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TRP Gene Family as a Prognostic Marker for Kidney Renal Clear Cell Carcinoma: Analysis of TCGA Data

Jiaxin Wang[1,2], Xin Zhang[1,2], Renhui Xiong[1,2], Qian Zhang[1,2], Zhong Luo[1,2], Guyue Hu[1,4], Lei Niu[1,2,3]*,

[1]School of Medicine, Xiamen University, Xiamen, China
[2]Central Laboratory, Xiang'an Hospital of Xiamen University, School of Medicine, Xiamen University, Xiamen, China
[3]Cancer Center and Department of Breast-Thyroid-Surgery, Xiang'an Hospital of Xiamen University, Xiamen, China
[4]Department of Ultrasound, Xiang'an Hospital of Xiamen University, Xiamen, China

Research Article

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***For Correspondence:**
Lei Niu, Cancer Center and Department of Breast-Thyroid-Surgery, Xiang'an Hospital of Xiamen University, Xiamen, China
E-mail: 3851702@qq.com

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ABSTRACT

The Transient Receptor Potential (TRP) channels are a group of gated cation channels with multiple activation properties. The connection between the TRP gene family and tumor progression has been identified. It is still unclear, nevertheless, how they influence the progression and prognosis of Kidney renal clear cell carcinoma (KIRC). Single- and multi-factor analyses of KIRC patients' data from The Cancer Genome Atlas (TCGA) database were performed, and the Least Absolute Shrinkage and Selection Operator (LASSO) algorithm was established and tested. The changes in TRP family expression have been investigated through tumor stages to determine whether they associate with poor outcomes. Analyze functional enrichment of essential TRP members. Our results revealed TRP gene family could be used as an essential prognostic marker in KIRC. Additionally, an abnormally high expression of TRPV3 has a strong connection with a poor prognosis and could shorten the survival time of patients and lead to the cancer progression.

Keywords: Transient receptor potential channels; Kidney renal clear cell carcinoma; Prognostic model; Neuronal communication; Bioinformatics

INTRODUCTION

Cancer continues to be the leading cause of mortality in a large number of nations around the world. According to GLOBOCAN the latest data, there were 9,958,000 cancer fatalities globally [1-3]. Kidney cancer, one of the most prevalent solid malignancies of the urinary tract, is ranked 14th among all cancers by the Global Cancer Observatory, causing over 173,000 deaths annually [3,4]. The latest oncology analysis data showed that the incidence of kidney cancer continued to rise by roughly 1% per year between 2016 and 2020 [1,5,6]. Depending on WHO staging on histology, 90% of these cases are Kidney renal clear cell carcinoma (KIRC) [7]. Even though surgical resection remains the primary method of therapy for restricted KIRC, some patients could still have distant metastases after surgery. Additionally, some patients failed to respond to radiation and chemotherapy, so their treatment outcomes and prognosis are quite unsatisfactory, leading to the 5-year relative survival rate of advanced KIRC less than 10% [8-10]. It is essential to avoid underestimating the potential future costs of kidney cancer to the global health system and the economy. Consequently, it is urgent to come up with novel targets to enhance the precision of prognosis predictions and treatment outcomes in kidney cancer.

The Transient Receptor Potential (TRP) channels are a family of gated cation channels with multimodal activation properties [11]. Only worms, flies, and zebrafish contain TRPN (Drosophila NOMPC), which does not encode any mammalian proteins. TRP channels are characterized by the homotetrameric organization of subunits with six transmembrane segments (S1-6) [12,13]. TRP is a versatile signaling molecule that is expressed widely in various tissues and cells [14,15]. A TRP channel could be activated by multiple ways; nearly all TRPs are responsive to chemical signals, while some are also sensitive to physical impulses (temperature, voltage, pressure and tension) [11,12,16,17]. Therefore, the individual cell with TRP channel can react swiftly to changes in the surroundings. Additionally, the TRP is unique among known ion channel families due to its peculiar cation selectivity, with some TRPs functioning as non-selective cation channels on the plasma membrane and others controlling the release of calcium ions (Ca^{2+}) from intracellular organelles [11].

Since most TRP channels are located on the cell surface, they are ideal targets for ion channel drugs that require no cellular entry to exert their effects [17]. Consistent attention is being paid to TRP gene family in the study of chronic pain, neurological and psychiatric disorders, respiratory diseases, urological, dermatological, and diabetic illness, and growing evidence pointed out the involvement of TRP gene family in tumor initiation, progression, invasion, and dissemination [11,13,18-20]. Tumors possessing malignant characteristics, such as hypoxia, oxidative stress, and red-hot state activation, are notoriously challenging to treat, however, TRP gene family has the potential to be used as a therapeutic target for cancer because of their deep links to signal transduction related to the Hypoxia-Inducible Factor 1α (HIF-1α) protein, enhanced Reactive Oxygen Species (ROS) tolerance [13]. Furthermore, Ca^{2+} related channels have been demonstrated to play essential roles in tumor growth, metastasis, and angiogenesis, while TRP channels could control Ca^{2+} concentrations by either stimulating calcium entry pathways in the plasma membrane or excreting calcium from internal stores like the endoplasmic reticulum and mitochondria [21-23]. In recent years, Transient Receptor Potential Canonical (TRPC) channels have also been identified to be involved in cancer cells proliferation, invasion and chemo resistance [24,25]. In digestive tract malignancies, TRP

