MEDS Clinical Medicine (2023)
Clausius Scientific Press, Canada

DOI: 10.23977/medsc.2023.040209
ISSN 2616-1907 Vol. 4 Num. 2

Analysis of the expression and clinical significance of PGM5 in clear cell renal cell carcinoma based on the TCGA database

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Keywords: Clear cell renal cell carcinoma, PGM5, TCGA database

Abstract: To analyze the expression characteristics and clinical relevance of PGM5 in clear cell renal cell carcinoma based on the TCGA database. The differential expression of PGM5 and the association between PGM5 and clinical diagnosis and prognosis in clear cell renal cell carcinoma were analyzed based on the TCGA, GEIPIA, and Linkedomics databases. The R language was used to process and analyze data. The genes related to the expression of PGM5 were analyzed with the WebGestalt tool. The analysis based on the TCGA showed that the expression level of PGM5 mRNA in ccRCC tissues was lower than that in normal renal tissues. The Linkedomics database analysis showed that PGM5 mRNA is different expressed in the pathological Stage of the clear cell takes cell carcinoma ($P < 0.05$). The expression level tended to be downregulated with the happens of the pathological stage. PGM5 mRNA was differentially expressed in different T, N, and M stages ($P<0.05$). Survival analysis showed that PGM5 expression level was significantly associated with overall survival and disease-free survival in ccRCC patients. The diagnostic accuracy showed that the PGM5 expression had excellent diagnostic value by ROC curve analysis for distinguishing between ccRCC tissues and normal renal tissues. In addition, the expression level of PGM5 was positively correlated with that of PAPB, and PARM1, respectively, and negatively correlated with those of PMM2, NME1, and PDCD5. The enrichment analysis of genes associated with PGM5 expression was positively correlated with DNA replication and cell cycle and negatively correlated with those of cGMP-PKG and Rap1 signaling pathways. The expression level of PGM5 mRNA is associated with the pathological stage, T stage, N stage, M stage, and prognosis in patients with clear cell renal cell carcinoma. Therefore, PGM5 has the potential to become a diagnostic marker, as well as a therapeutic target of clear cell renal cell carcinoma.

1. Introduction

Renal cell carcinoma (RCC) is a common and fatal malignant tumor, accounting for more than 2% of all adult cancers. The incidence and mortality of renal cancer are also increasing year by

year.Eighty percent of kidney cancers are subclassified as clear cell renal cell carcinoma (ccRCC) based on histological and cytogenetic features[1].With the improvement of medical technology, significant progress has been made in the diagnosis and treatment of renal cell carcinoma, but the prognosis of patients has not been improved.In recent years, with the in depth research of tumor molecular biology, targeted therapy has become a new diagnosis and treatment strategy in the current clinical application[2]. Therefore, it is extremely important to find new molecular targets for the clinical diagnosis, treatment and prognosis monitoring of ccRCC.

PGM5, also known as phosphoglycosidase-associated protein (PGM-rp) or Aciculin, is located on human chromosome 9 (9q21.11) and consists of two closely related 60 kDa and 63 kDa isoforms[3].PGM5 is one of the five proteins in the α-D-phosphohexamutase superfamily in the human genome.They are represented by phosphoglucomutase 1 (PGM1), which regulates glucose homeostasis through the interconversion of glucose 1-phosphate and glucose 6-phosphate, and four other proteins, called PGM2, PGM2L1, PGM3, and PGM5[4].According to the existing literature, the PGM family has been reported to be associated with cancer proliferation, invasion and metastasis[5–7].PGM5 promotes the conversion of glucose 1-phosphate (G1P) to glucose 6-phosphate (G6P) and inhibits breast cancer cell proliferation and migration by regulating aerobic glycolysis[8].miR-1293 promotes the proliferation, migration and invasion of LUAD cells by targeting PGM5[9].PGM5 is lowly expressed in pca and the low expression of PGM5 and its associated gene signature are associated with poor clinical outcome and high Gleason score.In vitro experiments showed that overexpression of PGM5 significantly inhibited the proliferation and migration of prostate cancer cells[6].The above studies suggest that PGM5 is closely related to tumor progression.However, the expression of PGM5 in ccRCC and its prognosis have not been reported in detail.Therefore, in this study, we used TCGA database mining analysis to understand the expression of PGM5 in ccRCC, analyze its relationship with the clinicopathological characteristics of ccRCC, and explore its potential diagnostic and prognostic value.

