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Development of a SETD2-related immune prognostic signature in clear cell renal cell carcinoma

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Abstract

Background: Renal cell carcinoma (RCC) is a malignant tumor of urinary system, and clear cell RCC (ccRCC) is the major pathological subtype. A high-frequency mutation in *SETD2* gene is related to the occurrence, development, and poor prognosis of RCC.

Objective: The research of immune-related genes (IRGs) is important to the success of immunotherapy in RCC. The aim of this study was to develop *SETD2*-related immune prognostic signature (IPS) potentially useful in the prognosis prediction of ccRCC.

Methods: The expression profile, mutation profile, and clinical data related to ccRCC were obtained from the TCGA (Cancer Genome Atlas) and cBioPortal databases. The data of IRGs were downloaded from the ImmPort database.

Results: An IPS with 5 genes (*PDIA2*, *PAEP*, *AMELX*, *GREM2*, and *INHA*) was constructed by analyzing the correlation between prognosis data and IRGs associated with ccRCC patients with wild type and mutant *SETD2* genes. The clinical utility of the IPS and its relationship with immune microenvironment were also studied.

Conclusions: According to the results of this study, the IPS can be a promising biomarker of ccRCC to guide its prognosis and treatment.

Abbreviations: AUC = area under the curve, ccRCC = lear cell renal cell carcinoma, DEG = differentially expressed gene, DEIRG = differentially expressed immune-related gene, DRG = downregulated gene, ICI = immune checkpoint inhibitor, IPS = immune prognostic signature, IRG = immune-related gene, PDEIRG = prognostic DEIRG, ROC = receiver operating characteristic, TIC = tumor-infiltrating immune cell, URG = upregulated gene.

Keywords: clear cell renal cell carcinoma, immune prognostic signature, individualized medicine, *SETD2* mutation

1. Introduction

Renal cell carcinoma (RCC) is a malignant tumor that originates from renal tubular epithelial cells. RCC is the most common renal tumor, and clear cell RCC (ccRCC) is the major pathological subtype, accounting for 75% to 80% of all cases.[1] The prevalence of RCC has raised sharply in most countries in recent years, and 400,000 new RCC cases are diagnosed annually throughout the world, which contributes to over 175,000 deaths. According to its incidence and mortality, RCC ranks third in urologic malignancies worldwide.[2,3] Although great progress has been made in the diagnosis and treatment of RCC, there is still no effective biomarker to predict the prognosis of RCC patients.[4] Meanwhile, RCC shows tolerance to both chemotherapy and radiotherapy. Therefore, it is necessary to investigate prognostic factors of RCC patients.

In 2015, an immune checkpoint inhibitor, nivolumab, was approved for RCC patients[5] and opened a new era of immunotherapy for RCC patients. Since then, the interest in the development of immune checkpoint inhibitors (ICIs) for the treatment of RCC patients has been significant. Hence, several new types of standalone ICI and combination chemotherapy of ICI and targeted drugs have achieved therapeutic success in RCC patients.[6–8] However, insensitivity to immunotherapy has been a challenge in some RCC patients. Notably, it has been shown that immune-related genes (IRGs) are not only related to effects of immunotherapy but also to prognosis of ccRCC patients.[9,10] Therefore, application of IRG to predict the effects of immunotherapy is feasible theoretically and has clinical significance.

Mutations or deletions of the short arm of chromosome 3 often occur in ccRCC. According to TCGA database, *SETD2*

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Data will be available from the corresponding author upon reasonable request.

Ethics approval and consent to participate are not applicable. This study applies the data of public database.

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located at 3p21 is the third most frequently mutated gene in ccRCC patients.[4] In a TCGA cohort of patients with localized RCC, the frequency of *SETD2* mutation was 13%, while in patients with metastatic RCC, the frequency increased to >30%.[11,12] *SETD2* is the only chromatin regulatory gene present on chromosome 3p, which is significantly associated with disease recurrence, suggesting that *SETD2* may play an important role in tumorigenesis.[13] In another study, researchers further analyzed the status of *SETD2* mutation in ccRCC. Compared to patients with wild-type *SETD2* gene, those with mutated *SETD2* gene showed shorter disease-free survival and higher risk of disease recurrence.[14] Therefore, *SETD2*, which showed high-frequency mutation in ccRCC, can promote the occurrence of ccRCC through a variety of mechanisms,[15–17] which are further explained in detail in the Discussion section..

Therefore, it is very important to develop a prognosis model for patients with the mutated *SETD2* gene to guide the prognosis and treatment of RCC patients. Additionally, it is of great significance for individualized and precise medical treatment. In this study, the effects of the mutated *SETD2* gene on IRGs were analyzed and an immune prognostic signature (IPS) based on 5 IRGs to *SETD2* was developed. The clinical utility of IPS in patients with ccRCC and the relationship of IPS with tumor-infiltrating immune cells (TICs) were also analyzed.

2. Materials and Methods

2.1. Database

Transcriptome RNA-seq data and the corresponding clinical data of 539 ccRCC cases were downloaded from the TCGA database (https://portal.gdc.cancer.gov). Mutation information was obtained from the cBioPortal for Cancer Genomics website (http://www.cbioportal.org). The list of IRGs was obtained from the Immport database (https://www.immport.org). The data of immune cells including $CD4^+$ T cells, B cells, dendritic cells, $CD8^+$ T cells, neutrophils, and macrophages in the samples of ccRCC patients were harvested from the TIMER database (https://cistrome.shinyapps.io/timer). We used R programming language version 3.6.2 (https://www.r-project.org/) to process all data in this study.

2.2. Survival analysis

Packages "survival" and "survminer" in R language were used to analyze survival, followed by plotting the survival curve based on theee Kaplan–Meier method. A P value of <.05 was considered statistically significant.