channels are usually dysregulated, especially the Transient Receptor Potential Vanilloid (TRPV), Transient Receptor Potential Melastatin (TRPM), and TRPC subfamilies, which can lead to changes in cancer hallmark characteristics such increased proliferation, migration, invasion, and the inability to trigger apoptosis [26-29]. Moreover, the survival time of patients were lower the more TRPM2 mRNA was expressed in pancreatic malignant tissue. Furthermore, it was discovered that PANC-1 cells, a human pancreatic cancer cell line, proliferated, migrated, and invaded more readily when TRPM2 was expressed [30].

TRPV subfamily is a class of ligand-gated ion channels with sensory receptors that are primarily activated when subjected to thermal, mechanical, or chemical stimuli. Several investigations in recent years have proven that TRPV channels contributed to the development of different tumors [31-33]. TRPV4 regulated angiogenesis and vascular growth in malignancies, human phase I research has also investigated at the TRPV6 calcium channel inhibitor SOR-C13 in patients with advanced solid tumors of epithelial origin [33,34]. By preventing tumor cell proliferation, overexpression of the TRPV1 ion channel encouraged the utilization of cisplatin in the therapy of Esophageal Squamous Cell Carcinoma (ESCC) [35]. TRPC channels are clustered as tetramers around a single central ionophore, and the pore is non-selectively permeable to cations and gated. Different TRPC and TRPV subtypes have emerged as significant regulators of vascular tone and blood flow pressure in the TRP family [36]. Variety of human diseases could be caused by changes in expression and function of TRPM subfamilies, making them a promising target for novel therapeutic, drug-design, and diagnostic approaches [37]. For people with solid tumors, the TRPM8 activator, D-3263, might be a good choice because it could induce apoptosis in cancer cells [20].

Consequently, the investigation of TRP gene family is crucial for both fundamental research and drug discovery, and the results on TRP gene family could undoubtedly have a major effect on public health, especially malignancies. In our study, we analyzed the expression of TRP gene family and their prognostic significance in KIRC, we anticipated to lay the foundation for further research into and practical application of TRP gene family as a potential biomarker for the diagnosis and prognosis of KIRC.

MATERIALS AND METHODS

Correlation analysis of the TRP gene family in KIRC

We downloaded the clinical information of 541 patients from the TCGA database (https://portal.gdc.com). The median expression level of TRP family gene family was selected as the cut-off value of the KIRC dichotomy so that each patient was divided into high-expression group and low-expression group. We further investigated these factors by univariate and multivariate Cox regression analyses to find 7 related genes. The univariate and multivariate Cox regression analysis were used to show the p value, Hazard Ratio (HR), and 95% CI of each variable through the “forestplot” R package. All analysis methods and the R package were implemented by R version 4.2.1 the survival outcomes included the Overall Survival (OS), Progression-Free Survival (PFS), and Disease-Specific Survival (DSS).

Construction of prognostic signature

We enrolled the 7 genes into the Univariate Cox regression analysis with Least Absolute Shrinkage and Selection Operator (LASSO) algorithm. Based on these genes, the risk score formula was established as follows: Risk core=(-0.2716*expression of TRPM3)+(0.6982* expression of TRPV3)+(-0.0745*expression of TRPM7)+(-0.1054*_x0002_expression of TRPA1)+(-0.1068*expression of TRPV4)+(-0.0138*expression of TRPM8)+(0.2481*expression of TRPM2). A 6-gene prognostic signature was screened based on the minimum criteria.

To evaluate the predictive efficiency, we calculated the Receiver Operating Characteristic (ROC) curve and COX analysis by using Kaplan-Meier analysis. Kaplan–Meier Plotter (http://kmplot.com) is an online database of cancer

clinical gene chips published online, which is constructed based on the gene chip and high-throughput expression profile from public databases. We used the Kaplan–Meier plotter to analyze the prognostic correlation of NEK expression levels in BC. The t-SNE analysis were performed to illustrate the patients in different risk groups by using the "Rstne" R package.

Mutation profiles of the TRP gene family

The method "Wil-cox. Test" was applied to analyze the differential TRPM3, TRPV3, TRPM7, TRPA1, TRPV4 and TRPM2 expression in KIRC. "*", "**", "***", indicate P value<0.05, <0.01, <0.001, respectively. The box plot was further designed using the R-package "ggpubr". Genomic Alterations of TRP gene family in KIRC and we used the cBioPortal (http://www.cbioportal.org/) database to assess the spectrum and types of gene mutations in the TRP gene family.