2. Materials and Methods

2.1 TCGA database (GDC (cancer.gov)) was used to analyze the expression level of PGM5 in normal renal tissues and ccRCC

The Cancer Genome Atlas (TCGA)[10]Is a project overseen jointly by the National Cancer Institute and the National Human Genome Research Institute.More than 20,000 primary cancers were molecular-characterized and matched to normal samples from 33 cancer types.It has the advantages of high data quality, rich omics data, large sample size, and comprehensive clinical information.R language was used to perform statistical analysis and visualization of ccRCC transcript data in TCGA.

2.2 The expression level of PGM5 in tissues was analyzed by The Human Protein Atlas, a public database

The Human Protein Atlas (HPA) provides information on the tissue and cellular distribution of 26,000 human proteins. The expression of each protein was examined in detail in 64 cell lines, 48 human normal tissues, and 20 tumor tissues by immunoassays using highly specific antibodies. The immunohistochemical results of PGM5 in normal renal tissues and renal cell carcinoma tissues were downloaded from the official website, and the expression of PGM in tissue protein was analyzed.

2.3 Linkedomics database was used to analyze the relationship between PGM5 expression and clinicopathological features of ccRCC

Linkedomics database contains multi-omics data and clinical data of 32 cancer types from TCGA and other platforms, which can be used to analyze the relationship between clinical phenotype and gene expression of tumors in TCGA database[11].In this study, the data of PGM5 expression, pathological stage, T stage, N stage and M stage were downloaded from the Linkedomics database as the clinicopathological characteristics of ccRCC. GraphPad Prim 9.4 was used to analyze the relationship between PGM5 expression and clinicopathological characteristics of ccRCC.

2.4 GEIPIA website was used to analyze the relationship between PGM5 expression in ccRCC and its prognosis

GEPIA is an interactive web server newly developed by Zefang Tang et al., Peking University, to analyze RNA-sequencing expression data from 9,736 tumors and 8,587 normal samples from the TCGA and GTEx projects[12].The relationship between PGM5 expression level and prognosis of patients was analyzed by this platform.

2.5 Linkedomics database was used to screen the genes related to PGM5

Go to the Linkedomics website (LinkedOmics: In the first step, select the tumor type "ccRCC", in the second step, select the data type "RNAseq", the data platform "HiSeq RNA", in the third step, select the target "PGM5", in the fourth step, select the target database type "RNAseq", and the data platform "HiSeq RNA". In the fifth step, select "Pearson Correlation test" as the statistical method, and finally submit it. Genes positively and negatively correlated with PGM5 were analyzed.

2.6 The WebGestalt tool was used to analyze the enriched pathways of PGM5-related gene sets

Web Gestalt 2017 supports 12 organisms from a variety of databases and technology platforms, 324 gene identifiers, and 150937 functional categories from public databases and computational analyses. Omics data with gene identifiers not supported by WebGestalt and functional categories not included in the WebGestalt database can also be uploaded for enrichment analysis[13].