2.3. Identification of prognostic differentially expressed IRGs

Differential expression analysis was conducted using the "EdgeR" R package. The |log2 fold change| >2 and $P < .05$ were set as the cutoff values to screen for differentially expressed genes (DEGs). The DEGs and IRGs were combined to get differentially expressed immune-related genes (DEIRGs).

The samples with a survival time of <90 days were removed from DEIRGs, and the survival time was integrated with the survival status by univariate Cox regression analyses. The 17 genes associated with prognosis, called PDEIRGs, were obtained from the analyses and were shown in the forest plots.

2.4. Heatmap and Volcano plot

R programming language and the "pheatmap" package were used to make heatmaps and volcano plots of DEGs and DEIRGs.

2.5. Construction of the IPS

Multivariate Cox analysis of PDEIRGs was performed, and 5 most suitable risk genes and their corresponding coefficients were obtained. A model based on the expression and coefficient

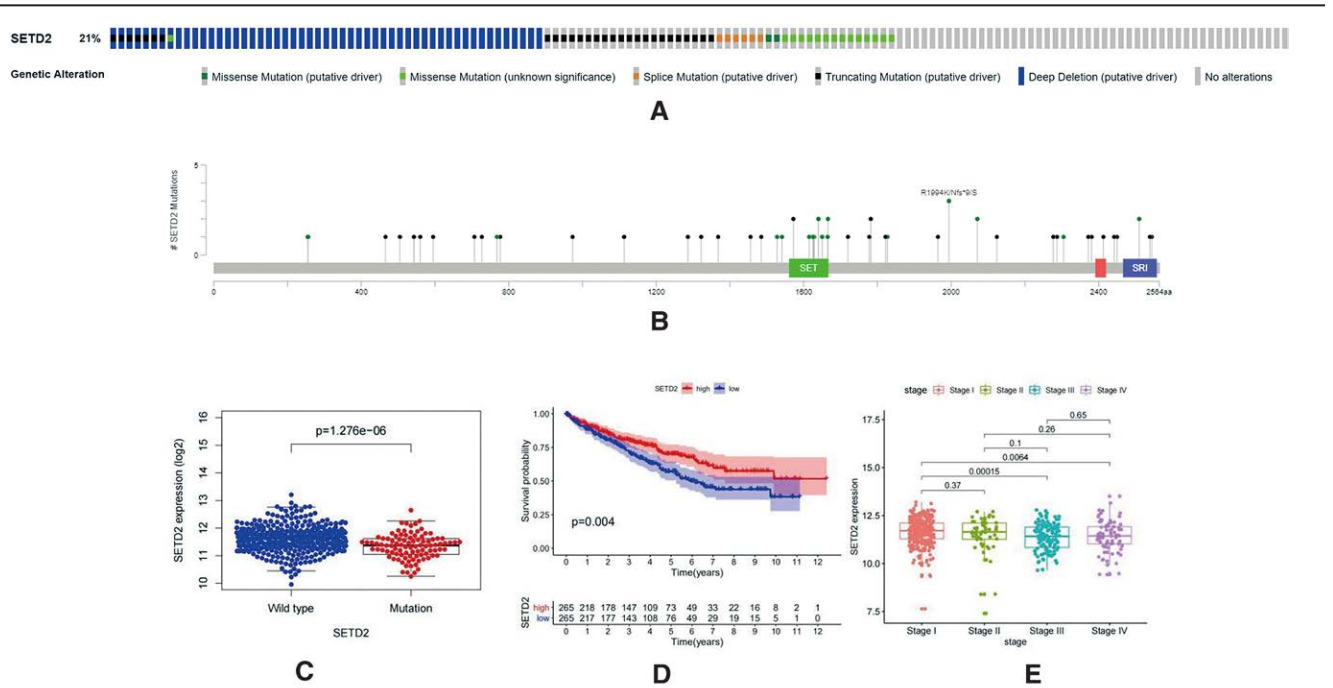

Figure 1. Mutation analysis. (A) Rate (21%) and types of mutations and (B) sites of mutation in *SETD2* gene in ccRCC patients. (C) The expression level of *SETD2* gene in mutant and wild-type groups ($P < .001$). (D) The Kaplan–Meier plot of *SETD2* expression ($P = .004$). (E) Correlation between levels of *SETD2* expression and clinical stages of ccRCC ($P < .01$). ccRCC = clear cell renal cell carcinoma.

