PANK1 is a Prognostic Biomarker Associated with Immune Infiltration of Clear Cell Renal Carcinoma

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Research Article

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Abstract

Background: PANK1 is expressed in some cancer types, but its role in clear cell renal carcinoma (ccRCC) is unclear. We aimed to demonstrate the relationship between PANK1 and ccRCC based on a cancer genomic atlas (TCGA) database.

Methods: The Kruskal-Wallis test, Wilcoxon signed rank test and logistic regression were used to analyze the relationship between the clinical pathological characteristics of ccRCC and the expression of PANK1. The ROC curve was used to describe the prognostic value of PANK1 using area under curve (AUC) scores. Kaplan-Meier method and Cox regression analysis were used to evaluate the factors affecting the prognosis of ccRCC. Gene set enrichment analysis (GSEA) and immuno-infiltration analysis were performed to identify a significantly related function of PANK1.

Results: PANK1 expression in renal clear cell carcinoma was different from that in stage N ($P=1.3E-03$), sex ($P=5.1E-07$), stage M ($P=8.3E-04$), residual tumor ($P<0.001$), T stage (T1 vs T4 ($P=6.5E-03$), T1 vs T3 ($P=6.9E-06$)), histological grade (G1 vs G4 ($P=3.6E-05$), G2 vs G4 ($P=2.1E-10$), G3 vs G4 ($P=1.7E-05$)), pathologic stage (STAGE 1 vs. STAGE4 ($P=1.4E-05$), STAGE1 vs. STAGE3 ($P=7.1E-05$)). The ROC curve suggest that PANK1 has significant diagnostic and prognostic capabilities (AUC =0.898). Low expression of PANK1 predicted poor overall survival (OS) ($P<0.001$), while that of PANK1 (HR:0.398; 95% CI:0.248-0.639, $P<0.001$) is OS-independent predictor in patients with ccRCC. GSEA and immune infiltration analysis showed that the expression of PANK1 is related to extracellular matrix receptor pathway, signaling pathway related to hypertrophic cardiomyopathy, cytokine-cytokine receptor interaction pathway, as well as complement and coagulation cascade pathway.

Conclusion: PANK1 expression is significantly associated with poor survival and immune infiltration of ccRCC, which may be a promising prognostic biomarker for ccRCC.

Introduction

Renal cell carcinoma is one of the most common cancers and also the most common tumor in the urinary system[1]. Statistics show that 350,000 people are diagnosed with renal cell carcinoma every year[2]. Clear cell carcinoma of the kidney is the most common one, accounting for about 85%[3, 4]. The survival rate of early renal cell carcinoma after treatment is 60–70%, while that of advanced renal cell carcinoma has a poor prognosis with a five-year survival rate less than 10%[5]. CcRCC are aggressive tumors with high metastasis rates[6, 7]. At the time of diagnosis, about one-third of the patients have metastasized[8], and another third of the patients may eventually metastasize[9, 10]. Patients with metastatic advanced clear cell carcinoma of the kidney are insensitive to both radiotherapy and chemotherapy[11, 12], and new targeted drugs are ineffective in patients with a large number of metastatic ccRCC[13, 14]. Recent immunotherapy with checkpoints has been shown to be effective for RCCs, but unfortunately only in a small number of people[15-17]. In addition, despite the continuous improvement in the treatment of renal cancer, the corresponding mortality rate of renal cancer is still increasing[18]. Therefore, finding new therapeutic targets for renal cancer is of great significance for improving the prognosis of patients with advanced renal cancer.

Pantothenate kinase (Pank) is a rate-determining enzyme for the biosynthesis of coenzyme a (CoA) [19]. The mouse pantothenate kinase (Pank1) gene consists of seven introns and eight exons, and is located on

chromosome 19 (19C2-3)[19]. Genes encoding pantothenate kinases (the Pank gene family) are also present in the human body and are involved in the metabolism of substances in the human body. PANK1, PANK2, PANK3 and PANK4 are four known isomers of pantothenate kinase[20]. A previous study has shown that the PANK family gene is associated with the prognosis of acute myeloid leukemia and pointed out that high expression of PANK2 may have a good effect on the prognosis of AML, while high expression of PANK4 indicates a poor prognosis[21]. Pantothenate kinase-related neurodegenerative disease is a rare hereditary neurodegenerative disease associated with nucleotide variations in the PANK2 gene encoded by mitochondrial human pantothenate kinase 2(PANK2) protein[22]. Pantothenate kinase-related neurodegenerative diseases are the main symptoms of pan-extrapyrmidal dysfunction and non-heme iron accumulation[22]. Although a few studies have suggested that PANK1 may play a key role in the occurrence of cancer[23], there is no literature exploring the correlation between PANK1 and renal clear cell carcinoma. Therefore, the purpose of this study was to elucidate the expression of PANK1 in ccRCC tissues and its potential therapeutic and prognostic value.