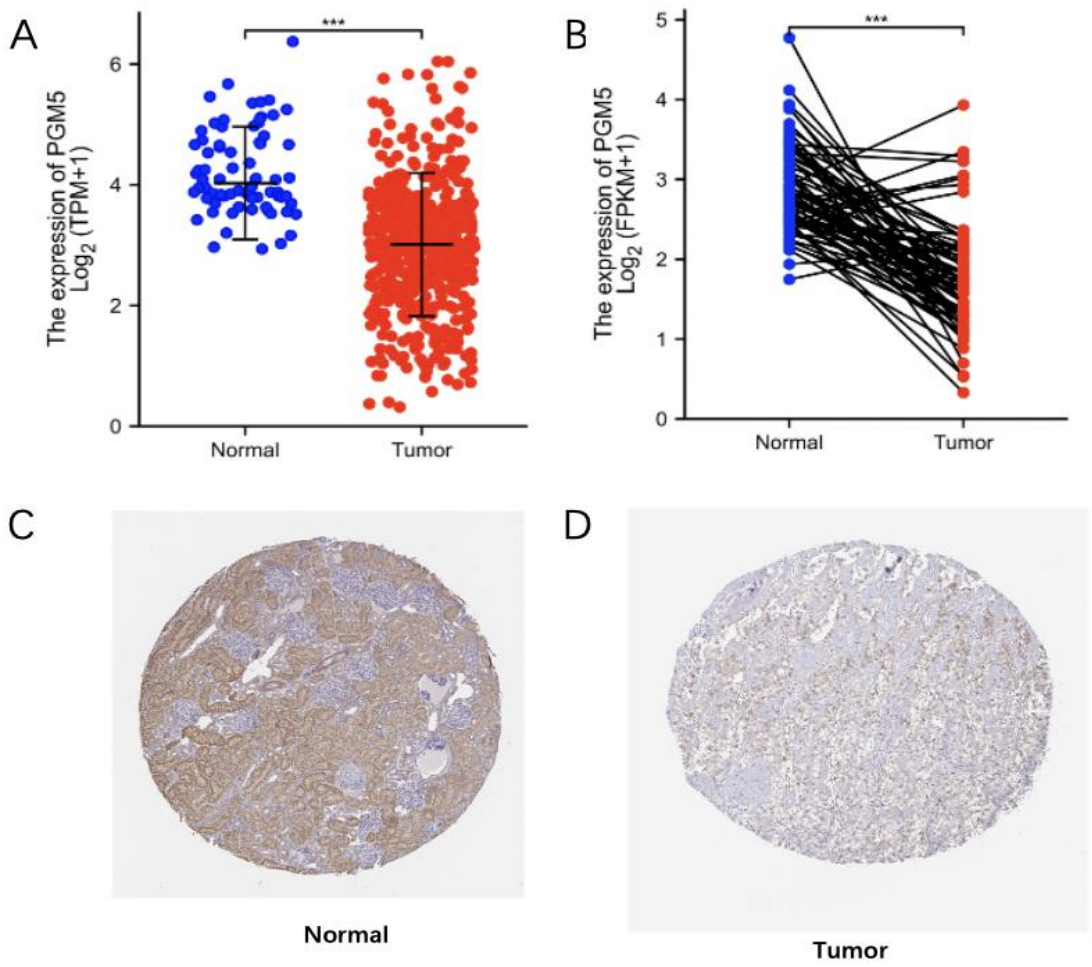
2.7 Statistical analysis

GraphPad Prim Version 9.4 statistical software was used for analysis, and t test was used for comparison between the two groups. $P<0.05$ was considered statistically significant.

3. Results

3.1 Expression of PGM5mRNA and protein in ccRCC tissues

The expression of PGM5 mRNA in normal kidney tissues (72 cases) and ccRCC tumor tissues (539 cases) in TCGA database was analyzed by R language.The immunohistochemical results of PGM5 in normal kidney tissues and ccRCC tissues were downloaded from the public database (The Human Protein Atlas).The results showed that PGM5 in ccRCC tissues was lower than that in normal kidney tissues at the mRNA (Figure 1A, B) and protein (Figure 1C, D) levels.

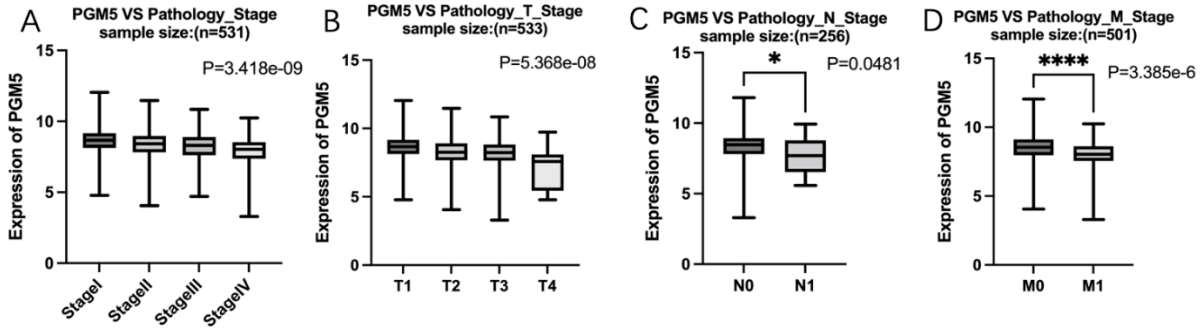


A. The expression level of PGM5 mRNA for unpaired sample B. The expression level of PGM5 mRNA for paired sample C. IHC for anti-PGM5 of normal renal tissue D. IHC for anti-PGM5 of renal carcinoma

Figure 1: The expression level of PGM5 mRNA and protein in ccRCC tumor tissue and normal kidney tissue.

