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Article

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The immune reprogramming mediated by MZB1 reveals the immune and prognostic features of clear cell renal cell carcinoma

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Abstract

Objective: Immune reprogramming plays a crucial role in establishing the tumor immune microenvironment (TIME). This study aims to explore potential regulatory factors of TIME and their impact on the prognosis and immunotherapy of clear cell renal cell carcinoma (ccRCC).

Methods: We obtained the RNA sequencing data of 529 ccRCC samples from The Cancer Genome Atlas (TCGA) database. The ESTIMATE algorithm and Kaplan-Meier survival curve analysis were applied to investigate the relationship between immune cell and stromal cell infiltration levels in all ccRCC samples and patient overall survival (OS). Immune genes significantly associated with ccRCC prognosis were identified through univariate Cox regression analysis and protein-protein interaction network analysis. The individual key immune genes were identified by the gene alteration...
analysis for further study, such as clinical feature correlation analysis, gene set enrichment analysis (GSEA), estimation of tumor-infiltrating immune cell (TIC) proportions, immune checkpoint correlation analysis, and drug sensitivity analysis. These processes were designed to discover the potential regulatory effects of the key immune genes in TIME. Finally, the expression of the key immune gene was confirmed using the UALCAN and Human Protein Atlas (HPA) databases.

**Results:** We obtained seven key genes significantly associated with the prognosis of ccRCC via comprehensive analysis, which were IL6, PLG, IGLL5, MZB1, CCL13, CD19, and POU2AF1. The gene alteration analyses indicated that MZB1 presented the highest mutation rate and was associated with the survival in 354 patients with ccRCC. And thus, we took the MZB1 for further study. Other analyses showed that MZB1 expression was up-regulated in ccRCC tumor samples and negatively correlated with survival. In the immune microenvironment of ccRCC, we found a higher level of immune infiltration of several TICs such as CD8+ T cells, Tregs, and macrophages. Furthermore, MZB1 expression was positively correlated with the infiltration level of eight TICs, including B memory cells, CD4+ T cells, CD8+ T cells, plasma cells, and Tregs. It also exhibited a positive correlation with six common immune checkpoint molecules, including PDCD-1, CTLA-4, and LAG3, including PDCD-1, CTLA-4, and LAG3. Drug sensitivity analysis suggested that high expression of MZB1 reduced the sensitivity to PD-1 immune checkpoint inhibitors, such as nivolumab and pembrolizumab. The GSEA enrichment analysis demonstrated that the MZB1 high-expression group was mainly associated with immune-related pathways such as NF-κB signaling, interferon reaction (IFNα, IFNγ), and IL2-STAT5 signaling. In contrast, the enrichment results of the MZB1 low-expression group were mainly associated with tumor metabolism, such as the bile acid metabolism, the fatty acid metabolism, the oxidative phosphorylation and other metabolic pathways. Finally, we found that MZB1 protein showed high expression in ccRCC patients in the UALCAN database, regrettably, the HPA immunohistochemistry database did not detect the expression of MZB1.

**Conclusion:** MZB1 promotes the formation of the tumor immune-suppressive
microenvironment by mediating immune reprogramming, including the recruitment of immunosuppressive TICs and the expression of immune checkpoint, and it is prospective to be a prognostic factor for ccRCC immunotherapy.

**Keywords** ccRCC; tumor immune microenvironment; immune reprogramming; immune checkpoints

1. Introduction

In recent years, the tumor microenvironment (TME) has emerged as a novel indicator of cancer progression and metastasis, in which the interaction between tumor cells and immune cells was a focus of attention [1]. It is well-known that clear cell renal cell carcinoma (ccRCC) is highly immune infiltrated, displaying distinct clinical and pathological characteristics under different immune cell infiltrations [2]. The immune microenvironment of ccRCC represents a complex immune status formed by the interaction of various immune cells, cytokines, and signaling pathways. One of its defining features is the presence of tumor-infiltrating immune cells (TICs), including T cells, B cells, natural killer cells, dendritic cells, macrophages, and myeloid-derived suppressor cells (MDSCs). These cells interact through a complex network of signaling pathways to establish a dynamic microenvironment with both anti-tumor immune stimulation and immune suppression [3,4].