Survival analysis of TRP gene family

In an effort to further explore the relationship about the expression level of TRP gene family with survival probability in KIRC patients, we used Kaplan–Meier Plotter tool to analyze the OS. Kaplan–Meier Plotter (http://kmplot.com) is an online database of cancer clinical gene chips published online, which is constructed based on the gene chip and high-throughput expression profile from public databases.

The expression of TRP gene family in different tumor grades

The RNAseq data and corresponding clinical information for KIRC were obtained from the TCGA database (https://portal.gdc.com). We envisaged whether the transcriptional level of the TRP gene family was connected to KIRC grades and TNM stages according to the World Health Organization (WHO) from TCGA database.

Evaluating the prognostic value of the gene signature

Receiver Operating Characteristic (ROC) curve was conducted to assess the predictive performance of the signature model by using "pROC version 1.18.0" and "ggplot2 version 3.3.6" in R version 4.2.1. Furthermore, we performed a time dependent ROC curve on survival data and calculated the Area Under the Curve (AUC) by using "timeROC version 0.4" and "ggplot2 version 3.3.6" in R version 4.2.1. We also visualized AUC of TRPM3, TRPV3, TRPM7, TRPA1, TRPV4, TRPM2 among 1-5 years and found that the prognosis of TRPV3 had the highest ROC and AUC results, so we chose TRPV3 for further research.

Correlation between TRPV3 and clinicopathology of KIRC

The high and low expression profiles of TRPV3 in 541 KIRC patients with clinical information was downloaded from RNAseq data in TCGA-KIRC in TCGA datasets (https://portal.gdc.cancer.gov) through the R package "stats".

Genes related to differential expression of TRPV3

In order to search for differential genes of TRPV3, we downloaded parameters from RNAseq data in TCGA-KIRC in TCGA datasets (https://portal.gdc.cancer.gov) and visualized with volcanic map by using "ggplot2 version 3.3.6" in R version 4.2.1. GSEA was performed to investigate the potential biological pathways that TRPV3 may be involved in KIRC. The GSEA enrichment analysis of this signature were mapped by using R packages "clusterProfiler version 4.4.4"and "ggplot2 version 3.3.6". The data came from MSigDB Collections.

Predicted functions and pathways of TRPV3

Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis and Gene Set Enrichment Analysis (GSEA) were performed based on the TRPV3 interacted genes. To deeply explore the molecular biological mechanism of TRPV3 in KIRC initiation and progression, we obtained selected each of 20 genes and applied GO, including Molecular Function (MF), Cell Component (CC) and Biological Process (BP), and KEGG enrichment analysis. R version 4.2.1 and ggplot2 version 3.3.6 were used for the statistical analysis and Bubble

diagrams visualization. The data were RNAseq data from TCGA-KIRC database in TCGA (https://portal.gdc.cancer.gov). After that, we screened out each of 20 genes that expressed positive correlation or negative correlation with TRPV3 and showed them with heatmap. Data processing and result visualization used R package "ggplot2 version 3.3.6".

RESULTS

The connection between the TRP gene family and the prognosis of KIRC

Univariate and multivariate Cox analysis were employed to analyze the association between the TRP gene family and KIRC prognosis (Table 1). The whole dataset containing survival information was randomly divided into the training set and the validation set in a 1:1 ratio. The univariate Cox regression analysis results showed that 7 genes have a statistically significant relationship with OS. Among them, The HR values of TRPV3 (HR (95% CI)=2.167 (1.636–2.869), P<0.001), TRPM8 (HR (95% CI)=1.396 (1.149–1.696), P=0.017), TRPM2 [HR (95%CI)=1.300 (1.091–1.549), P=0.003) were all higher than 1, indicating that the high expression of TRPV3, TRPM8 and TRPM2 genes was a risk factor for KIRC compared with the low expression group. However, the HR values of TRPM3 (HR (95%CI)=0.667 (0.579–0.767), p<0.001), TRPM7(HR (95%CI)=0.744 (0.618–0.897), P=0.002), TRPA1 (HR (95%CI)=0.697 (0.571–0.851), p<0.001) and TRPV4 (HR (95%CI)=0.774 (0.690–0.869), P<0.001) were all less than 1, suggesting that the high expression of TRPM3, TRPM7, TRPA1 and TRPV4 genes could be the protective factor of KIRC (Table 1).