In this study, the RNA-seq data of ccRCC in the Database of Cancer Genome Atlas (TCGA) were used to compare the expression difference of PANK1 between tumor tissues and normal samples, and to explore the correlation between the expression level of PANK1 and the clinical pathological characteristics of ccRCC. Next, we evaluated the prognostic value of PANK1 in ccRCC. In addition, gene set enrichment analysis (GSEA) was performed on the high-expression group and the low-expression group of PANK1 to reveal its potential function. Finally, by analyzing the correlation between the expression of PANK1 and immune infiltration, we comprehensively explored the potential mechanism of PANK1 in regulating the occurrence and development of ccRCC.

Method And Materials

RNA sequencing data and bioinformatics analysis

We used the TCGA database (<https://portal.gdc.cancer.gov/>) to collect RNA-seq data and clinical information from 539 patients with KIRC and included 72 cases with adjacent tissue matches. All procedures performed in this study were in compliance with the Declaration of Helsinki (revised in 2013).

Gene set enrichment analysis (GSEA)

In this paper, GSEA enrichment analysis were performed according to c2.cp.v7.2.symbols.gmt [Curated]. The gene set comes from the MSigDB Collections database (<https://www.GSEA-MSigDB.org/GSEA/MSigDB/Collections.jsp#c2>). The R package cluster profiler (version 3.6.0) **was used to** perform GSEA[24, 25] between high PANK1 and low PANK1 groups. According to the default statistics, the procedure is repeated 1000 times for each analysis, and is generally considered to satisfy the False discovery rate (FDR) < 0.25 and p. Adjust < 0.05 condition for significant enrichment.

Immune infiltration analysis of ssGSEA

The immunologic invasion analysis of renal clear cell carcinoma (RCC) was performed using R (version 3.6.3) and its corresponding R package GSVA [26](version 1.34.0) (<https://www.bioconductor.org/packages/release/bioc/html/GSVA.html>) SSGSEA algorithm, we quantified the level of infiltration of 24 immune cell types according to the Gene expression profiling available in the literature[27]. In order to explore the correlation between PANK1 and the infiltration level of 24 kinds of immune cells, the P value was determined by Spearman correlation analysis.

statistical analysis

All statistical analyses were performed using R (version 3.6.3). The Wilcoxon rank sum test, chi-square test, Fisher's exact test and logistic regression were used to analyze the relationship between clinical pathological features and PANK1. The Kaplan-Meier method was used to calculate the survival rate of patients with TCGA. Univariate and multivariate analyses were performed using a Cox proportional risk model to estimate the correlation between clinical and genetic clinical features and overall survival (OS). P-values less than 0.05 were considered statistically significant.

Results

PANK1 expression is correlated with ccRCC clinicopathological features

To identify the difference in PANK1 expression, we analyzed PANK1 expression levels in 539 ccRCC tissues and 72 adjacent normal renal tissues and we found low expression of PANK1 in ccRCC tissues ($P < 0.001$, Figure 1A). At the same time, we also analyzed the expression of PANK1 in 72 ccRCC tissues and their matched neighboring tissues. The results showed low expression of PANK1 in ccRCC tissues ($P < 0.001$, Figure 1B). Meanwhile, the expression levels of PANK1 in the normal samples from the GTEx combined TCGA database and the ccRCC samples from the TCGA database were compared. To determine the differential expression of PAK1 in tumor and normal tissues, transcriptional levels of PAK1 in different multiple cancer types and normal tissues were analyzed using TCGA and GTEx databases. This analysis showed higher expression of PANK1 in various types of cancer than in normal tissues (Figure S1). We downloaded the unified and standardized pan-cancer data set: TCGA Target GTEx (Pancan, $n = 19131$, $g = 60499$) from the UCSC(<https://xenabrowser.net/>) database, and further extracted the expression data of PANK1 gene in each sample. Further, we screened the sample sources as follows: Solid Normal, Primary Solid Tumor, Primary Tumor, Normal Tissue, Primary Blood Derived Cancer-Bone Marrow, The samples of Primary Blood Derived Cancer-Peripheral Blood are further subjected to $\log_2(x+0.001)$ transformation for each expression value. Finally, we also removed the cancer species with the number of samples less than three from a single cancer species, and finally obtained the expression data of 34 cancer species. We used the R software (version 3.6.3) to calculate the expression differences of normal samples and tumor samples in each tumor, and performed difference significance analysis using unpaired Wilcoxon Rank Sum and Signed Rank Tests. We observed significant up-regulation in 19 tumors such as GBMLGG(Tumor: 2.15 ± 0.99 , Normal: 1.31 ± 1.50 , $p = 4.2e-47$) $\square$ LGG(Tumor: 2.39 ± 0.90 , Normal: 1.31 ± 1.50 , $p = 1.5e-61$) $\square$ UCEC(Tumor: 2.09 ± 1.05 , Normal: 0.82 ± 0.70 , $p = 4.5e-8$) $\square$