In many cancers, tumor-infiltrating immune cells (TICs) commonly exhibit targeted effects on tumor cells and inhibition of tumor growth, displaying anti-tumor immune activity. However, in the immune microenvironment of ccRCC, these cells can demonstrate pro-cancer activity and participate in tumor development and metastasis [5]. For instance, CD4+ T cells can promote ccRCC proliferation through the TGFβ1/YBX1/HIF2α signaling axis [6]. MDSCs inhibit T cell function by producing immunosuppressive factors such as prostaglandin E2 and IL-10, as well as depleting arginine, thereby promoting tumor growth [7]. Additionally, the role of TICs in tumors is regulated by immune infiltration-related genes (IIRGs), which serve as effective indicators for individualized immune therapy [8,9].
In the past decade, anti-angiogenic targeted agents have been applied extensively in the treatment of metastatic ccRCC, among which inhibitors targeting vascular endothelial growth factor (VEGF) and inhibitors of the mammalian target of rapamycin (mTOR) are the most representative, such as sunitinib, axitinib, sorafenib, everolimus, temsirolimus and etc.\textsuperscript{[10]} Despite the fact that these molecularly targeted agents have made great progress in the management of metastatic ccRCC, the prognosis of some patients is still unsatisfactory due to individual differences and resistance to targeted therapies.\textsuperscript{[11]} One important reason for this is that TICs increase resistance to anti-angiogenic targeted agents, such as tyrosine kinase inhibitors (TKIs).\textsuperscript{[12,13]}

Recent studies have confirmed that ccRCC upregulates the expression of immune checkpoint molecules to promote the formation of an immune-suppressive microenvironment, such as programmed death-ligand 1 (PD-L1), which aids cancer cells in escaping from immune surveillance by inhibiting T cell activation.\textsuperscript{[14,15]} Currently, immune checkpoint inhibitors (ICIs) are considered a new and available treatment for treating patients with metastatic ccRCC. For example, immune checkpoint inhibitors targeting PD-1 and PD-L1 have been approved for clinical use and have shown significant improvement in patient prognosis.\textsuperscript{[16]} Most importantly, the combination of traditional targeted therapies in ccRCC with ICIs has demonstrated more pronounced anti-tumor effects in some clinical trials.\textsuperscript{[17]} Therefore, analyzing the regulatory network of the immune microenvironment in ccRCC and exploring new immune predictive factors and prognostic indicators are of great significance for individualized treatment of ccRCC patients\textsuperscript{[18]}.

2. Methods

2.1 Data Source

We collected transcriptomic RNA-seq data from 529 CCRCC tumor samples and 72 adjacent non-tumor samples from the TCGA database, as well as relevant clinical data.
2.2 Analysis of the Association between ESTIMATE Score, Overall Survival (OS), and Clinicopathological Characteristics

We utilized the R package "estimate" to calculate the infiltration proportions of immune and stromal components in all tumor samples. The immune infiltration was represented by the ImmuneScore, while the stromal infiltration was represented by the StromalScore. The scores are positively correlated with the degree of infiltration, with higher scores indicating higher infiltration levels. The ESTIMATEScore is a comprehensive score that combines both immune and stromal components, representing their combined proportion in the tumor microenvironment (TME).

Furthermore, based on the median score, we divided all tumor samples into high and low immune, stromal, and ESTIMATE score groups. We generated Kaplan-Meier curves to compare the overall survival (OS) between these two groups. Additionally, the relationship between prognostically relevant scores and clinicopathological parameters was assessed via the method of using the Kruskal-Wallis rank sum test.

2.3 Identification of Immune-Related Genes

Based on the median immune score or stromal score, the 529 ccRCC samples were divided into high and low score groups. We performed differential gene expression analysis using the "limma" R package to identify immune-related genes. The R package "pheatmap" was used to generate heatmaps of gene expression. Only FDR <0.05 and |Log(FC)|>1 were considered significant.

2.4 Enrichment Analysis of Immune-Related Genes

The immune-related genes obtained from the intersection of genes selected based on immune score and stromal score were considered as common immune genes. These genes were then subjected to Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis using R packages such as clusterProfiler, enrichplot, and ggplot2. Significantly enriched terms were identified when both p-
values and q-values were less than 0.05.

2.5 Protein-Protein Interaction (PPI) Network and Cox Regression Analysis

We established a protein-protein interaction (PPI) network of the common immune genes using the STRING database (http://string-db.org/). The relationship score was set to 0.7, and the network was further optimized using Cytoscape v.3.9.1 software. We used the CytoHubba plugin's degree analysis in Cytoscape to identify the top 30 genes as key immune genes. To identify immune genes significantly associated with ccRCC prognosis, we performed univariate Cox regression analysis based on the survival time of 529 ccRCC patients. This analysis was conducted using the "survival" R package, and a significance level of P<0.001 was considered significant. At last, We intersected the results of these two analyses to obtain the key immune genes associated with prognosis.