3.2 Correlation between PGM5 expression level and clinicopathological features of ccRCC patients

The relationship between PGM5 expression and clinicopathological features of ccRCC was analyzed by GraphPad software using the PGM5 expression data downloaded from TCGA database.The results showed that the expression level of PGM5 was different in the clinical pathological stage of ccRCC ($P<0.05$,n=531), and the difference was statistically significant, and with the higher pathological stage, the expression level of PGM5 had a downward trend (FIG. 2A).In addition, PGM5 expression was differentially expressed in different T stages ($P<0.05$,n=533) (Figure 2B), N stages ($P<0.05$,n=256) (Figure 2C), and M stages ($P<0.05$,n=501) (Figure 2D).The results of these analyses indicated that PGM5 was highly correlated with ccRCC progression.

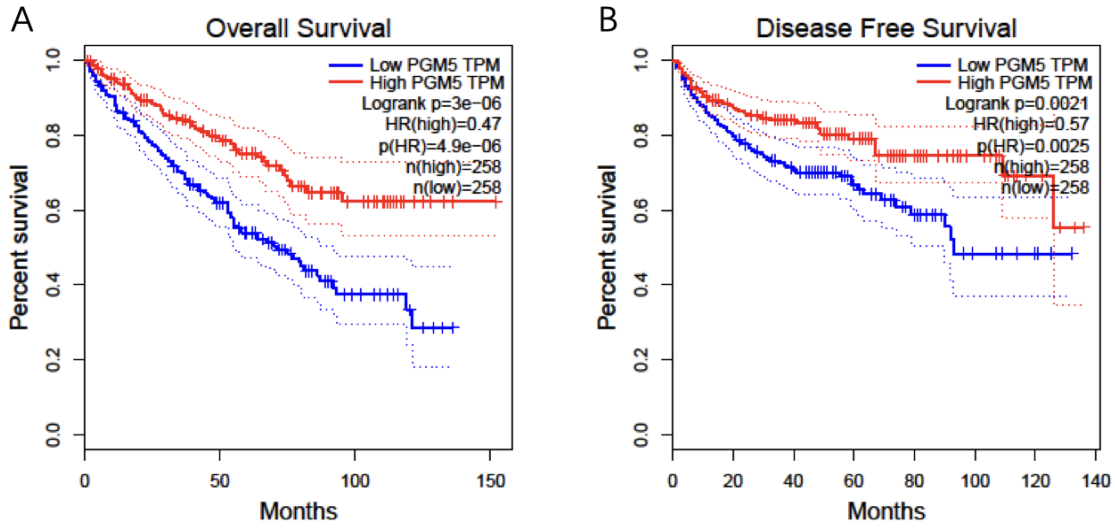


A: The expression level of PGM5 in different pathological stages. B. The expression level of PGM5 in different T stages. C. The expression level of PGM5 in different M stages. D: The expression level of PGM5 in N stages

Figure 2: The relationship between the expression level of PGM5 and clinicopathological characteristics of ccRCC patients

3.3 Relationship between PGM5 expression level and prognosis of ccRCC patients

Using GEIPIA platform analysis, it was found that PGM5 expression level was significantly correlated with overall survival (Logrank p=3e-06,n=258) and relapse-free survival (Logrank p=0.0021,n=258) of ccRCC patients (FIG. 3).Among them, the overall survival rate of patients with low PGM5 expression (n=258) was lower than that of patients with high PGM5 expression (n=258) (Figure 3A), and the relapse-free survival time of patients with high PGM5 expression (n=258) was longer than that of patients with low PGM5 expression (n=258) (Figure 3B).