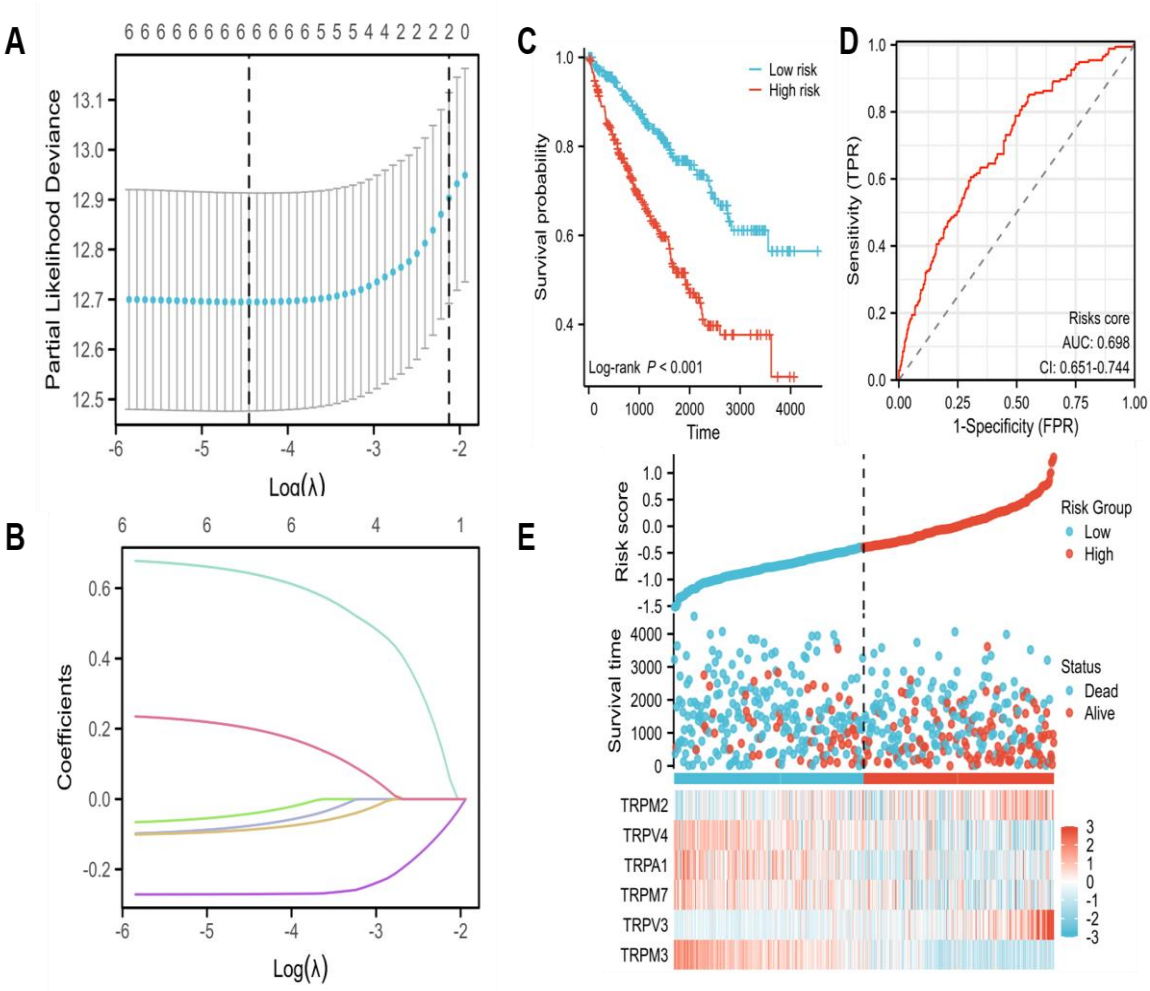
Table 1. Univariate and multivariate analysis of TRP in renal cancer.

Characteristics	Total(N)	Univariate analysis		Multivariate analysis	
		HR (95% CI)	P value	HR (95% CI)	P value
TRPV3	541	2.167 (1.636 - 2.869)	< 0.001	1.846 (1.295-2.631)	<0.001
TRPA1	541	0.697 (0.571 - 0.851)	< 0.001	0.811 (0.605-1.087)	0.162
TRPV4	541	0.774 (0.690 - 0.869)	< 0.001	0.908 (0.788-1.047)	0.185
TRPM2	541	1.300 (1.091 - 1.549)	0.003	1.311 (1.099-1.565)	0.003
TRPM3	541	0.667 (0.579 - 0.767)	< 0.001	0.739 (0.620-0.881)	<0.001
TRPM7	541	0.744 (0.618 - 0.897)	0.002	1.009 (0.779-1.305)	0.948
TRPM8	541	1.396 (1.149 - 1.696)	< 0.001	1.006 (0.782-1.295)	0.96

Subsequently, six significant genes were identified, followed by LASSO regression analysis of the TRP family to improve the prognostic ability of the model. Combining the results of Figures 1A and 1B , it was considered that the model fit the best when the penalty coefficient was 7, and the corresponding seven genes were selected into the model, which were TRPM3, TRPV3, TRPM7, TRPA1, TRPV4, TRPM8 and TRPM2. Furthermore, multivariate Cox regression analysis manifested that TRPM3 (HR (95%CI)=0.739 (0.620–0.881), P<0.001) and TRPV3[HR (95%CI) =1.846 (1.295–2.631), P<0.001) were considerably correlated with the prognosis of KIRC. Moreover, the corresponding regression coefficients were obtained, β1-β6, which was -0.2716, 0.6982, -0.0745, -0.1054, -0.1068, -0.0138, 0.2481. The risk score formula was established as follows: Riskscore=(-0.2716*expression of TRPM3) + (0.6982* expression of TRPV3)+(-0.0745*expression of TRPM7)+(-0.1054* expression of TRPA1)+(-0.1068*expression of TRPV4)+(-0.0138*expression of TRPM8)+(0.2481*expression of TRPM2). Moreover, according to the above formula, the risk score of each KIRC patient was directly calculated. And then, the patients were divided into high-risk group and low-risk group, which were grouped according to the median and interquartile range (M(IRQ)=-0.402). The results of the KM curve showed that the prognosis of the high-risk group was worse than that of the low-risk groups (Figure 1C, log-rank P<0.001; HR=2.483, 95% CI =1.844-3.344). ROC curves were