BRCA(Tumor:1.38±1.33,Normal:0.75±0.83,p=2.7e-20)□CESC(Tumor:1.76±1.08,Normal:1.21±0.70,p=0.02)□
 LUAD(Tumor:1.59±0.98,Normal:0.78±1.15,p=1.0e-25)□ESCA(Tumor:1.96±1.11,Normal:0.90±1.32,p=1.3e-25)□
 STES(Tumor:2.28±1.07,Normal:0.94±1.33,p=2.6e-94)□COAD(Tumor:3.29±0.82,Normal:1.39±2.04,p=5.7e-42)□
 COADREAD(Tumor:3.34±0.79,Normal:1.45±2.05,p=2.9e-48)□
 STAD(Tumor:2.42±1.02,Normal:1.07±1.38,p=2.7e-43)□LUSC(Tumor:1.50±0.96,Normal:0.78±1.15,p=2.7e-22)□
 BLCA(Tumor:1.49±1.04,Normal:0.90±0.78,p=1.8e-3)□OV(Tumor:2.11±1.24,Normal:0.91±0.75,p=9.5e-25)□
 PAAD(Tumor:1.14±0.79,Normal:-0.52±1.24,p=2.1e-45)□TGCT(Tumor:1.90±1.08,Normal:1.72±0.65,p=0.02)□
 UCS(Tumor:2.28±0.94,Normal:0.71±0.72,p=3.3e-16)□ALL(Tumor:-2.17±2.23,Normal:-4.53±2.20,p=7.7e-26)□
 LAML(Tumor:1.15±1.22,Normal:-4.53±2.20,p=2.3e-74) and we observed significant downregulation in 10
 tumors such as KIRP(Tumor:2.27±1.10,Normal:3.98±1.68,p=1.6e-38)□
 KIPAN(Tumor:2.61±1.32,Normal:3.98±1.68,p=1.1e-36)□HNSC(Tumor:1.13±1.04,Normal:1.61±0.94,p=2.6e-3)□
 KIRC(Tumor:2.75±1.41,Normal:3.98±1.68,p=7.5e-28)□LIHC(Tumor:3.73±1.05,Normal:4.11±1.10,p=1.5e-6)□
 WT(Tumor:3.21±0.84,Normal:3.98±1.68,p=9.3e-15)□SKCM(Tumor:0.91±1.14,Normal:2.90±0.77,p=3.2e-45)□
 THCA(Tumor:2.19±0.98,Normal:2.50±1.07,p=3.2e-8)□KICH(Tumor:2.96±1.04,Normal:3.98±1.68,p=1.4e-11)□
 CHOL(Tumor:2.46±1.12,Normal:4.94±0.46,p=2.3e-9) (Fig. S1). In addition, the receiver operating characteristic
 (ROC) curve was used to analyze the effectiveness of ccRCC expression levels in distinguishing ccRCC
 tissues from non-tumor tissues. The area under the curve (AUC) of PANK1 was 0.898, indicating that PANK1
 could be an ideal biomarker to differentiate ccRCC from non-neoplastic tissues (Figure 1C).

Patient characteristics are presented in Table 1, with 539 cases of primary ccRCC with clinical and gene expression data collected from the TCGA database. According to the average relative expression level of PANK1, patients with ccRCC were divided into high-expression group (n=270) and low-expression group (n=269). To evaluate the correlation between the expression of PANK1 and the clinical pathological features of patients with ccRCC. The chi-square test showed that the expression of PANK1 was related to gender (P<0.001), T stage (P<0.001), histological grade (P<0.001), pathological stage (P<0.001), N stage (P<0.05), and M stage (P<0.05).

Logistic regression was used to analyze the relationship between the clinical pathological characteristics of ccRCC and the expression level of PANK1.