2.6 Gene Alteration Analysis to Determine Individual Key Immune Gene for Further Study

The cBioPortal database (http://www.cbioportal.org/) is an excellent platform that provides various cancer genomics information. We selected 512 ccRCC patients from the TCGA-PanCancerAtlas database as the analysis dataset. The key immune genes associated with prognosis were subjected to genomic analysis in the database. We utilized the OncoPrint function module to examine the gene mutation status, and the Survival function module to analyze the correlation between gene mutations and patient survival. Based on the mutation rate of the gene and its association with prognosis, we determined the individual key immune gene for further study.
2.7 Relationship between Expression of Individual Key Immune Gene and OS, Clinicopathological Characteristics

Based on the median expression of the individual key immune gene, the 529 samples were divided into low expression and high expression groups. Kaplan-Meier survival analysis was performed to compare the overall survival (OS) between these two groups. The Wilcoxon rank-sum test was used to analyze the differential expression of the key immune gene between the tumor group and the non-tumor group. Additionally, the Kruskal-Wallis rank-sum test was employed to analyze the relationship between the key immune gene and clinicopathological parameters. ROC curves were used to assess the accuracy of this immune gene in predicting survival in ccRCC patients as well as survival time.

2.8 GSEA Enrichment Analysis of Individual Key Immune Gene

We performed GSEA enrichment analysis using the Hallmark and C7-V7.0 gene sets, both of which use the R packages cluster Profiler, enrichplot, and gseaplot2, and only gene sets with NOMp<0.05 and FDRq<0.06 were considered significant.

2.9 Correlation Analysis between Individual Key Immune Gene and Tumor-Infiltrating Immune Cell (TICs)

We assessed the infiltration percentage of 21 TICs in all tumor samples using the R package CIBERSORT, and then retained tumor samples with P<0.05 to analyze the correlation between the 21 TICs and individual key immune genes.
2.10 Correlation Analysis between Individual Key Immune Gene and Common Immune Checkpoints in ccRCC

We utilized the TISIDB database (http://cis.hku.hk/TISIDB/index.php) to investigate the relationship between a single key immune gene and common immune checkpoints. Additionally, we predicted the sensitivity of immunotherapy and targeted therapy-related drugs using the R package "oncopredict", which could evaluate the impact of this immune gene on ccRCC immunotherapy.

2.11 Validation of Differential Expression of Individual Key Immune Gene

The UALCAN database (path.uab.edu/analysis-prot.html) integrates oncogene protein expression data from the CPTAC database, through which we analyzed the differential expression of the individual key immune gene at the protein level in normal tissues and ccRCC tissues. Besides, The HPA database (https://www.proteinatlas.org/) contains a large amount of oncogene expression information in a variety of cancer tissues and normal organ tissues, through which the expression of this gene in ccRCC is verified by immunohistochemical data.

2.12 Statistical Analysis

Kaplan-Meier survival curves were used to analyze the relationship between survival rate and Immune/Stromal/ESTIMATE scores in ccRCC patients. Wilcoxon rank-sum test or Kruskal-Wallis rank-sum test was employed to analyze the correlation between Immune/Stromal/ESTIMATE scores and clinicopathological characteristics. Univariate Cox regression analysis was performed to screen for genes significantly associated with prognosis. The effects of MZB1 expression on survival and other clinicopathological characteristics were analyzed using Wilcoxon rank sum or Kruskal-Wallis rank sum tests; the relationship between MZB1 and 21 TICs was assessed using Spearman correlation analysis. Statistical significance was set at p < 0.05. All of the
3. Results

3.1 Relationship between ESTIMATE Scores and OS

We divided tumor samples into high and low score groups based on the median scores of the three assessments, and subsequently constructed Kaplan-Meier curves to analyze the relationship between each score and patient survival. The results showed that patients with a higher immune score had significantly worse overall survival (OS) compared to those with a lower score (Figure 1-A). In contrast, there was no significant relationship between stromal and ESTIMATE scores and OS, suggesting that immune cell infiltration is a better prognostic indicator for ccRCC.

![Figure 1](image)

Figure 1  A-C: The relationship between immune score, stromal score, and ESTIMATE score with the survival of ccRCC patients.