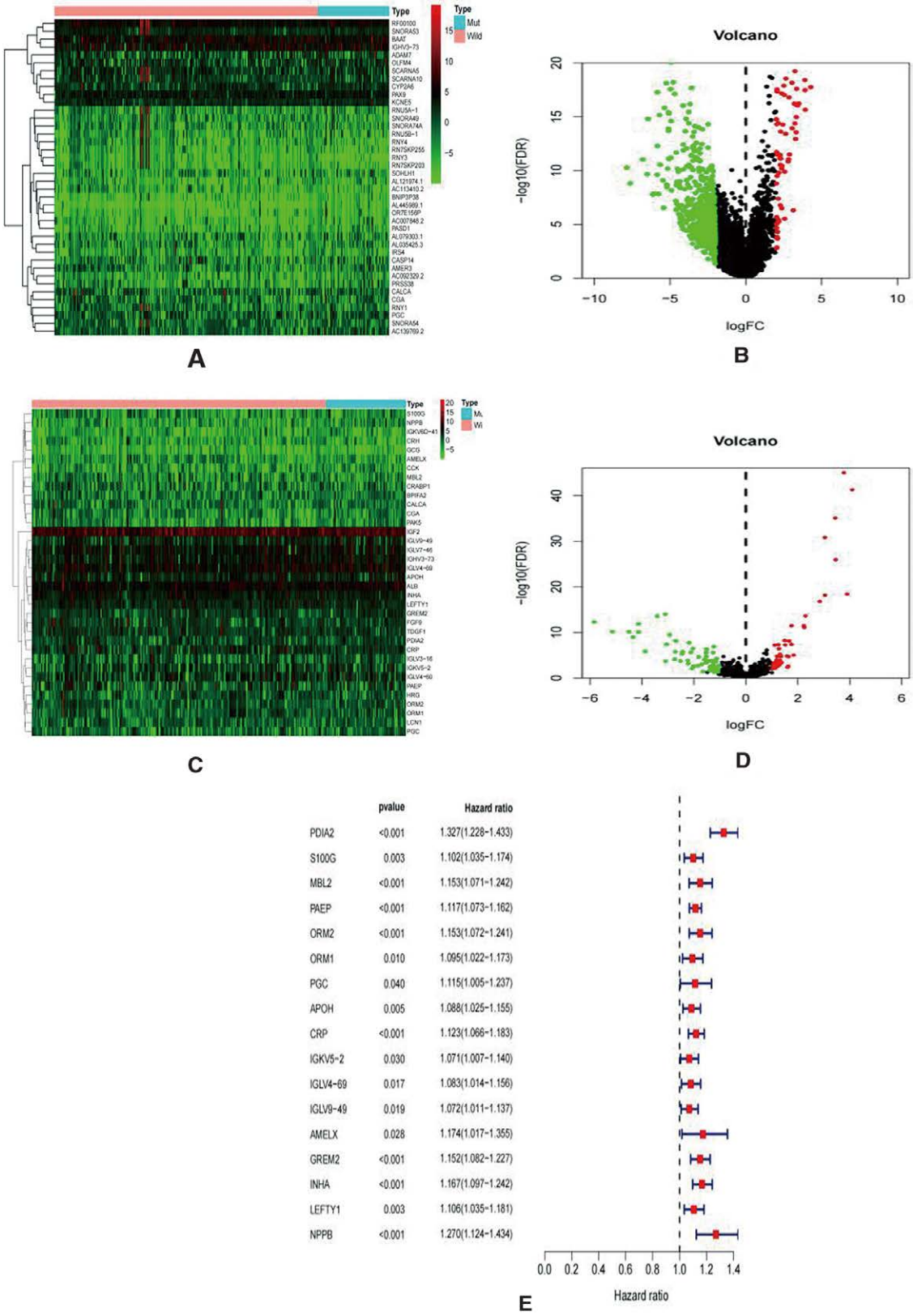

Figure 2. Identification of PDEIRGs. (A) Heatmap of the 40 most significant differentially expressed genes based on logFC (20 upregulated and 20 downregulated genes) between mutant and wild-type *SETD2* groups. (B) Volcano plot of DEGs. (|logFC| >2, FDR < 0.05). (C) Heatmap of DEIRGs. (D) Volcano plot of DEIRGs (|logFC| >1, FDR < 0.05). (E) Forest plot for PDEIRGs. (*P* < .05). DEG = differentially expressed gene, DEIRG = differentially expressed immune-related gene, PDEIRG = prognostic DEIRG.

of risk genes was built and the ccRCC patients were divided into 2 groups according to the risk score.

Receiver operating characteristic (ROC) curves were depicted to evaluate the sensitivity and specificity using the "survivalROC" R package. Area under the curve (AUC) values were calculated from the ROC curves. An AUC value of >0.60 indicated the predictive value was acceptable, and an AUC value of >0.75 indicated the predictive value was excellent.

3. Results

3.1. Analysis of mutation in SETD2 gene in ccRCC

The transcriptome and clinical data of ccRCC cases were extracted from the TCGA-KIRC database. Following the data obtained from the cBioPortal for Cancer Genomics website, a total of 96 mutant *SETD2* samples and 355 wild-type *SETD2* samples were analyzed (Fig. 1A). Mutation types comprised missense mutation, splice mutation, truncating mutation, and deep deletion. The sites of mutation are shown in Figure 1B. Compared with the wild group, the expression of mutant *SETD2* gene group was lower (Fig. 1C). The survival analysis and analysis of clinical features were conducted in line with the median score of *SETD2* expression, which showed that lower expression of *SETD2* gene was associated with poorer prognosis and higher TMN stage (Fig. 1D, E). These results indicated that mutations in *SETD2* gene not only affected its level of expression but also affected the prognosis and clinical progress of ccRCC patients. Therefore, ccRCC patients with *SETD2* mutation may benefit from early intervention.

3.2. Identification of PDEIRGs

Based on the EdgeR algorithm, 648 DEGs were obtained between mutant and wild-type *SETD2* groups, including 98 upregulated genes and 550 downregulated genes (Fig. 2A, B). To identify genes associated with the immune system called IRGs, the Immport database was used. The analysis of the Immport database revealed 36 DEIRGs (differentially expressed IRGs), including 11 upregulated genes and 25 downregulated genes (Fig. 2C, D). A correlation study of DEIRGs with clinical data using a univariate Cox analysis revealed 17 PDEIRGs (prognostic DEIRGs; Fig. 2E).