applied to assess the accuracy of established models for predicting OS in patients with KIRC. As shown in Figure 1D, the AUC values of 1 year were 0.698, representing the robustness and accuracy of the model in predicting patient prognosis as shown in Figures 1A-1E.

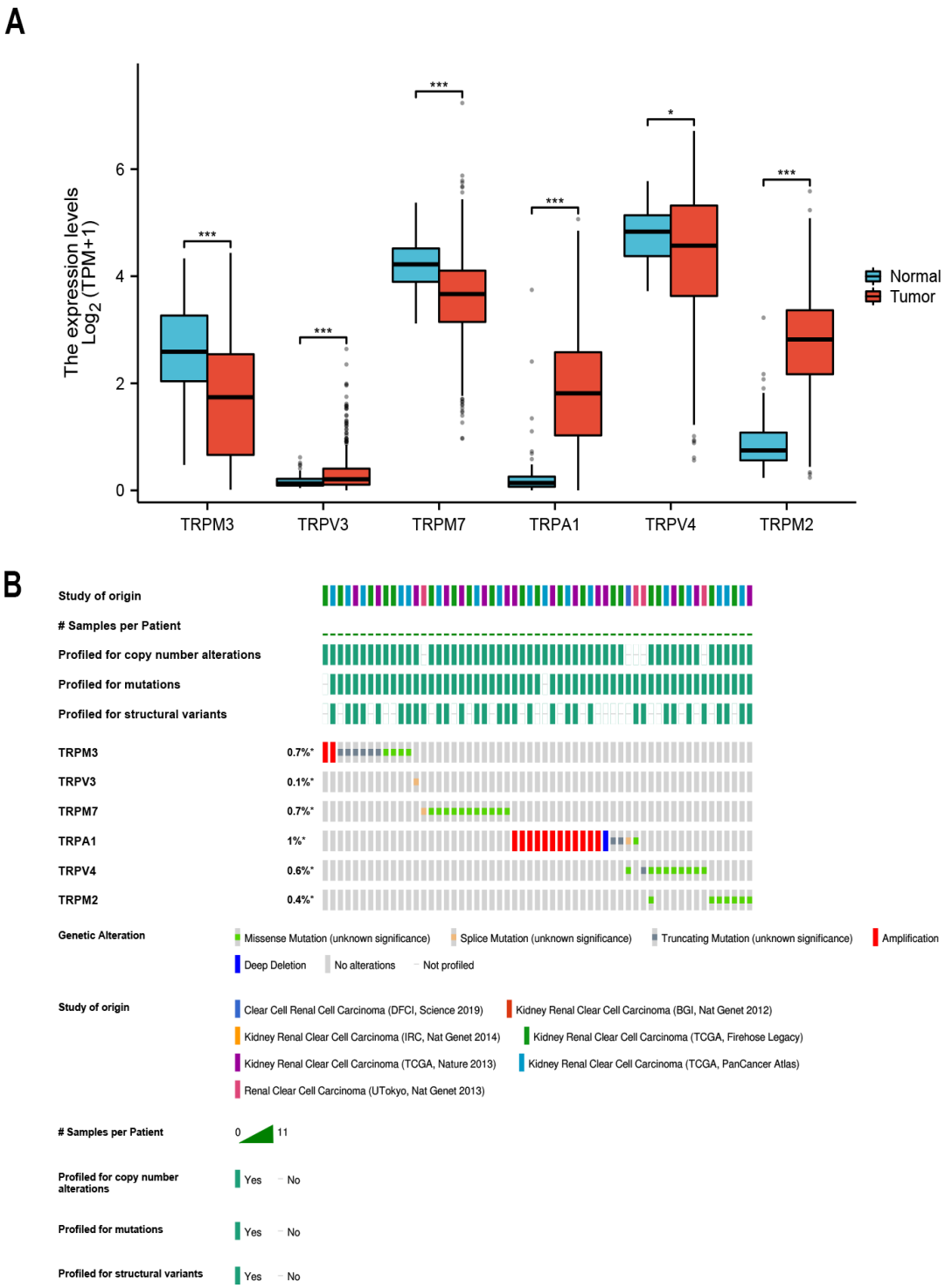
Figure 1. Selection of TRP gene family associated with the survival of KIRC by Lasso regression analysis. A) Cross-validation for selecting risk genes for the LASSO model. The left vertical line represents the 6 genes finally identified; B) Lasso coefficient profiles of the 6 risk genes from Lasso model. The lines stand for coefficient of Lasso; C) The KM analysis of different expressions of risk score; D) The ROC curve of different expressions of risk score; E) Heatmap result of six genes expressed in risk score.