The results suggested that PANK1 had a significant correlation with gender (P<0.001), T stage (P<0.001), histological grade (P<0.001), pathological stage (P<0.001), N stage (P=0.012), and M stage (P=0.007) (Table 2, Figure 2).

PANK1 expression associated with poor prognosis in ccRCC patients

The association of PANK1 expression with PFS in ccRCC patients was assessed by Kaplan-Meier analysis and showed a negative association of PANK1 expression with poor OS in ccRCC patients (P<0.001, Figure

3A). In addition, to expand our observation to pan-cancer levels, the relationship between expression of PANK1 and patient survival was further analyzed in a variety of cancer types other than ccRCC. As shown in Figure S2, a significant association between PANK1 expression and poor OS was also observed in patients with colon cancer (COAD), renal papillary cell carcinoma (KIRP), brain low-grade gliomas (LGG), mesothelioma (MESO), pancreatic cancer (PAAD), and rectal adenocarcinoma (READ).

Cox univariate and multivariate analysis of prognostic factors in ccRCC

Table 3 shows the Cox univariate and multivariate analysis results for OS in patients with ccRCC. $P < 0.01$ variables in the Cox univariate regression model were age ($P < 0.001$), T stage ($P < 0.001$), N stage ($P < 0.001$), M stage ($P < 0.001$), pathological stage ($P < 0.001$), histological grade ($P < 0.001$), and PANK1 ($P < 0.001$). Multivariate analysis further revealed that age ($P = 0.023$), M stage ($P < 0.001$), pathological stage ($P = 0.042$), histological grade ($P = 0.014$), and PANK1 ($P < 0.001$) were independent prognostic factors for OS in patients with ccRCC (Table 3, Figure 4). A nomogram was developed, that is able to predict 1, 3 and 5-year OS using the PANK1 gene and other clinical features of KIRC (including age, gender, T stage, N stage, M stage, pathological stage, and histologic grade). To read the nomogram, a vertical line up to the top point row to assign points for each variable should be drawn. Then, the total points for a patient can be added up, and one can obtain the probability of 1, 3 and 5-year OS by drawing a vertical line from the total points row. The calibration plots for the probabilities of 1, 3 and 5-year OS showed good agreement between the predicted OS by nomogram and actual OS of ccRCC patients (Figure 5).

Correlation signal path of PANK1 based on GSEA

The KEGG signaling pathway associated with PANK1 was identified using the GSEA method. GSEA showed significant differences in MSigDB enrichment (c5) ($\text{Padj} < 0.05$, $\text{FDR} < 0.25$). According to the screening conditions, we screened four significantly related signaling pathways from the KEGG signaling pathway enriched in GSEA, namely, the extracellular matrix receptor pathway, the signaling pathway related to hypertrophic cardiomyopathy, the cytokine-cytokine receptor interaction pathway, and the complement and coagulation cascade pathway (Table 4, Figure 6).

Correlation between expression of PANK1 and immune infiltration

We further analyzed the correlation between the expression of PANK1 and the immune invasion of ssGSEA using Spearman R. The results showed that the expression of PANK1 was negatively correlated with the infiltration levels of aCD, B cells, CD8+T cells, cytotoxic cells, macrophages, natural killer (NK) CD56 bright cells, plasma cell-like dendritic cells (pDC), T cells, Tem cells, Th1 cells, Th2 cells, and Treg cells ($P < 0.001$), and positively correlated with the infiltration levels of eosinophils, neutrophils, and Th17 cells ($P < 0.001$). (Table 5, Figure 7 and Figure S3)

Discussion

In this study, we investigated the expression of PANK1 in ccRCC and its correlation with the diagnosis and prognosis of ccRCC. According to our results, PANK1 is an important gene related to substance metabolism. Some studies have shown that insulin resistance caused by high-fat diet is an important factor leading to obesity, type 2 diabetes and cancer, and the PANK1 gene participates in the pathogenic process of many diseases caused by insulin resistance[28]. Besides, studies have shown that for leptin-deficient mice, knocking out the PANK1 gene can reduce the incidence of hyperglycemia and hyperinsulinemia, and improve the systemic material metabolism[29]. It has been reported that the PANK series genes, including PANK1, are dysfunctional in several cancer types and play an important role in the occurrence and development of cancer. For example, high PANK2 expression may have a favorable effect on the prognosis of patients with acute myeloid leukemia, while high PANK4 expression indicates a poor prognosis of patients with acute myeloid leukemia[21]. All these studies indicate that the genes of the PANK family may play different roles in a variety of cancer types. This study showed a decrease in PANK1 levels in ccRCC tissues, which was associated with a poor prognosis for our patient. In addition to ccRCC, survival analysis showed that PANK1 can also be used as a prognostic indicator for colorectal cancer, pancreatic cancer, mesothelioma, glioblastoma, and renal papillary cell carcinoma[21, 28, 29].