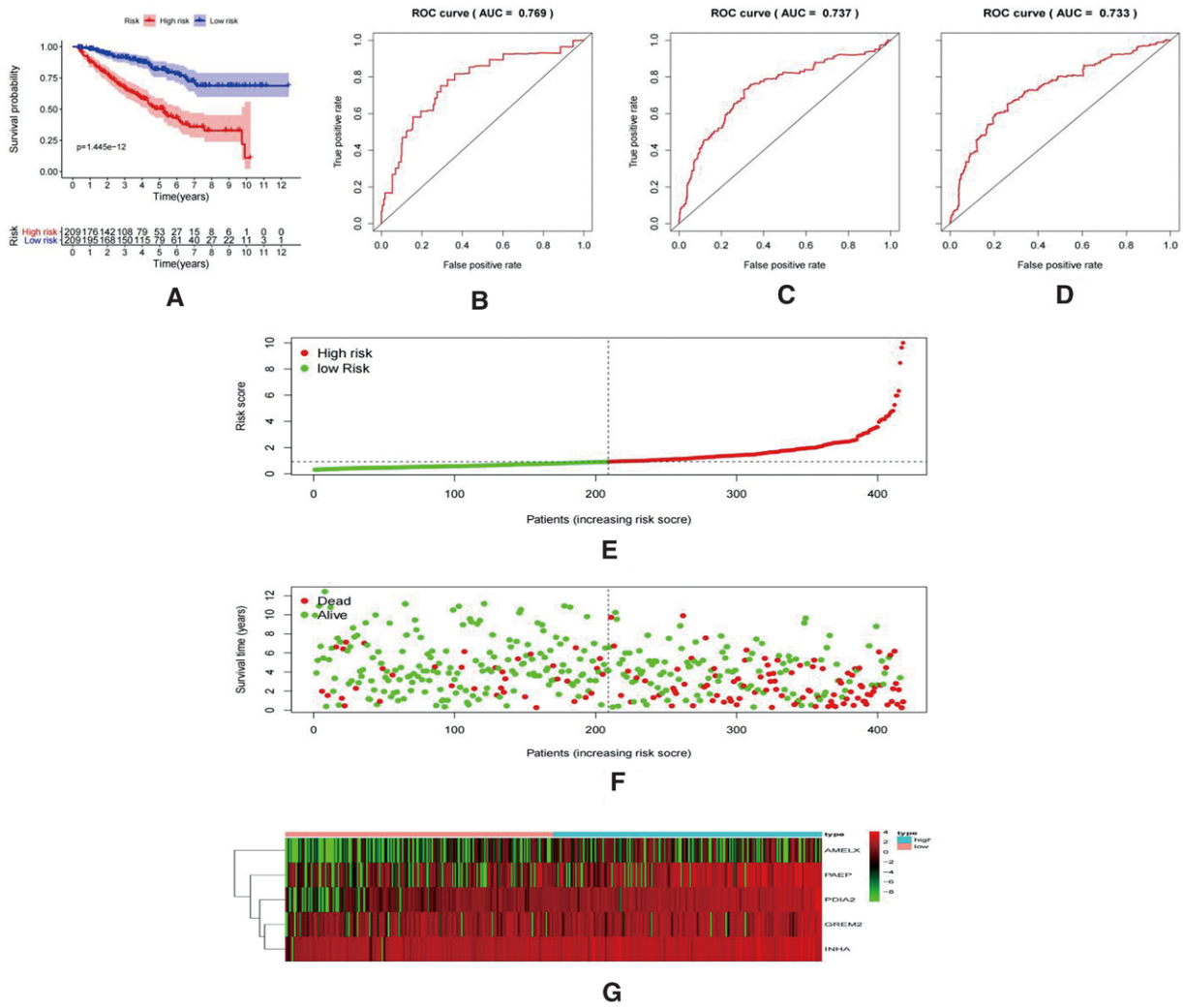

Figure 3. Prognostic validation analysis of the IPS. (A) The Kaplan–Meier plot for HR and LR groups ($P < .001$). (B–D) Time-dependent ROC curves of IPS in 1, 3, and 5 years. (E) Risk score distribution of ccRCC patients. (F) Scatter plots for survival status. The red dots mean dead patients, and the green dots mean surviving patients. (G) Heatmap for expressions of risk genes. (E–G) The left and right sides indicate LR and HR groups, respectively. AUC = area under the curve, ccRCC = clear cell renal cell carcinoma, HR = high risk, IPS = immune prognostic signature, LR = low risk, ROC = receiver operating characteristic.

3.3. Construction and verification of IPS

The multivariate Cox analysis of PDEIRGs showed 5 genes with the highest risk, *PDIA2, PAEP, AMELX, GREM2,* and *INHA*, which predicted a poor outcome for ccRCC patients. The risk value based on the expression levels and coefficient values of each gene was calculated using the following formula:

Risk score = (0.2292 * expression of *PDIA2*) + (0.0610 * expression of *PAEP*) + (0.1904 * expression of *AMELX*) + (0.0491 * expression of *GREM2*) + (0.0937 * expression of *INHA*).

Each sample was ranked using the above formula to divide the samples into HR (high-risk) and LR (low-risk) groups by the median score. The survival analysis showed that ccRCC samples in the LR group had longer survival than those in the HR group (Fig. 3A). The time-dependent ROC curves were used to determine the accuracy of the signature predicting survival rates over 1 year, 3 years, and 5 years. The AUC values for the prognostic signature were 0.769 for 1 year, 0.737 for 3 years, and 0.733 for 5 years (Fig. 3B–D). The classification of the samples based on their risk scores gave the risk curve (Fig. 3E). The analysis of the survival status of patients showed that patients in the HR group had higher mortality (Fig. 3F). The heatmap for expressions of risk genes in the 2 groups showed that the expression level increased with the increase of risk scores (Fig. 3G).

3.4. Independent prognostic value of IPS

To verify the independent prognostic value of IPS, univariate and multivariate Cox regression analyses were performed. The univariate analysis indicated that the prognosis of ccRCC patients was associated with the variation in age, Fuhrman grade, clinical stage of the disease, tumor size (T), distance of metastases (M),and the number of lymph nodes showing metastases (N) and risk score, while the variation in gender was not statistically significant (Fig. 4A). The multivariate analysis indicated that the variation in age, risk scores, and distance of metastases (M) were independently related to the survival rate of ccRCC patients (Fig. 4B). Therefore, IPS could be used as an independent prognostic factor to predict the prognosis of ccRCC patients.