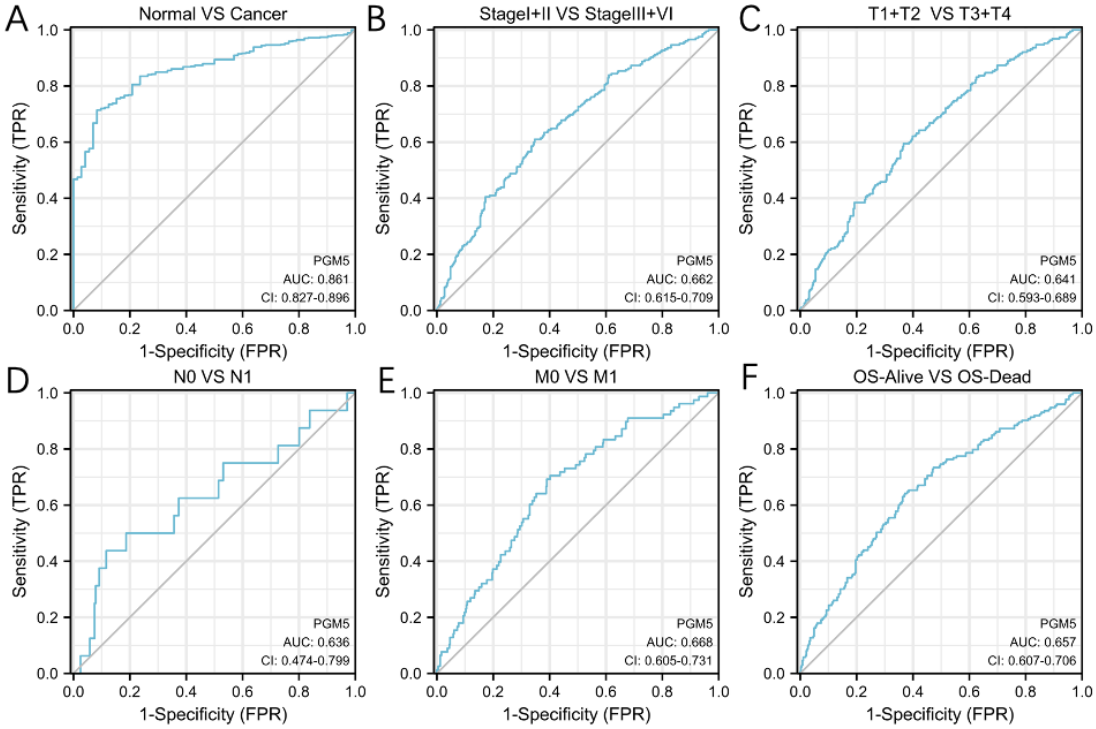
A: The OS survival curve of ccRCC patients. B: The DFS survival curve of ccRCC patients.

Figure 3: The relationship between the expression level of PGM5 and the prognosis of ccRCC patients

3.4 Diagnostic value of PGM5 expression in ccRCC patients

Download the TCGA (https://portal.gdc.cancer.gov/) KIRC (renal clear cell carcinoma) in the project level 3 HiSeq- FPKM RNAseq data format. R was used to draw the diagnostic ROC curve.

The results showed that PGM5 expression level had a certain diagnostic value in distinguishing normal renal tissues from ccRCC tissues (AUC=0.861, CI: 0.827-0.896, Figure 4A).The diagnostic value of PGM5 expression for clinical pathology of ccRCC was as follows: StageI+II VS StageIII+IV (AUC=0.662, CI: 0.615-0.709, Figure 4B), T1+T2 VS T3+T4 (AUC=0.641, CI: 0.641). 0.593-0.689, Figure 4C), N0 VS N1 (AUC=0.636, CI: 0.474-0.799, Figure 4D), M0 VS M1 (AUC=0.668, CI: 0.605-0.731, Figure 4E), OS-Alive VS OS-Dead (AUC=0.657, CI: 0.607-0.706, Figure 4F).



A. PGM5 discriminated between ccRCC and paired normal tissues (AUC=0.861). Receiver operating characteristic curve sub analysis was performed concerning the following subgroups of patients with ccRCC:(B) Tumor-Node-Metastasis stage,(C) T stage,(D) lymph node metastasis, (E) distant metastases, (F) overall survival.

Figure 4: The Diagnostic Significance of the PGM5 Expression and Relationship between Clinical Characteristics in ccRCC.

3.5 Analysis of PGM5 related genes

Linkedomics database was used for PGM5 related gene analysis, and a total of 20159 related genes were detected with P<0.01 as the cut-off (FIG. 5). Among them, the expression of PGM5 in ccRCC was positively correlated with PAPB (r=0.66), PARM1 (r=0.6583) and Loc572558 (r=0.6556) genes (Figure 6).The expression of PGM5 in CCRCC was negatively correlated with PMM2 (r=-0.4837), NME1 (r=-0.4730) and PDCD5 (r=-0.4725) genes (Figure 7).