mRNA expressions of TRP gene family in patients with KIRC

To investigate the expression level of distinct TRP gene family members in patients with KIRC, mRNA expressions were evaluated through the Wil-cox. Test. The results demonstrated that the mRNA expression levels of TRPV3, TRPA1, TRPV4 and TRPM2 were up-regulated in KIRC tissues (Figure 2A), while it is noteworthy that the mRNA expression level of TRPM3 was down-regulated in tumor tissues. The mRNA expression of TRPM7 had no difference between KIRC and normal kidney tissues. Besides, the cBioPortal database was utilized to examine the frequency and types of gene mutation in the TRP gene family (Figure 2B). The data revealed that TRPA1 had amplification with 1% in KIRC, while other genes mutated with missense mutation. Furthermore, we have examined the mutation rates of various genes. The results showed that the mutation rates of TRPM3, TRPV3, TRPM7, TRPA1, TRPV4, and TRPM2 were 0.7%, 0.1%, 0.7%, 1%, 0.9%, and 0.4%, respectively as shown in Figures 2A and 2B.

Figure 2. The expression status of TRP gene family in KIRC. A) Boxplots of 6 selected genes in KIRC; B) The mutation frequency plot of TRP gene family in the cBioPortal database. **Note:** *indicating P<0.05; **, P<0.01;***, P<0.001.

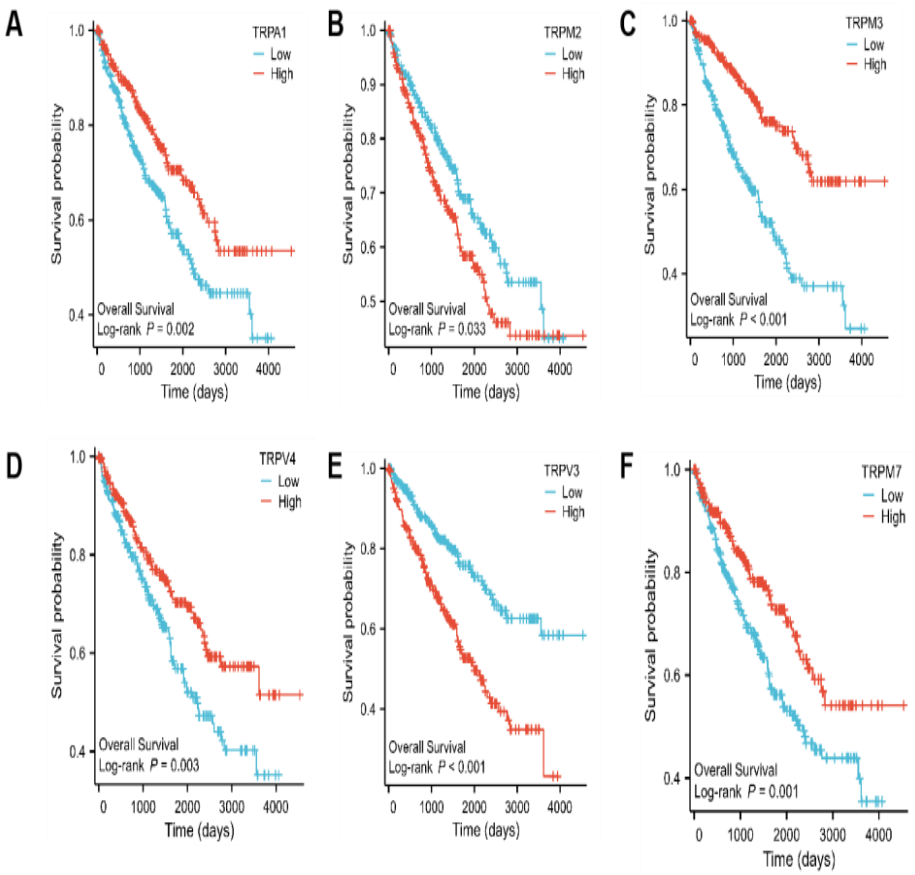


The relationship between TRP gene family and the prognosis of KIRC patients

The association between the expression of TRP gene family and the prognosis of KIRC patients was depicted by the Kaplan-Meier curve. As illustrated in Figure 3, in TRPM2 (P=0.033) and TRPV3 (P<0.001) (Figures 3B and 3E), the

prognosis of the high expression group was worse than that of the low expression group, which was statistically significant. Notably, in TRPA1 (P=0.002), TRPM3 (P<0.001), TRPV4 (P=0.003) and TRPM7 (P=0.001) (Figures 3A-3F), the high expression group had a good prognosis, and the difference was statistically significant as well as shown in Figures 3A-3F.