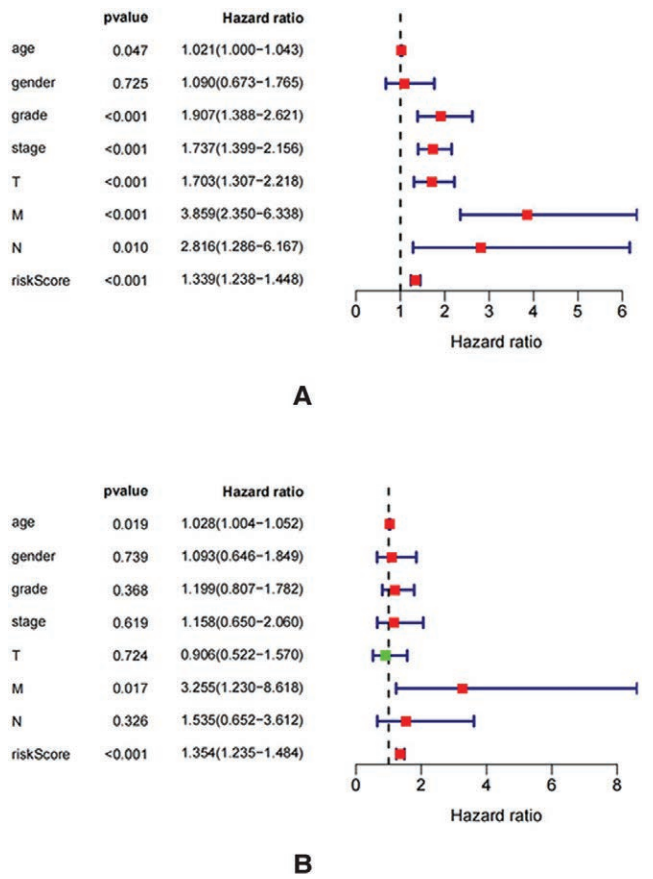

Figure 4. Independent prognostic analysis. (A) Univariate Cox regression analysis. (B) Multivariate Cox regression analysis. A *P* value of <.05 was considered statistically significant.

3.5. Clinical correlation analysis of IPS

In order to verify the relationship between IPS and clinical progression of ccRCC, a correlation analysis between risk scores of IPS and clinical variables (age, gender, grade, stage, T, M, N) was performed in this study. Based on the analysis, 3 variables, including Fuhrman grade, clinical stage and T stage, showed the highest statistical significance ($P < .05$) and were positively correlated with the risk score of IPS (Fig. 5A–C). These results indicated that IPS was related to oncogenesis and tumor progress, and a high score of IPS was associated with poor prognosis for ccRCC patients.

3.6. Correlation of IPS with TICs

TIMER database (https://cistrome.shinyapps.io/timer) containing the TICs content was downloaded and the correlation of IPS with the immune microenvironment was investigated. According to the proportions of TICs in HR group and LR groups, it was found that the risk score was positively correlated with the number of $CD4^+$ T cells and neutrophils and negatively correlated with the number of $CD8^+$ T cells, B cells, macrophages, and dendritic cells (Fig. 6A–F). However, there was significant correlation between the risk score of IPS and the number of $CD8^+$ T cells only ($P < .05$). These results indicated that there might be suppression of infiltration by immune cells, especially $CD8^+$ T cells, in the HR group, leading to poor prognosis.

4. Discussion

Previous data support the conclusion that RCC ranks the third among tumors in the urinary system, which is related to a great number of deaths yearly. However, the biomarkers for the prognosis of RCC remain to be determined. With the widespread application of immunotherapy in RCC treatment, IRGs have also received great attention. IRGs can reflect the prognosis of tumor and the effect of immunotherapy. Therefore, this study was designed to determine a signature using IRGs to predict the prognosis of ccRCC.

SETD2, also known as *SET2, KMT3A,* and *HYPB*, is located in the first band of region 2 (3p21.31) of the short arm of human chromosome 3. *SETD2* protein can catalyze histone H3 lysine 36 (H3K36) in vivo to change it from dimethyl state to trimethyl state (H3K36me3), which is the specific catalytic enzyme of H3K36me3.[18] The ccRCC tumors with mutant *SETD2* show chromatin accessibility changes in the H3K36me3-labeled region, leading to extensive defects in transcription processing and increasing intron retention, which may lead to functional loss of protein products.[15] Another mechanism of *SETD2* mutation underlying the promotion of tumorigenesis is closely related to its role in the maintenance of genomic stability. *SETD2* is involved in genetic processes, for example, DNA homologous recombination and mismatch repair. Mutations in *SETD2* gene increase the number of false transcriptional initiation and abnormal gene transcription, which lead to a decrease in genomic stability and further promote the occurrence of tumor.[19,20] In addition, a study found that mutations in the *SETD2* gene can inhibit the apoptosis of