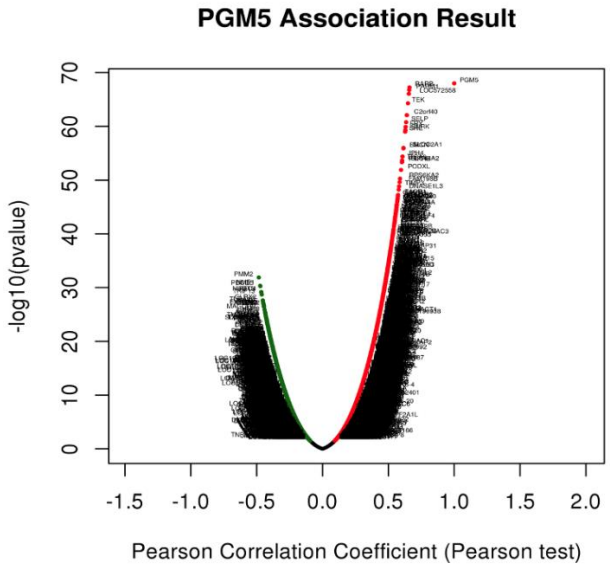


Figure 5: The scatter plot of gene distribution related to PGM5

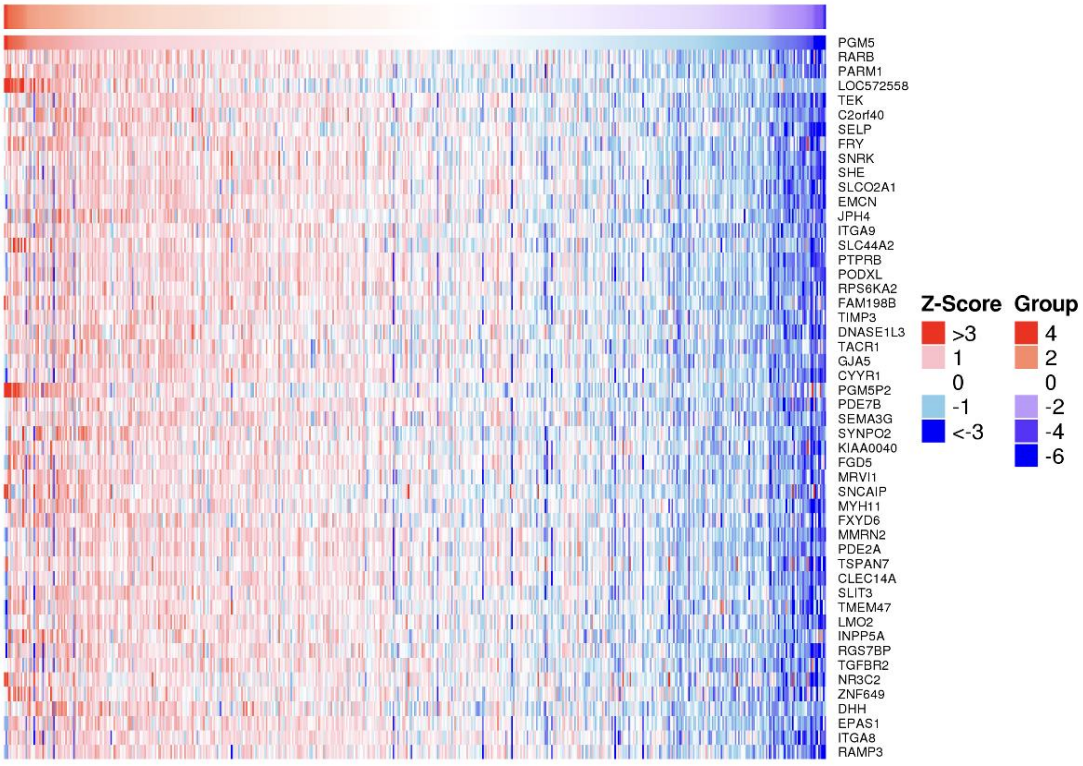


Fig6. The heat map of genes positively correlated with PGM5（top 50）

Figure 6: The heat map of genes positively correlated with PGM5(top 50)

