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

Xingyuan Wang, MM, PhD*a, Zhinan Xia, MMa, Zhiyuan Li, MBBSa, Cheng Zhang, PhDa,*

Abstract

Background: Renal cell carcinoma (RCC) is a malignant tumor of the 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 = clear cell renal cell carcinoma, DE = differentially expressed gene, DEIRG = differentially expressed immune-related gene, DRG = downregulated gene, ICI = immune checkpoint inhibitor, IPS = immune-related 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. 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. 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. 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 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. 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. 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
located at 3p21 is the third most frequently mutated gene in ccRCC patients.\textsuperscript{[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\%.\textsuperscript{[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.\textsuperscript{[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.\textsuperscript{[14]} Therefore, SETD2, which showed high-frequency mutation in ccRCC, can promote the occurrence of ccRCC through a variety of mechanisms,\textsuperscript{[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 the 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 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 “survival-ROC” 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 expression 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.

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.[13] 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.[13] 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...
ccRCC, and the downregulation of the \( \text{SETD2} \) gene in ccRCC patients is negatively correlated with a higher level of mir-106b-5p expression.\(^{[16]} \) In conclusion, mutations in \( \text{SETD2} \) gene occur frequently in ccRCC patients. The low expression of \( \text{SETD2} \) 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, \( \text{SETD2} \) was selected as the target gene of this study and IPS was constructed using \( \text{SETD2} \)-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 \( \text{SETD2} \) 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 (\( \text{PDIA2}, \text{PAEP}, \text{AMELX}, \text{GREM2}, \text{and} \text{INHA} \)) 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.

\( \text{PDIA2} \), a member of the PDI protein family, was first found in phage proteins of pancreas.\(^{[21]} \) Researchers discovered that tissue destruction regulated by \( \text{PDIA2} \)-specific T cells is controlled by CTLA-4, which is expressed on regulatory T cells or \( \text{PDIA2} \)-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.[13] 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, breast cancer, and ccRCC patients has also been widely reported.[11] The high expression level of PAEP proteins in ovarian cancer, breast cancer, and ccRCC patients has also been widely reported.[11] 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.[26] 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.[27] 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.[28] INHA has also been reported to be associated with adenocortical carcinoma.[11,32] 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**

Conceptualization: Xingyuan Wang, Cheng Zhang
Data curation: Zhiyuan Li
Formal analysis: Xingyuan Wang, Zhinan Xia
Funding acquisition: Cheng Zhang
Investigation: Xingyuan Wang, Zhinan Xia
Methodology: Xingyuan Wang, Zhinan Xia
Project administration: Xingyuan Wang, Zhinan Xia
Resources: Xingyuan Wang, Zhinan Xia
Software: Xingyuan Wang, Zhinan Xia
Supervision: Xingyuan Wang, Zhinan Xia

**Validation:**

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

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