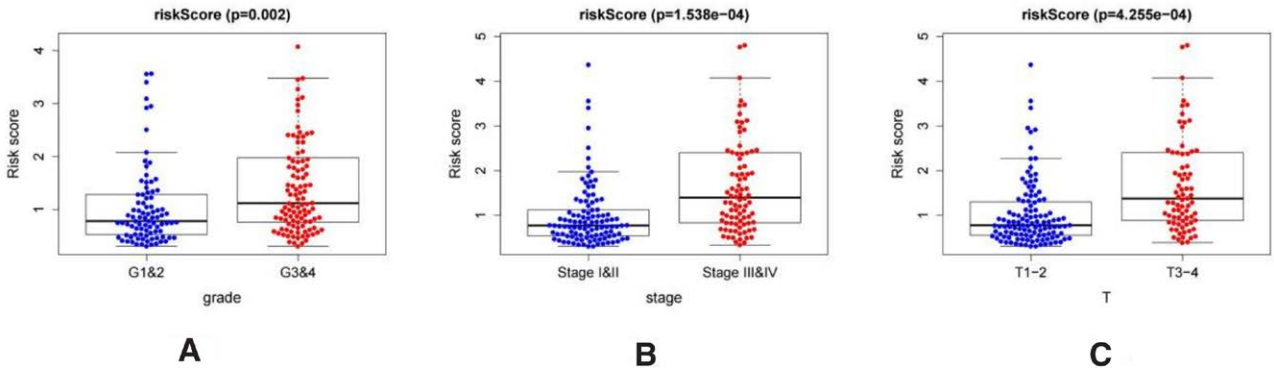

Figure 5. Clinical correlation analysis of IPS. (A) Risk scores and Fuhrman grade. (B) Risk scores and clinical stage. (C) Risk scores and T stage. A *P* value of <.05 was considered statistically significant. IPS = immune prognostic signature.

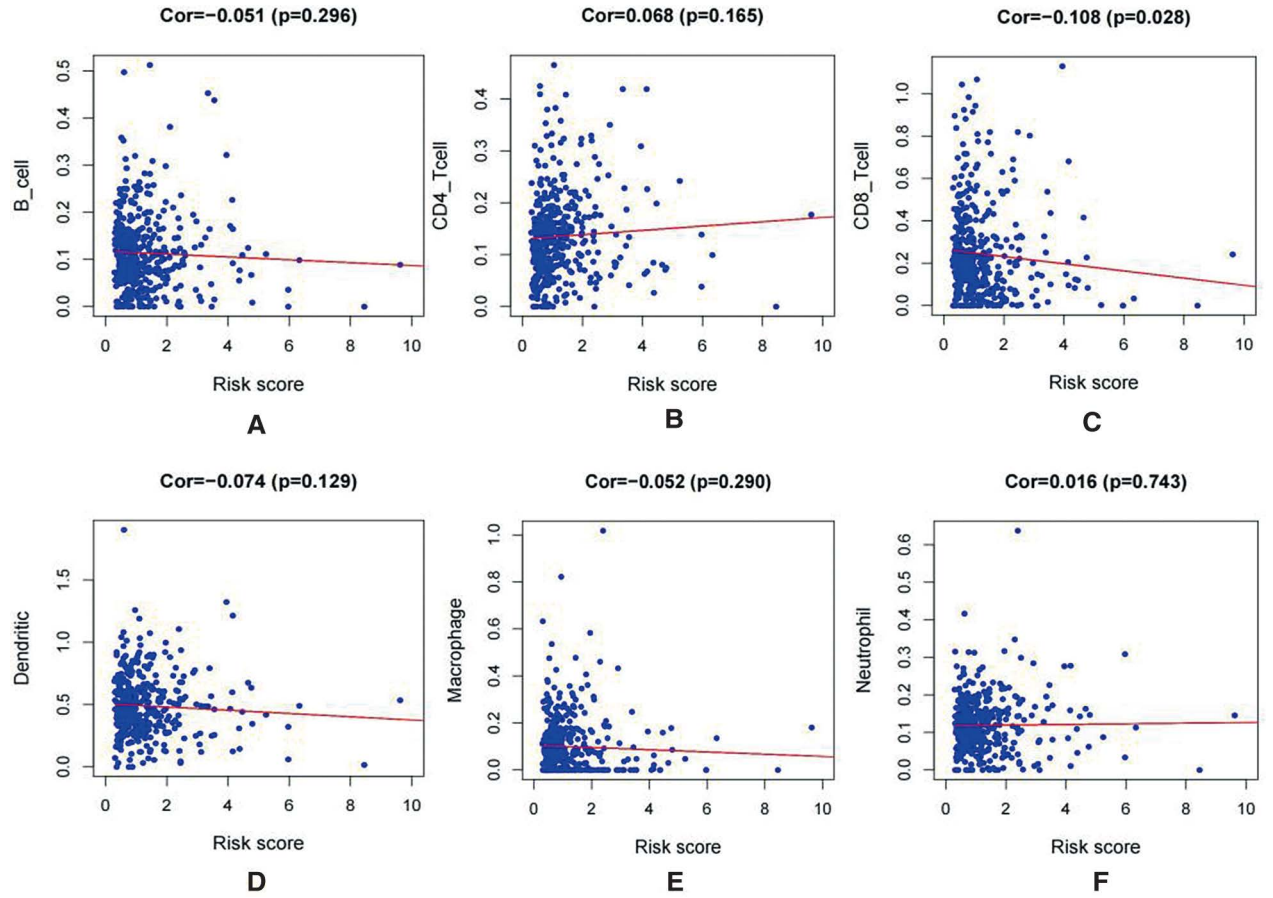

Figure 6. Correlation analysis between IPS risk scores and TICs contents. (A) B cells, (B) $CD4^+$ T cells, (C) $CD8^+$ T cells, (D) dendritic cells, (E) macrophages, and (F) neutrophils. IPS = immune prognostic signature, TIC = tumor-infiltrating immune cell.

ccRCC, and the downregulation of the *SETD2* gene in ccRCC patients is negatively correlated with a higher level of mir-106b-5p expression.[16] In conclusion, mutations in *SETD2* gene occur frequently in ccRCC patients. The low expression of *SETD2* caused by mutation is related to the recurrence and poor prognosis of ccRCC and can promote the occurrence of ccRCC through a variety of mechanisms. Therefore, *SETD2* was selected as the target gene of this study and IPS was constructed using *SETD2*-related IRGs.