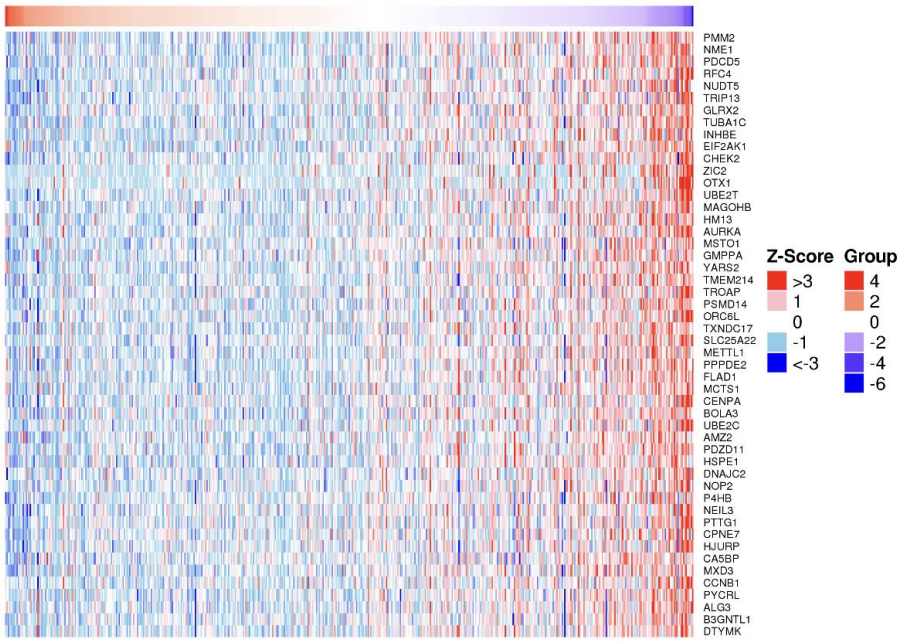


Figure 7: The heat map of genes negatively associated with PGM5(top 50)

3.6 Enrichment pathway analysis of genes related to PGM5 expression

PGM5 enrichment analysis showed that genes negatively related to PGM5 expression were significantly enriched in DNA replication and cell cycle. Positively correlated genes were significantly enriched in cGMP-PKG, Apelin, Rap1, cell adhesion molecules and other signaling pathways