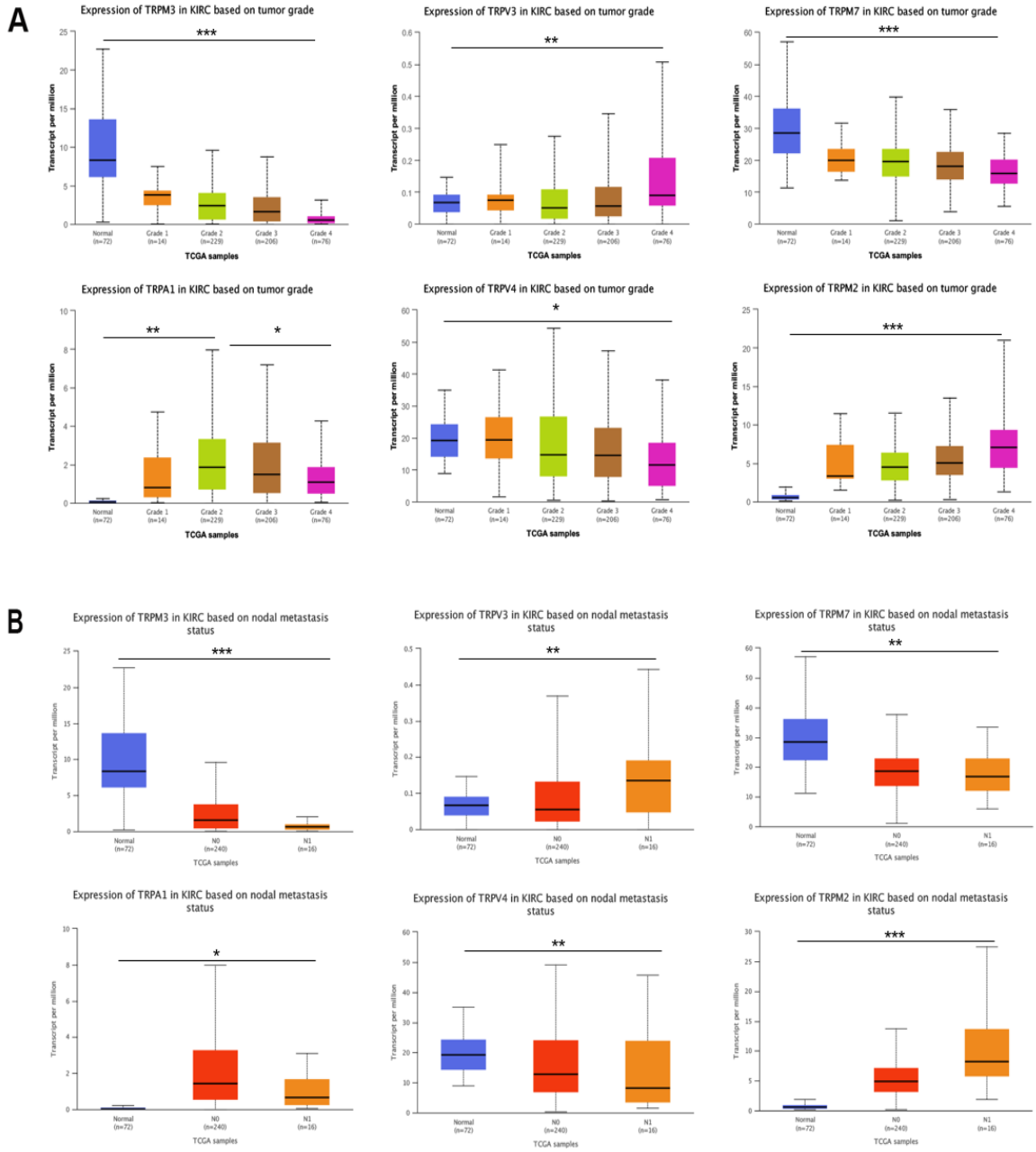
Figure 3. The overall survival of 6 genes in KIRC. A) KM curve for KIRC patients based on TRPA1 expression; B) KM curve for KIRC patients based on TRPM1 expression; C) KM curve for KIRC patients based on TRPM3 expression; D) KM curve for KIRC patients based on TRPV4 expression; E) KM curve for KIRC patients based on TRPV3 expression; F) KM curve for KIRC patients based on TRPM7 expression.



The expression of TRP gene family between different clinical characteristics

After a comprehensive expression analysis of each TRP gene family member, the relationships between the expression of TRP members and tumor grade and nodal metastasis in KIRC were further investigated *via* the UALCAN database. According to Figure 4, the mRNA levels of TRPM3, TRPV3, TRPM7, TRPA1, TRPV4, TRPM2 were all bound to tumor grades. In general, the mRNA expressions of TRPV3 and TRPM2 have been found higher as tumor grade increased, whereas the mRNA expression of TRPM3, TRPM7, TPRV4 tended to be lower with increasing tumor grade. The highest mRNA levels of TRPA1 were observed in tumor grade III. Additionally, the connection between TRP gene family and nodal metastasis status displayed that TRPV3 and TRPM2 were usually highly expressed in patients with lymph node metastasis, while TRPM3, TRPM7, TRPA1,TRPV4 were usually low expressed in patients with lymph node metastasis (Figure 4B).