The clinical data as well as gene expression and mutation of ccRCC patients were collected from TCGA and cBioPortal databases. The ccRCC patients were classified into mutant and wild-type *SETD2* groups, and an analysis of the differentially expressed genes between the 2 groups was performed. The IRGs were obtained from the ImmPort database and the correlation of DEIRGs with prognosis was analyzed. Finally, screening of the databases revealed 5 best genes (*PDIA2, PAEP, AMELX, GREM2*, and *INHA*) suitable to construct the IPS. At the same time, these genes are also expected to become new molecular targets for immunotherapy and may play a crucial role in the prediction of prognosis of patients with ccRCC.

PDIA2, a member of the PDI protein family, was first found in phage proteins of pancreas.[21] Researchers discovered that tissue destruction regulated by *PDIA2*-specific T cells is controlled by CTLA-4, which is expressed on regulatory T cells or *PDIA2*-specific effector cells. CTLA-4 checkpoint inhibitor

is used in emerging cancer immunotherapy; therefore, further research on *PDIA2* may provide new ideas for tumor immunotherapy.[22] The *PAEP* gene encodes *PAEP* protein, a secreted glycoprotein, which was originally isolated from human placenta and amniotic fluid.[23] The *PAEP* protein plays an important role in the establishment of maternal immune tolerance by inhibiting the activity of T cells.[24,25] In addition, the high expression level of *PAEP* proteins in ovarian cancer and breast cancer patients has also been widely reported.[26,27] Notably, *AMELX* gene has already been used as a prognostic gene marker in breast cancer and is included in the construction of a prognostic model.[28] One study has shown that the silencing of *GREM2* gene inhibits the activation of JNK signaling pathway, thereby inhibiting tumor progression. Therefore, the *GREM2*-mediated JNK signaling pathway is expected to become a new therapeutic target for gastric cancer chemotherapy.[29] In addition, further studies have pointed out that in hepatocellular carcinoma, *GREM2* shows a significantly higher expression level compared with the adjacent tumor. Thus, *GREM2* has the potential to become a new molecular marker.[30] *INHA* has also been reported to be associated with adrenocortical carcinoma.[31,32]

The construction of IPS was carried out and the reliability and stability of IPS were analyzed and verified. The IPS can be used as an independent prognostic factor. The risk score of IPS was related to Fuhrman grade, clinical stage and T stage of ccRCC patients, and was positively correlated with the clinical progress of tumor. Therefore, the IPS can predict the prognosis of ccRCC patients based on the level of risk scores, helping to identify HR patients in the early stage of the disease.

Currently, several immunotherapies are considered to work by stimulating the immune response of TME.[33,34] It was reported that the composition of TICs was related to the therapeutic effects of immunotherapy.[35] These reports confirmed the function of TICs in the treatment of RCC patients. According to the TIMER database, the correlation between TICs and IPS was analyzed, and the results indicated that $CD8^+$ T cells showed a significant correlation with IPS. As the risk score in the studied patients increased, the infiltration of $CD8^+$ T cells decreased, which was linked to poor prognosis and tolerance to immunotherapy. Hence, these results can provide guidance about treatment plans for RCC patients. The current study provided new insights into the prognosis and treatment of ccRCC patients. The selected genes of IPS should be further used in the basic research to confirm their potential value in the prognosis of ccRCC patients. Since the data used in this study were obtained from online databases, the reported findings have some limitations. Therefore, further well-detailed experiments are necessary.

In conclusion, this study provided new insight into the prognostic mechanism of *SETD2* mutation from the perspective of immunology. The authors also reported that IPS can be a promising biomarker for ccRCC in clinical applications. Based on the results of this study, the patients can be divided into different subgroups according to the risk score, providing valuable information for guiding the prognosis and individualized medication of ccRCC.

Author contributions

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Writing – review & editing: Xingyuan Wang, Zhinan Xia, Cheng Zhang