3.2 Correlation between Immune Score and Clinicopathological Characteristics

Figure 2 illustrates the relationship between immune cell infiltration and clinicopathological characteristics. The results show that the immune score is positively correlated with tumor pathological grade (Figure 2-C, p < 0.05). In terms of TNM staging, the immune score is associated with T-stage and M-stage (Figure 3-D, F). In addition, the immunity score was also related to gender, and there was no significant
difference between the immunity score of older and younger patients. These findings suggest that immune cell infiltration is involved in the progression of ccRCC, including invasion and metastasis.

Figure 4  A, B: The relationship between immune score and age, gender. C: The relationship between immune score and tumor pathological grade. D, E, F: The relationship between immune score and tumor TNM staging.

3.3 Identification and Enrichment Analysis of TIME-Related Genes

We obtained 637 up-regulated and 387 down-regulated genes from the ImmuneScore group, 283 up-regulated and 584 down-regulated genes from the StromalScore group, from which 363 co-immune genes were acquired by taking the intersection, including 75 up-regulated and 288 down-regulated genes. Furthermore, enrichment analysis of these 363 genes revealed that GO enrichment was primarily associated with B-cell proliferation, CCR chemokines, JAK-STAT signaling pathway, and organic substance transport. KEGG enrichment analysis showed relevance to viral protein-cellular cytokine interactions, complement activation, JAK-STAT signaling pathway, IgA immune response and other immune pathways. These enrichment results collectively demonstrate the immune relevance of this set of genes.
3.4 Intersection Analysis of PPI Network and Univariate Cox Regression

We constructed a PPI network of 363 immune genes through the STRING database, with the minimum interaction score set at 0.7, and optimized the PPI network using Cytoscape software, after which the top 30 genes were taken as the key immune genes according to the degree score by Cytohubba plug-in. We performed univariate COX regression analysis based on the survival time of 529 ccRCC patients, which was considered to be significantly associated with prognosis at p-value < 0.001, and then, the genes obtained from the PPI network analysis and the univariate COX regression analysis were intersected to obtain seven key immune genes, which were IL6, PLG, IGLL5, MZB1, CCL13, CD19, POU2AF1.
3.5 Gene Alteration Analysis Identifies MZB1 as a Single Key Immune Gene for Further Investigation

To determine a single key immune gene for further investigation, we performed gene alteration analysis on the 7 immune genes significantly associated with prognosis. The results showed that among 448 ccRCC patients, IL6, PLG, MZB1, CCL13, CD19, and POU2AF1 exhibited genomic alterations. MZB1 had the highest mutation rate and was correlated with VHL gene mutations. Furthermore, MZB1 mutations were also associated with disease survival rates in ccRCC patients, suggesting that MZB1 mutations impact patient prognosis. It is well known that the VHL gene is closely associated with the pathogenesis of ccRCC. Therefore, we further explored the regulative actions of MZB1 in the immune microenvironment.
3.6 Relationship between MZB1 Expression and Survival, Clinical Pathological Characteristics in ccRCC

To further elucidate the expression of MZB1 in ccRCC, we conducted differential expression analysis between tumor and non-tumor samples. The results revealed that MZB1 is upregulated in ccRCC (Figure 6-A). Furthermore, survival analysis demonstrated a negative correlation between MZB1 expression and overall survival in ccRCC patients (Figure 6-F), and the ROC curve accurately predicted survival at 1, 3, and 5 years (Figure 6-H). Regarding clinical pathological parameters, MZB1 expression increased with tumor pathological grade and TNM staging progression (stage I vs stage II, stage III; T1 vs T3; N0 vs N1; M0 vs M1; P<0.05), suggesting that MZB1 is involved in the occurrence and progression of ccRCC.
3.7 Protein expression levels and immunohistochemical analysis of MZB1 in ccRCC

Figure 7 displays the expression of MZB1 protein in normal kidney and ccRCC. We retrieved the expression data of MZB1 in 84 normal kidney tissues and 110 ccRCC tissues from the UALCAN database. The results showed that MZB1 protein is upregulated in ccRCC (Figure 7-A, statistical difference value $P < 0.05$). Additionally, the HPA database provided immunohistochemical data for MZB1 in 3 normal kidney tissues and 12 ccRCC specimens. Unfortunately, all tumor and normal samples showed no positive staining cells. It has been reported that MZB1 is upregulated in ccRCC and influences patient prognosis\[19\]. Taking all these results into consideration, we conclude that MZB1 protein is highly expressed in ccRCC, which is consistent with the previous analysis.
3.8 The immune landscape of MZB1