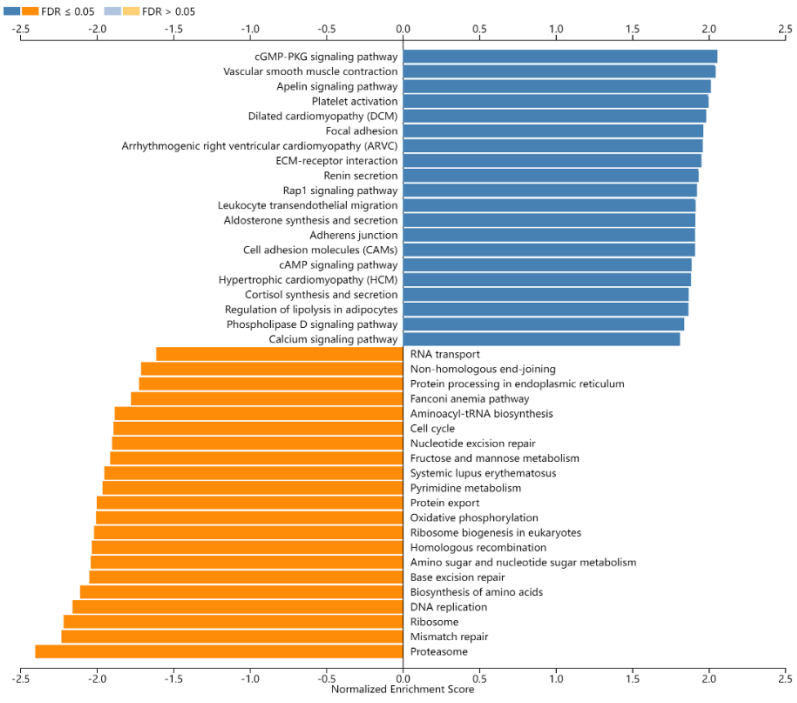


Figure 8: The enrichment analysis of PGM5-related genes

4. Conclusions and Discussion

With the development of big data, more and more genes related to tumor progression have been discovered, such as REST[14]And PKMYT1[15]And C1QTNF6[16].In recent years, PGM5 has been found to be associated with cancer.As early as 2017, PGM5 was found to be a target molecule for differentiating precancerous lesions from rectal cancer[17].PGM5 can also be used as a diagnostic and prognostic marker for HCC[18].In vitro experiments showed that overexpression of PGM5 inhibited tumor proliferation and migration in prostate cancer cells.Low PGM5 expression in pca patients promotes tumor progression and has a poor prognosis[6].PGM can also be regulated by miR-1293 as a downstream target molecule to promote the proliferation, migration and invasion of LUAD cells[9].MiR-1224-3p promotes proliferation and migration of breast cancer cells through PGM5-mediated aerobic glycolysis[8].However, the expression of PGM5 in ccRCC and its relationship with tumor progression have not been reported.

In order to explore the expression of PGM5 in ccRCC and its relationship with tumor, we found that the expression of PGM5 in ccRCC tissues was lower than that in normal renal cell carcinoma tissues through TCGA and HPA databases.At the same time, we also analyzed the relationship between the expression level of PGM5 and the clinicopathological characteristics of ccRCC patients. The expression level of PGM5 was lower in the higher pathological stage, T stage, N stage and M stage, and the difference was statistically significant ($P<0.05$).These results suggest that the progression and metastasis of ccRCC may be related to the low expression of PGM5.By drawing the diagnostic ROC curve, we found that PGM5 had a certain diagnostic value in distinguishing normal renal tissues from ccRCC tissues.In addition, the overall survival rate and recurrence-free survival rate of patients with low PGM5 expression were significantly lower than those with high PGM5 expression ($P<0.05$), indicating that the low expression of PGM5 may be an adverse prognostic factor for ccRCC patients.

To further explore the potential mechanism of PGM5 in ccRCC.We analyzed the genes related to PGM5 expression using Linkedomics database and found that the expression of PGM5 was positively correlated with PAPB, PARM1, Loc572558 and TEK genes.PMM2, NME1 and PDCD5 genes were negatively correlated with PGM5 expression, suggesting that these molecules may be the upstream and downstream molecules of PGM5.Parm-1 is specifically up-regulated in T-CD8+ leukemia.Transient transfection of mouse and human Parm-1 cDNA resulted in anchored and serum-independent growth of NIH/3T3 cells and enhanced cell proliferation in vitro[19].Long non-coding RNA LOC572558 inhibits bladder cancer cell proliferation and tumor growth by regulating AKT-MDM2-p53 signaling axis[20].Knocking out TEK promoted ccRCC cell proliferation and migration in vitro, and we found that TEK promoted apoptosis by regulating AKT phosphorylation, thereby inhibiting cell proliferation[21].Knockdown of PMM2 expression in renal cancer cells inhibited the migration and invasion of cancer cells, suggesting that overexpression of PMM2 may promote tumorigenesis[22].The metastasis suppressor NME1 controls the invasive transition of breast cancer by regulating MT1-MMP surface clearance[23].Therefore, further analysis of the relationship between these related genes and PGM5 is particularly important to reveal the mechanism of PGM5 in ccRCC.We also used the online tool WebGestalt to analyze and predict the signaling pathways that PGM5 might regulate, and found that the genes related to PGM5 expression were related to DNA replication, cell cycle, cGMP-PKG, Rap1 and cell adhesion molecule signaling pathways.It is speculated that low expression of PGM5 may promote the progression and metastasis of ccRCC by regulating DNA replication, cell cycle and cGMP-PKG signaling pathway.

This study analyzed the expression and clinical significance of PGM5 in ccRCC through TCGA database. It was found that PGM5 was lowly expressed in ccRCC tissues and was closely related to the prognosis of ccRCC, and the possible molecular mechanism and signaling pathway of PGM5 in

ccRCC were explored. Therefore, PGM5 may be a prognostic marker and potential therapeutic target for ccRCC.TCGA database has the advantages of large sample size and high reliability, which lays a preliminary foundation for further study of the relationship between PGM5 and ccRCC. In the future, we can continue to explore the potential mechanism of PGM5 in regulating ccRCC cell cycle and proliferation by increasing cell and animal level experiments.

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