Reference

[1] Clark DJ, Dhanasekaran SM, Petralia F, et al. Integrated proteogenomic characterization of clear cell renal cell carcinoma. Cell. 2019;179:964–983.e31.
[2] Bray F, Ferlay J, Soerjomataram I, et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018;68:394–424.
[3] Ferlay J, Colombet M, Soerjomataram I, et al. Estimating the global cancer incidence and mortality in 2018: GLOBOCAN sources and methods. Int J Cancer. 2019;144:1941–53.
[4] Dizman N, Philip EJ, Pal SK. Genomic profiling in renal cell carcinoma. Nat Rev Nephrol. 2020;16:435–51.
[5] Motzer RJ, Escudier B, McDermott DF, et al. Nivolumab versus everolimus in advanced renal-cell carcinoma. N Engl J Med. 2015;373:1803–13.
[6] Motzer RJ, Tannir NM, McDermott DF, et al. Nivolumab plus ipilimumab versus sunitinib in advanced renal-cell carcinoma. N Engl J Med. 2018;378:1277–90.
[7] Motzer RJ, Penkov K, Haanen J, et al. Avelumab plus axitinib versus sunitinib for advanced renal-cell carcinoma. N Engl J Med. 2019;380:1103–15.
[8] Rini BI, Plimack ER, Stus V, et al. Pembrolizumab plus axitinib versus sunitinib for advanced renal-cell carcinoma. N Engl J Med. 2019;380:1116–27.
[9] Mikami S, Mizuno R, Kondo T, et al. Clinical significance of programmed death-1 and programmed death-ligand 1 expression in the tumor microenvironment of clear cell renal cell carcinoma. Cancer Sci. 2019;110:1820–8.
[10] Ueda K, Suekane S, Kurose H, et al. Prognostic value of PD-1 and PD-L1 expression in patients with metastatic clear cell renal cell carcinoma. Urol Oncol-Semin Ori. 2018;36:499.e499–16.
[11] Ho TH, Choueiri TK, Wang K, et al. Correlation between molecular subclassifications of clear cell renal cell carcinoma and targeted therapy response. Eur Urol Focus. 2016;2:204–9.
[12] Hsieh JJ, Chen D, Wang PI, et al. Genomic biomarkers of a randomized trial comparing first-line everolimus and sunitinib in patients with metastatic renal cell carcinoma. Eur Urol. 2017;71:405–14.
[13] Hakimi AA, Ostrovnaya I, Reva B, et al. Adverse outcomes in clear cell renal cell carcinoma with mutations of 3p21 epigenetic regulators BAP1 and SETD2: a report by MSKCC and the KIRC TCGA research network. Clin Cancer Res. 2013;19:3259–67.
[14] Sato Y, Yoshizato T, Shiraishi Y, et al. Integrated molecular analysis of clear-cell renal cell carcinoma. Nat Genet. 2013;45:860–7.
[15] Simon JM, Hacker KE, Singh D, et al. Variation in chromatin accessibility in human kidney cancer links H3K36 methyltransferase loss with widespread RNA processing defects. Genome Res. 2014;24:241–50.
[16] Xiang W, He J, Huang C, et al. miR-106b-5p targets tumor suppressor gene SETD2 to inactive its function in clear cell renal cell carcinoma. Oncotarget. 2015;6:4066–79.
[17] Chen R, Zhao W-Q, Fang C, et al. Histone methyltransferase SETD2: a potential tumor suppressor in solid cancers. J Cancer. 2020;11:3349–56.
[18] Edmunds JW, Mahadevan LC, Clayton AL. Dynamic histone H3 methylation during gene induction: HYPB/Setd2 mediates all H3K36 trimethylation. EMBO J. 2008;27:406–20.
[19] Pfister SX, Ahrabi S, Zalmas L-P, et al. SETD2-dependent histone H3K36 trimethylation is required for homologous recombination repair and genome stability. Cell Rep. 2014;7:2006–18.
[20] Li F, Mao G, Tong D, et al. The histone mark H3K36me3 regulates human DNA mismatch repair through its interaction with MutSα. Cell. 2013;153:590–600.
[21] Walker AK, Soo KY, Levina V, et al. N–linked glycosylation modulates dimerization of protein disulfide isomerase family A member 2 (PDIA2). FEBS J. 2013;280:233–43.
[22] Ise W, Kohyama M, Nutsch KM, et al. CTLA-4 suppresses the pathogenicity of self antigen–specific T cells by cell-intrinsic and cell-extrinsic mechanisms. Nat Immunol. 2010;11:129–35.
[23] Seppala M, Taylor RN, Koistinen H, et al. Glycodelin: a major lipocalin protein of the reproductive axis with diverse actions in cell recognition and differentiation. Endocr Rev. 2002;23:401–30.

[24] Tseng L, Mazella J. Endometrial cell specific gene activation during implantation and early pregnancy. Front Biosci-Landmrk. 2002;7:1566–74.
[25] Ren S, Liu S, Howell PM Jr, et al. Functional characterization of the progestagen-associated endometrial protein gene in human melanoma. J Cell Mol Med. 2010;14:1432–42.
[26] Ho M-L, Kuo W-K, Chu LJ, et al. N-acetylgalactosamine-6-sulfatase (GALNS), similar to glycodelin, is a potential general biomarker for multiple malignancies. Anticancer Res. 2019;39:6317–24.
[27] Hautala LC, Greco D, Koistinen R, et al. Glycodelin expression associates with differential tumour phenotype and outcome in sporadic and familial non-BRCA1/2 breast cancer patients. Breast Cancer Res Tr. 2011;128:85–95.
[28] Zhang Z, Li J, He T, et al. Bioinformatics identified 17 immune genes as prognostic biomarkers for breast cancer: application study based on artificial intelligence algorithms. Front Oncol. 2020;10:330–330.
[29] Ran A, Guan L, Wang J. Wang Y. GREM2 maintains stem cell-like phenotypes in gastric cancer cells by regulating the JNK signaling pathway. Cell Cycle. 2019;18:2414–31.
[30] Wang L, Ding Q, Zhao L, et al. Decreased BMP-7 and p-Smad1/5/8 expression, and increased levels of gremlin in hepatocellular carcinoma. Oncol Lett. 2018;16:2113–8.
[31] Hofland J, Steenbergen J, Voorsluijs JM, et al. Inhibin alpha-subunit (INHA) expression in adrenocortical cancer is linked to genetic and epigenetic INHA promoter variation. PLoS One. 2014;9:e104944 –e104944.
[32] Longui CA, Lemos-Marini SHV, Figueiredo B, et al. Inhibin alpha-subunit (INHA) gene and locus changes in paediatric adrenocortical tumours from TP53 R337H mutation heterozygote carriers. J Med Genet. 2004;41:354–9.
[33] Afonso J, Santos LL, Longatto-Filho A, et al. Competitive glucose metabolism as a target to boost bladder cancer immunotherapy. Nat Rev Urol. 2020;17:77–106.
[34] Paucek RD, Baltimore D, Li G. The cellular immunotherapy revolution: arming the immune system for precision therapy. Trends Immunol. 2019;40:292–309.
[35] Gajewski TF, Schreiber H. Fu Y-X. Innate and adaptive immune cells in the tumor microenvironment. Nat Immunol. 2013;14:1014–22.