A major focus of this work will be to predict the potential mechanisms of PANK1 in regulating the development of ccRCC. PANK1 was found to be involved in the extracellular matrix receptor interaction through GSEA experiments. Many articles have suggested that ECM is related to the occurrence and development of various tumors. For example, studies have suggested that most differential genes related to breast cancer are related to extracellular matrix [30]. Changes in the density and composition of the extracellular matrix (ECM) play an important role in tumors; The stiffness and degradation of the extracellular matrix contribute to the growth and progression of tumors [31]. Previous studies have shown that the accumulation and remodeling of extracellular matrix (ECM) is considered to be the key to fibrotic diseases such as uterine fibroids [32]. Anti-fibrosis therapy can normalize the tumor microenvironment[33]. Other studies have shown that ECM is related to such diseases as gastric cancer[34], colorectal cancer[35], tongue cancer[36], and pancreatic cancer[37]. The evidence provided in the above literature suggests that PANK1 may play a role in a variety of tumors. This is also basically consistent with the above-mentioned relationship between PANK1 and the prognosis of multiple types of tumors. The formation of tumor microenvironment is related to cytokines. In the process of cancer cell formation, cancer cells release various cytokines to the surrounding, and recruit and reprogram many other types of cells to establish a tumor microenvironment[38]. We also found in this GSEA analysis that PANK1 was related to cytokines and the interaction between cytokines. The above evidences suggested that PANK1 might be related to the construction of tumor microenvironment. The complement system is an ancient and critical effector mechanism of the innate immune system, consisting of the central components of the entire cascade (C1–C9), regulators and inhibitors, proteases and newly assembled enzymes, receptors for a variety of activation products and complement components, and their products[39]. Complement can be activated within seconds of infection or stimulation. Complement activation can produce allergic peptide, cell detoxification compounds, and antibacterial compound. These generated molecules in turn activate pro-inflammatory mediators and recruit effector cells, thereby providing an immediate barrier against invading microorganisms or modified self-cells,

including tumor cells[40, 41]. In addition to supplementing the cascade system, the coagulation and fibrinolysis system is also an enzyme-dependent cascade system present in the blood. Coagulation and fibrinolysis system are the main vascular injuries that play a role when bleeding. Weakened clotting and fibrinolysis systems can cause uncontrolled bleeding. Excessive coagulation and fibrinolysis can lead to the occurrence of thrombotic diseases. In this study, we found that PANK1 was related to the complement system and coagulation reaction, suggesting that PANK1 might play a role in some immune diseases and hematological system diseases. This idea needs further experimental verification.

Another important aspect of this study is to investigate the relationship between the expression of PANK1 and different levels of immune infiltration in ccRCC. From ssGSEA analysis, we can find that PANK1 has a negative correlation with Th2,TFH and other immune cells that can promote tumor progression, indicating that PANK1 has the effect of inhibiting tumor, that is, patients with high expression of PANK1 have a better prognosis, which is consistent with the better prognosis of patients with renal clear cell carcinoma with high PANK1 in our study. In addition, PANK1 has a positive correlation with immune cells such as Th17 that inhibit tumor progression, which is also consistent with the above trend. However, there is also a negative correlation between PANK1 and CD8+T cells, Treg cells and other immunosuppressive immune cells, but there is no other evidence that PANK1 can aggravate the tumor.

To the best of our knowledge, this is the first effort to explore the relationship between PANK1 and ccRCC, although with some limitations. First, the current research is mainly based on bioinformatics analysis, which can be further strengthened through experimental research. Second, the number of healthy subjects used as controls differs greatly from the number of cancer patients. Last but not least, retrospective studies still have their own limitations, especially inconsistent interventions and lack of some information. Therefore, further studies are needed to further validate our findings.

Conclusion

In conclusion, we observed an increase in PANK1 expression in ccRCC associated with a better clinical prognosis in patients with ccRCC. PANK1 may participate in the development of ccRCC by affecting the extracellular matrix, cytokine-related interactions, and immune components such as complement. This study partially reveals the role of PANK1 in ccRCC and provides potential biomarkers for the diagnosis and treatment of renal clear cell carcinoma.

