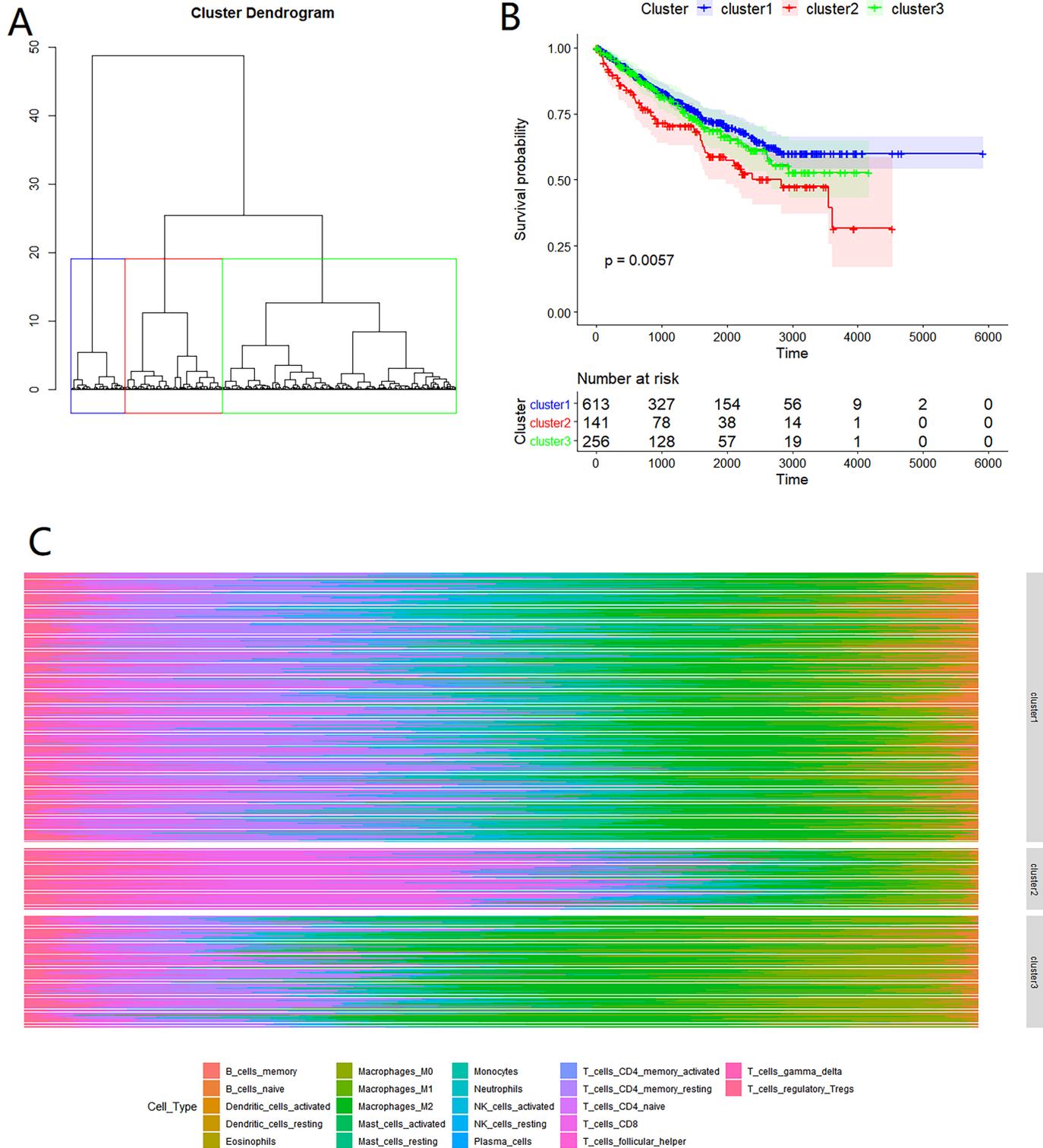


Figure 5

Immune clusters were associated with the prognosis of renal cancer patients. (A) Euclidean distance model identified three different immune clusters (cluster 1, cluster 2 and cluster 3). (B) Survival curves of

cluster 1, 2 and 3. Cluster 1 presented superior survival outcome, while cluster 2 presented inferior survival outcome ($p = 0.0057$). (C) The 3 clusters exhibited different compositions of 22 TIIcs subsets.

Supplementary Files

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- [FigS1.tif](#)