To further confirm the correlation between MZB1 expression and the immune microenvironment, we used the CIBERSORT algorithm to analyze the proportions of tumor-infiltrating immune cells (TICs) and constructed immune cell infiltration profiles for 21 different immune cell types in all tumor samples. The results showed that several kinds of TICs, including B cells, CD8+ T cells, CD4+ T cells, regulatory T cells (Tregs), NK cells, dendritic cells (DCs), and macrophages, exhibited different degrees of immune infiltration in ccRCC (Figure 8-A). According to the results of the correlation analysis between the expression of MZB1 and the TICs, there were 12 kinds of TICs associated with the expression of MZB1. Among them, 8 TICs showed a positive correlation with MZB1 expression, including B memory cells, CD4+ T cells, CD8+ T cells, plasma cells, Tregs, follicular helper T cells, and resting mast cells. On the other hand, 4 TICs were negatively correlated with MZB1 expression, including monocytes, resting NK cells, M2 macrophages, and resting mast cells (Figure 8-C). These results suggest that ccRCC has a highly immunologically infiltrated microenvironment, and MZB1 is an influential factor in the immune function of a variety of TICs, such as B cells, CD8+ T cells, regulatory T cells (Tregs), NK cells, and macrophages.
We divided ccRCC tumor samples into high MZB1 expression group and low MZB1 expression group according to the median MZB1 expression, and respectively performed GSEA enrichment analysis within two gene sets, Hallmark and C7-V7.0. The results demonstrated that, the high-MZB1 expression group in the Hallmark gene set was mainly associated with immune-related pathways of action, such as allograft rejection, complement production, interferon response, IL2-STAT5 signaling and other immune-related pathways (Figure 11-A). In addition, high-MZB1 expression was also associated with common cancer signaling pathways, such as the G2 checkpoint of the cell proliferation cycle, NF-κB apoptosis signaling pathway, and the epithelial-mesenchymal transition signaling pathway. In contrast, the low-MZB1 expression group was mainly associated with tumor metabolism signaling pathways, such as bile acid metabolism, fatty acid metabolism, and oxidative phosphorylation and etc(Figure 12-B). In the C7 immune gene set, the number of immune pathways enriched in the MZB1 high-expression group was significantly higher than that in the low-expression group. These results suggest that MZB1 is a vital regulator of the immune microenvironment in ccRCC.
3.9 Effect of MZB1 on common immune checkpoints and immunotherapeutic drugs

The immune checkpoint pathway is a key mechanism for tumors to evade immune surveillance, and we analyzed the relationship between MZB1 and immunosuppression related immune checkpoints of ccRCC via the TISIDB database, including PDCD1, LAG3, TIGIT, CTLA4, IL10, and HAVCR2. The results showed a positive correlation between MZB1 expression and these six major immune checkpoints (Figure 14 A-F). In terms of drug sensitivity analysis, high expression of MZB1 could down-regulate the sensitivity of PD-1 checkpoint inhibitors such as navulizumab and pembrolizumab (Figure 11 A-B), and it also had an inhibitory effect on the sensitivity of traditional anti-angiogenic targeted drugs(Figure 11 C-F). Consequently, we suggest that MZB1 is an important factor in the formation of the tumor immunosuppressive microenvironment as well as a potential observable of ccRCC immune efficacy.
Figure 15  A-F: the correlation of MZB1 with common immune checkpoints of PDCD1, LAG3, TIGIT, CTLA4, IL10, and HAVCR2

Figure 16 A-B: Impact of MZB1 expression on the sensitivity of navulizumab and pabolizumab; C-F: Impact of MZB1 expression on the sensitivity of common ccRCC-targeted drugs such as cabozantinib, lenvatinib, sunitinib, axitinib(* represents p-value < 0.05).