Abbreviations

Abbreviations	Full name
TCGA-ACC	Adrenocortical carcinoma
TCGA-BLCA	Bladder Urothelial Carcinoma
TCGA-BRCA	Breast invasive carcinoma
TCGA-CESC	Cervical squamous cell carcinoma and endocervical adenocarcinoma
TCGA-CHOL	Cholangiocarcinoma
TCGA-COAD	Colon adenocarcinoma
TCGA-COADREAD	Colon adenocarcinoma/Rectum adenocarcinoma Esophageal carcinoma
TCGA-DLBC	Lymphoid Neoplasm Diffuse Large B-cell Lymphoma
TCGA-ESCA	Esophageal carcinoma
TCGA-FPPP	FFPE Pilot Phase II
TCGA-GBM	Glioblastoma multiforme
TCGA-GBMLGG	Glioma
TCGA-HNSC	Head and Neck squamous cell carcinoma
TCGA-KICH	Kidney Chromophobe
TCGA-KIPAN	Pan-kidney cohort (KICH+KIRC+KIRP)
TCGA-KIRC	Kidney renal clear cell carcinoma
TCGA-KIRP	Kidney renal papillary cell carcinoma
TCGA-LAML	Acute Myeloid Leukemia
TCGA-LGG	Brain Lower Grade Glioma
TCGA-LIHC	Liver hepatocellular carcinoma
TCGA-LUAD	Lung adenocarcinoma
TCGA-LUSC	Lung squamous cell carcinoma
TCGA-MESO	Mesothelioma
TCGA-OV	Ovarian serous cystadenocarcinoma
TCGA-PAAD	Pancreatic adenocarcinoma
TCGA-PCPG	Pheochromocytoma and Paraganglioma
TCGA-PRAD	Prostate adenocarcinoma
TCGA-READ	Rectum adenocarcinoma
TCGA-SARC	Sarcoma

Abbreviations	Full name
TCGA-STAD	Stomach adenocarcinoma
TCGA-SKCM	Skin Cutaneous Melanoma
TCGA-STES	Stomach and Esophageal carcinoma
TCGA-TGCT	Testicular Germ Cell Tumors
TCGA-THCA	Thyroid carcinoma
TCGA-THYM	Thymoma
TCGA-UCEC	Uterine Corpus Endometrial Carcinoma
TCGA-UCS	Uterine Carcinosarcoma
TCGA-UVM	Uveal Melanoma
TARGET-OS	Osteosarcoma
TARGET-ALL	Acute Lymphoblastic Leukemia
TARGET-NB	Neuroblastoma
TARGET-WT	High-Risk Wilms Tumor
TCGA	the cancer genome atlas
GTE _x	Genotype-Tissue Expression
KEGG	Kyoto Encyclopedia of Genes and Genomes
ROC	receiver operating characteristic
AUC	area under the curve
ccRCC	Clear cell carcinoma of kidney
GSEA	Gene Set Enrichment Analyses
NES	normalized NS
Padj	adjust P value
FDR	false discovery rate

Declarations

Ethical Statement

The author is responsible for all aspects of the work and ensures that questions relating to the accuracy or completeness of any part of the work are properly investigated and resolved. All procedures performed in this study were in compliance with the Declaration of Helsinki (revised in 2013).

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Authors' contributions

Software, Formal analysis, writing original draft, visualization by Baishun Ma, Supervision by Pu Wang. All authors reviewed the manuscript. All authors read and approved the final manuscript.

Consent for publication

Not applicable.

Competing interests

The authors declare that there is no competing interests about this article.

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Tables

Table 1

Correlation between PANK1 expression and clinicopathological characteristics in renal clear cell cancer in TCGA.

Characteristic	Low expression of PANK1	High expression of PANK1	p
n	269	270	
Age, n (%)			0.212
<=60	142 (26.3%)	127 (23.6%)	
>60	127 (23.6%)	143 (26.5%)	
Gender, n (%)			< 0.001
Female	73 (13.5%)	113 (21%)	
Male	196 (36.4%)	157 (29.1%)	
T stage, n (%)			< 0.001
T1	119 (22.1%)	159 (29.5%)	
T2	34 (6.3%)	37 (6.9%)	
T3	107 (19.9%)	72 (13.4%)	
T4	9 (1.7%)	2 (0.4%)	
N stage, n (%)			0.009
N0	122 (47.5%)	119 (46.3%)	
N1	14 (5.4%)	2 (0.8%)	
M stage, n (%)			0.010
M0	203 (40.1%)	225 (44.5%)	
M1	50 (9.9%)	28 (5.5%)	
Pathologic stage, n (%)			< 0.001
Stage I	115 (21.5%)	157 (29.3%)	
Stage II	25 (4.7%)	34 (6.3%)	
Stage III	74 (13.8%)	49 (9.1%)	
Stage IV	53 (9.9%)	29 (5.4%)	
Histologic grade, n (%)			< 0.001
G1	2 (0.4%)	12 (2.3%)	
G2	103 (19.4%)	132 (24.9%)	
G3	105 (19.8%)	102 (19.2%)	
G4	56 (10.5%)	19 (3.6%)	
Age, mean $\pm$ SD	60.05 $\pm$ 12.74	61.2 $\pm$ 11.41	0.268

Table 2

PANK1 expression associated with clinicopathologic characteristics of renal clear cell cancer in TCGA (logistic regression).

Characteristics	Total(N)	Odds Ratio(OR)	P value
Age (>60 vs. ≤60)	539	1.259 (0.898-1.767)	0.182
Gender (Male vs. Female)	539	0.517 (0.360-0.741)	<0.001
Pathologic stage (Stage III&Stage IV vs. Stage I&Stage II)	536	0.450 (0.314-0.642)	<0.001
T stage (T2&T3&T4 vs. T1)	539	0.554 (0.393-0.778)	<0.001
N stage (N1 vs. N0)	257	0.146 (0.023-0.539)	0.012
M stage (M1 vs. M0)	506	0.505 (0.303-0.827)	0.007
Histologic grade (G3&G4 vs. G1&G2)	531	0.548 (0.388-0.773)	<0.001

Table 3

Associations with overall survival and clinicopathological characteristics in TCGA patients using Cox regression.

Characteristics	Total(N)	Univariate analysis		Multivariate analysis	
		Hazard ratio (95% CI)	P value	Hazard ratio (95% CI)	P value
Age	539				
<=60	269	Reference			
>60	270	1.765 (1.298-2.398)	<0.001	1.645 (1.072-2.524)	0.023
Gender	539				
Female	186	Reference			
Male	353	0.930 (0.682-1.268)	0.648		
T stage	539				
T1	278	Reference			
T2&T3&T4	261	2.917 (2.095-4.061)	<0.001	0.683 (0.320-1.458)	0.325
N stage	257				
N0	241	Reference			
N1	16	3.453 (1.832-6.508)	<0.001	1.256 (0.622-2.533)	0.525
M stage	506				
M0	428	Reference			
M1	78	4.389 (3.212-5.999)	<0.001	2.487 (1.499-4.126)	<0.001
Pathologic stage	536				
Stage I&Stage II	331	Reference			
Stage III&Stage IV	205	3.946 (2.872-5.423)	<0.001	2.227 (1.030-4.813)	0.042
Histologic grade	531				
G1&G2	249	Reference			
G3&G4	282	2.702 (1.918-3.807)	<0.001	1.873 (1.136-3.088)	0.014
PANK1	539				
Low	270	Reference			
High	269	0.360 (0.261-0.498)	<0.001	0.398 (0.248-0.639)	<0.001

Table 4

KEGG pathway enriched in high- and low-PANK1 groups by using GSEA.

ID	NES	p.adjust	qvalues
KEGG_HYPERTROPHIC_CARDIOMYOPATHY_HCM	-1.651683298	0.03139541	0.026800027
KEGG_ECM_RECEPTOR_INTERACTION	-1.567551718	0.03139541	0.026800027
KEGG_CYTOKINE_CYTOKINE_RECEPTOR_INTERACTION	-1.524368336	0.03139541	0.026800027
KEGG_COMPLEMENT_AND_COAGULATION_CASCADES	-1.704641039	0.03139541	0.026800027

Table 5

The correlation between PANK1 expression and 24 immune cells was detected by Spearman correlation method.

Molecular	Immune cell	R(Spearman)	P(Spearman)
PANK1	aDC	-0.190	<0.001
PANK1	B cells	-0.181	<0.001
PANK1	CD8 T cells	-0.179	<0.001
PANK1	Cytotoxic cells	-0.219	<0.001
PANK1	DC	-0.132	0.002
PANK1	Eosinophils	0.310	<0.001
PANK1	iDC	-0.026	0.545
PANK1	Macrophages	-0.159	<0.001
PANK1	Mast cells	0.065	0.134
PANK1	Neutrophils	0.333	<0.001
PANK1	NKCD56bright cells	-0.292	<0.001
PANK1	NKCD56dim cells	-0.120	0.005
PANK1	NK cells	-0.106	0.014
PANK1	pDC	-0.223	<0.001
PANK1	T cells	-0.144	<0.001
PANK1	T helper cells	0.092	0.032
PANK1	Tcm	0.133	0.002
PANK1	Tem	-0.158	<0.001
PANK1	TFH	-0.096	0.026
PANK1	Tgd	0.020	0.643
PANK1	Th1 cells	-0.284	<0.001
PANK1	Th17 cells	0.296	<0.001
PANK1	Th2 cells	-0.261	<0.001
PANK1	TReg	-0.386	<0.001

Supplemental

Supplemental Figure S1 is not available with this version.