4. Discussion

The progression of cancer occurs concurrently with the changes in the surrounding stroma, and cancer cells can direct the reprogramming of surrounding cell functions via the secretion of various cytokines, chemokines, and other factors, which shapes the microenvironment conducive to their survival and metastasis. Therefore, the progression of cancer is considered to be the outcome of complicated interactions between tumor cells and their microenvironment[20]. Not only are immune cells an
essential component of the tumor stroma, but they are also the leading regulators of the tumor immune microenvironment (TIME). Research has confirmed a significant correlation between TIME and the occurrence and progression of ccRCC. Tumor-infiltrating immune cells (TICs) play a crucial role in influencing the progression of ccRCC and its response to immunotherapy, and they possess significant prognostic value.\[21\]

In our research, we found that immune scoring is significantly associated with poor prognosis in patients with ccRCC. Furthermore, as the immune score increases, tumor progression becomes more evident. These findings suggest that the degree of immune cell infiltration impacts the progression of ccRCC and indicates compromised patient prognosis. Generally, there is a close association between cancer development and genetic mutations. It is known that the core pathogenic gene of ccRCC is VHL, and inactivation of this gene leads to activation of hypoxia-inducible factor (HIF), which in turn induces overexpression of a variety of target genes involved in glycolysis, tumor cell migration, and angiogenesis, exacerbating cancer progression and metastasis\[22\]. Our analysis revealed that MZB1 mutations were associated with VHL gene expression and that the high expression of MZB1 was negatively correlated with the survival of ccRCC patients. In addition, MZB1 was associated with the TNM stage and pathological grading of tumors, and as the tumor progressed, the expression of MZB1 increased. These results suggest that MZB1 a critical factor impacting the progression and prognosis of ccRCC.

The human MZB1 gene, also called plasma cell-induced endoplasmic reticulum resident protein (pERp1), is located on chromosome 5 in the q31.2 region, with four exons. In mammals, the sequence of MZB1 shows only 21.6% overall conservation\[23\]. pERp1 is mostly found in congenital B cells, including splenic marginal zone (Mz) B cells, peritoneal B1 cells, and antibody-secreting cells\[24\]. It can be directly activated by B lymphocyte-induced maturation protein-1 (Blimp1), which regulates B cell differentiation into plasma cells. Additionally, it enhances the interaction between immunoglobulin (Ig) heavy chains and the chaperone protein GRP94, thereby promoting IgM and IgA secretion and playing a crucial role in humoral immune
Some studies have found that MZB1 is also expressed in plasmacytoid dendritic cells (pDCs), where it induces the secretion of large amounts of interferon-alpha (IFNα) through the unfolded protein response (UPR) mediated by ATF6. This response pathway is positively regulated by Toll-like receptors 7/9 (TLR7/9) \(^{[27,28]}\). In addition, this IFNα also stimulates B cells to transform into plasma cells to increase antibody secretion and enhance the immune response\(^{[28,29]}\). In the tumor microenvironment, cancer cells can secrete various cytokines (such as transforming growth factor β (TGFβ)) to impede the secretion of IFNα from tumor-infiltrating pDCs, which ultimately confers immunosuppressive properties to the pDCs, and this transformation of immunosuppressive properties can be seen in many cancers such as breast cancer, lung cancer, and ovarian cancer\(^{[27,30,31]}\). In some studies, it has been found that up-regulation of MZB1 expression is associated with poorer prognosis in various cancers, including lung adenocarcinoma, pancreatic cancer, follicular lymphoma, and estrogen receptor-positive breast cancer, and it also exhibits immune relevance in these tumors\(^{[32–34]}\).