Figures

Figure 1

PANK1 expression and clinicopathological features of ccRCC. (A) Wilcoxon rank sum test was used to analyze the difference expression of PANK1 in ccRCC tissues and adjacent renal tissues. (B) Wilcoxon signed rank sum test was used to detect the difference expression of PANK1 in ccRCC tissues and adjacent renal tissues. (C) ROC curve showed the efficiency of PANK1 expression level to distinguishing ccRCC tissue from non-tumor tissue. X-axis represents false positive rate, and Y-axis represents true positive rate.

Figure 2

Association between PANK1 expression and clinicopathologic characteristics in ccRCC. (A) Wilcoxon rank sum test was used to compare the relationship between the expression of PANK1 and age of ccRCC patients in TCGA database. (B) Wilcoxon rank sum test was used to compare the relationship between the expression of PANK1 and gender of ccRCC patients in TCGA database. (C) Wilcoxon rank sum test was used to compare the relationship between the expression of PANK1 and Pathologic stage of ccRCC patients in TCGA database. (D) Wilcoxon rank sum test was used to compare the relationship between the expression of PANK1 and

Histologic grade of ccRCC patients in TCGA database. (E) Wilcoxon rank sum test was used to compare the relationship between the expression of PANK1 and T stage of ccRCC patients in TCGA database. (F) Wilcoxon rank sum test was used to compare the relationship between the expression of PANK1 and N stage of ccRCC patients in TCGA database. (G) Wilcoxon rank sum test was used to compare the relationship between the expression of PANK1 and M stage of ccRCC patients in TCGA database. (H) Wilcoxon rank sum test was used to compare the relationship between the expression of PANK1 in normal and tumor sample of ccRCC in TCGA database.

Figure 3

PANK1 expression and prognosis in patients with ccRCC. Kaplan-Meier curve was drawn using the R package survminer to evaluate the prognostic value of PANK1 in OS of ccRCC patients. PANK1 expression value was divided into high and low expression group according to median value. (A) All patients with clear cell renal carcinoma in TCGA were selected for the study. (B) All patients with clear cell carcinoma of the kidney in TCGA who were younger than or equal to 60 years of age were selected for the study. (C) All patients with clear cell carcinoma of the kidney in TCGA who were older than 60 years of age were selected for the study. (D) All of the women with clear cell renal carcinoma in TCGA were selected for the study. (E) All male patients with clear cell renal carcinoma in TCGA were selected for the study. (F) All patients with clear cell carcinoma of the kidney who did not have lymph node metastasis in TCGA were selected for the study. (G) All patients with clear cell carcinoma of the kidney in TCGA who did not have distant metastasis were selected for the study. (H) All patients with clear cell carcinoma of the kidney in TCGA with stage T1 were selected for the study. (I) All patients with T2-T4 stage of clear cell renal carcinoma in TCGA were selected for the study. (J) All patients with pathologic stage 1-2 renal clear cell carcinoma in TCGA were selected for the study. (K) All patients with pathologic stage 3-4 renal clear cell carcinoma in TCGA were selected for the study. (L) All patients with clear cell carcinoma of the kidney in TCGA with histologic stage 3-4 were selected for the study.

Figure 4

Forest plot for multivariate COX regression of clinicopathologic features and overall survival in patients with TCGA renal clear cell carcinoma.

A

B

Figure 5

Nomogram and calibration for PANK1 and other clinical characteristics in patients with TCGA renal clear cell carcinoma. (A) Nomogram for PANK1 and other clinical characteristics in patients with TCGA renal clear cell carcinoma. (B) Calibration analysis of the above nomogram for 1 year overall survival ,3 year overall survival and 5-year overall survival.

Figure 6

Enrichment plot from the GSEA. The data set was on the left significantly enriched in red area (PANK1 high expression group). NES, normalized NS; Padj, adjust P value; FDR, false discovery rate.

Figure 7

The expression level of PANK1 was related to the immune infiltration in the tumor microenvironment. (A) The forest plot shows the correlation between PANK1 expression level and 24 immune cells. The size of dots indicates the absolute value of Spearman r . (B) The Wilcoxon rank sum test was used to analyze the difference of 24 immune cells infiltration level between PANK1 high and low expression groups

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