Although the immune system is capable of recognizing and eliminating tumor cells, tumors have evolved multiple mechanisms to establish an immunosuppressive microenvironment evading immune surveillance\(^{[15]}\). In ccRCC, several mechanisms have been discovered to contribute to the formation of an immunosuppressive microenvironment, including alterations in antigen presentation, induction of immune checkpoint pathways, and recruitment of immune inhibitory cells\(^{[15,35]}\). Our research has found a higher infiltration level of macrophages, CD4\(^+\) T cells, Tregs, and CD8\(^+\) T cells in ccRCC. Additionally, the expression of MZB1 correlates positively with various tumor immune infiltrating cells such as B memory cells, CD4\(^+\) T cells, CD8\(^+\) T cells, plasma cells, and Tregs, while it is negatively correlated with monocytes, resting NK cells, M2 macrophages, and resting mast cells. ccRCC is considered a highly immunogenic malignancy that recruits immune inhibitory cells like regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSCs) into the tumor microenvironment, mediating immune dysfunction and creating an immune suppressive microenvironment favorable for tumor growth\(^{[36,37]}\). In general, immune
infiltration in ccRCC is mainly composed of T cells and MDSCs, with a higher proportion of CD4$^+$ T, CD8$^+$ T cells, and Tregs infiltrating. This aligns with our research findings, suggesting that MZB1 may be a potential regulatory factor in the ccRCC immune microenvironment. CD8$^+$ T cells play a leading role in TIME as they have immune surveillance functions to recognize and kill cancer cells, playing a dominant role in the tumor immune microenvironment (TIME). Researches finds that high levels of activated CD8$^+$ T cells show better prognosis in many cancers. However, in the tumor immune microenvironment of ccRCC, the infiltration of CD8$^+$ T cells is associated with poor prognosis, possibly due to T cell exhaustion. Additionally, Tregs are a subset of T cells with immune-suppressive properties which can inhibit the activation of effector T cells by producing inhibitory cytokines such as TGF-β, IL-10, IL-35 and upregulates the expression of immune checkpoint CTLA-4, promoting tumor immune evasion and the formation of an immunosuppressive microenvironment. Interestingly, CD4$^+$ T cells, NK cells, and macrophages have dual regulatory effects on the proliferation of ccRCC tumor cells. Depending on their polarization status, CD4+ T cells can simultaneously exhibit both pro-tumor and anti-tumor activities. NK cells and macrophages can promote cancer progression by secreting cytokines and growth factors that support tumor growth and angiogenesis. In addition, NK cells can also inhibit the activity of other anti-tumor immune cells to help tumors undergo immune escape. Based on the above discussion, we hypothesized that MZB1 may exhibit immunosuppressive effects in the ccRCC immune microenvironment by recruiting immune inhibitory cells.

In order to research the potential molecular functions of MZB1 in the immune microenvironment of ccRCC, we conducted GSEA enrichment analysis on the expression subgroups of MZB1. The results showed that the high expression group of MZB1 was mainly associated with immune-related signaling pathways, such as NF-κB signaling, interferon response (IFNα, IFNγ), IL2-STAT5 signaling, and others. While the low expression group of MZB1 was primarily associated with tumor metabolism pathways, including bile acid metabolism, fatty acid metabolism, oxidative phosphorylation, and others. In the human body, IFN-α is primarily produced by...
plasmacytoid dendritic cells (pDCs) in response to toll-like receptor 7/9 (TLR7/9) stimulation. It is commonly used in the treatment of diseases such as malignant melanoma, chronic leukemia, and Kaposi sarcoma\(^{[45]}\). Studies have found that tumor-infiltrating pDCs lose their responsiveness to TLR stimulation in many types of cancer, leading to reduced or absent production of IFNα. Importantly, tumor-infiltrating pDCs can also recruit regulatory T cells (Tregs), promoting the formation of a tumor immune-suppressive microenvironment that favors tumor growth\(^{[46,47]}\). It is worth noting that in the comprehensive treatment of RCC, there exists a synergistic effect between IFN-α and VEGF-targeted drugs. Genes involved in IFNγ signaling play an important role in the prognosis of RCC\(^{[48]}\). For example, IRF family genes adjusts the cell cycle and induces apoptosis in RCC cells, while RBCK1 promotes RCC cell proliferation by ubiquitinating and degrading p53\(^{[49,50]}\).

Current studies have confirmed that the signal transducer and activator of transcription (STAT) signaling pathway was involved in the progression of various human cancers, including ccRCC. Upregulation of STAT protein family expression can promote cancer cell proliferation, migration, and immune suppression, thereby promoting tumor growth and survival\(^{[51]}\). IL-2-induced STAT5 signaling restricts the differentiation of helper T cells 17 (Th17) by depressing the secretion of IL-17\(^{[52]}\). Additionally, STAT5 can promote the generation of regulatory T cells (Tregs) by regulating the function of forkhead box protein P3 (FoxP3)\(^{[53]}\). It is important to note that the activation of the STAT5 signaling pathway relies on the phosphorylation of various tyrosine kinases (such as JAK2, Bcr-Abl, Flt3, and Src). Combination therapy with STAT5 inhibitors and various TKIs has shown synergistic effects and alleviate resistance to treatment in patients undergoing TKI therapy\(^{[54,55]}\).

Furthermore, the NF-κB signaling pathway is one of the pro-cancer pathways caused by the activation of hypoxia-inducible factor (HIF) due to the inactivation of VHL. NF-κB induces the expression of cyclin D1, ultimately resulting in cancer cell proliferation, invasion, and angiogenesis\(^{[56]}\). Additionally, the IL6-STAT3 pathway also can promote the progression of ccRCC by inducing the expression of cyclin D1\(^{[57]}\). These pathways are collectively activated and regulated by HIF as well as associated
with a worse prognosis in ccRCC\textsuperscript{[58,59]}. Studies have found that NF-κB can regulate T cell activation and homeostasis, contributing to the formation of immune tolerance. For example, NF-κB can enhance FOXP3 expression to promote Treg differentiation and upregulate PD-1 expression in macrophages to facilitate immune suppression\textsuperscript{[58,60]}. These findings indicate that MZB1 may play a critical role in modulating the immune microenvironment of ccRCC through its involvement in these pathways.

The immune checkpoint pathway is a key mechanism for tumors to escape immune surveillance, and ccRCC tumor cells can upregulate the expression of immune checkpoint molecules such as programmed cell death protein 1 (PD-1) and cytotoxic T lymphocyte-associated antigen 4 (CTLA-4). These molecules can depress the activity of effector T cells and antigen-presenting cells, promoting immune escape\textsuperscript{[14]}. Immune checkpoint inhibitors (ICIs) are currently approved as first-line treatments for metastatic ccRCC, and their main role is to activate anti-tumor immune responses by blocking inhibitory molecules on T cells or their ligands on tumor cells\textsuperscript{[15]}. The common immune checkpoint molecules in ccRCC, such as PD-1 and CTLA-4, are expressed on activated effector T cells and can be activated by ligands expressed on tumor cells or other cells in the tumor microenvironment (TME), contributing to tumor promotion. Inhibitors of these checkpoints, such as navulizumab, pabolizumab, and atilizumab, have been licensed for the treatment of ccRCC and other cancers, showing the prospects of improving patients' prognosis\textsuperscript{[17,61]}. Similar immune checkpoints include LAG-3 (lymphocyte activation gene 3) and TIGIT (T-cell immunoglobulin and ITIM structural domain), which are checkpoint molecules expressed on NK and T cells that can interact with many ligands expressed on tumor cells, such as CD155, thus supporting immune escape\textsuperscript{[62]}. It was reported that blocking TIGIT could enhance anti-tumor immune responses\textsuperscript{[15,63]}. LAG-3 is also upregulated in ccRCC and blocking this checkpoint with antibodies can enhance T-cell activity and inhibit tumor growth\textsuperscript{[64]}. Notably, immune suppressive cells recruited in the ccRCC immune microenvironment can produce cytokines favorable for cancer cell growth and upregulate the expression of immune checkpoints, leading to resistance to ICI treatment\textsuperscript{[65]}. Our research revealed a positive correlation between MZB1 and the expression of multiple immune
checkpoint molecules, including PDCD-1, CTLA-4, and LAG3. Additionally, upregulation of MZB1 expression decreased the sensitivity to PD-1 immune checkpoint blockade and VEGF-targeted drugs, indicating the potential of MZB1 as an immunotherapeutic target for ccRCC.

With intensive research on immune infiltration of various tumors, cancer treatment has transformed from the traditional strategy of solely targeting oncogenic genes to a more comprehensive approach focusing on the tumor microenvironment. We analyzed the unique immune infiltration status of ccRCC and excavated potential markers regulating the immune microenvironment to provide valuable insights for personalized treatment of patients with metastatic ccRCC. However, there are still limitations to this study. Although we have initially revealed the regulatory role of MZB1 in the tumor immune microenvironment through analysis of public databases and confirmed the distinct clinical and biological significance of the ccRCC tumor immune suppressive microenvironment, it still requires relevant biological experiments to support our findings and help achieve clinical translation. Additionally, the role of MZB1 in other cancers exhibits heterogeneity, and the underlying reasons remain to be extensively explored, which would contribute to the precise and personalized treatment of cancer.

5. Conclusion

The progression and metastasis of cancer are closely related to the complex interplay between tumor cells and the immune microenvironment. Based on the above discussions, we believe that MZB1-mediated immune reprogramming, such as recruiting immunosuppressive tumor-infiltrating cells (TICs) and upregulating the expression of immune checkpoints, promotes the formation of an immune suppressive microenvironment in ccRCC and impacts patient prognosis. Furthermore, interferon response, IL2-STAT5 signaling transduction, NFkB signaling pathway, and metabolic pathways such as fatty acid metabolism and oxidative phosphorylation may be potential molecular functions of MZB1. Therefore, MZB1 is expected to be a prognostic predictor for ccRCC immunotherapy.
6. References


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