Figure 4. Correlation between expression of TRP gene family and clinicopathology in KIRC patients. A) Correlation of 6 TRP gene family and cancer grade; B) Correlation of 6 TRP gene family and nodal metastasis. **Note:** *, indicating P<0.05; **, P<0.01; ***, P<0.001.



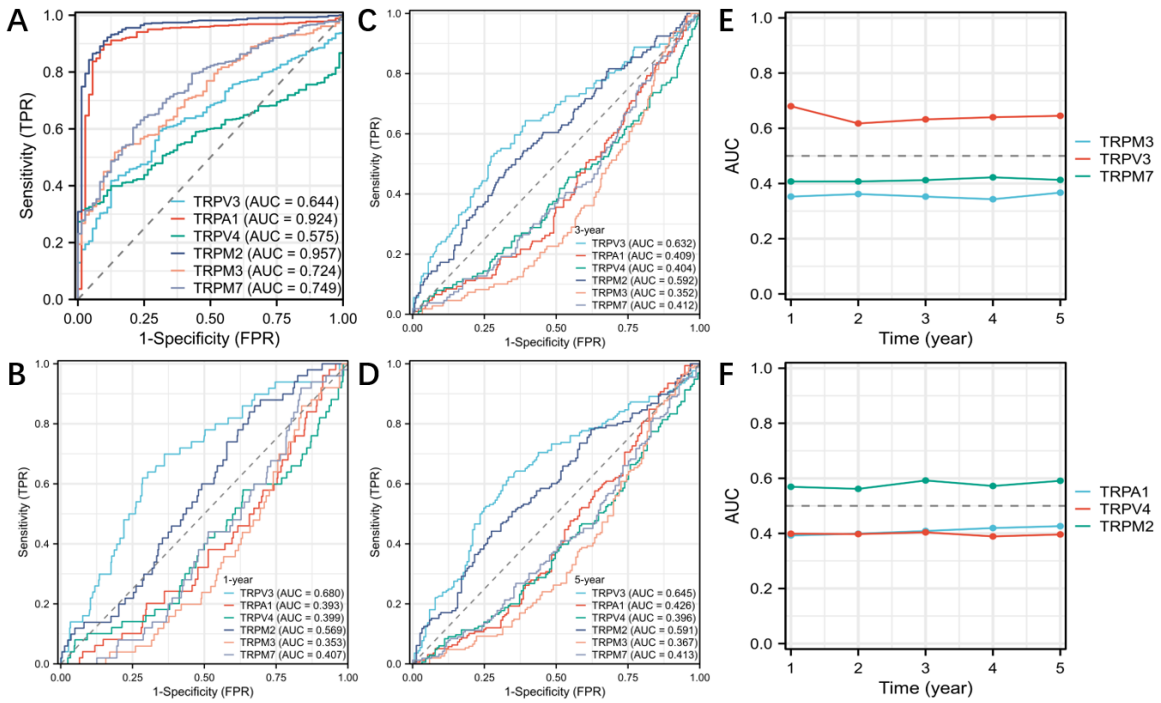
Predictive Role of TRP gene family in risk group

To evaluate the diagnostic value of these 6 genes, we constructed a ROC curve by plotting the sensitivity and specificity of the gene expression levels in distinguishing diseased and healthy individuals. The AUC of the ROC curve could be a measurement of diagnostic accuracy, with higher values meaning better performance (Figure 5A). Similarly, the prognostic performance of 6 genes could also be determined using ROC curves, where the gene expressions were plotted against the probability of survival or disease progression at different time points, such as 1, 3, and 5 years. The AUC of these curves could indicate the predictive power of genes expression levels for long-term outcomes. The results from Figures 5B-5D indicated that TRPV3 had the highest AUC values in predicting patient prognosis at three years. Specifically, the AUC values for 1, 3, and 5 years were 0.632, 0.660, and 0.645,

respectively. What's more, the AUC curve displayed in the Figures 5E and 5F, and the results reflected the predictive accuracy of TRPV3 in terms of the AUC. The X-axis represented the time point (1 to 5 years), while the Y-axis denoted the AUC values. The graph suggested that TRPV3 had the highest AUC values at all-time points, revealing that it could be a robust predictor of patient prognosis.

The highest total area under the curve was found for RNF7 (AUC=75 %, CI=0.705-0.800), which indicates that RNF7 has a good ability to discriminate correctly between tumor and non-tumor samples. B-L. The ROC curve of the RNF7 expression with clinical pathology (age, pathologic T, N, M, and tumor stage as well as the higher grade) and all of the AUC value is significant. ROC, receiver operating characteristic; AUC, area under the curve.

Figure 5. ROC curves and AUC analysis to test the validity of 6 TRP family genes in discriminating tumor and predicting prognosis in the TCGA cohorts. A) The ROC curve of 6 TRP family genes; B) One-year prognostic ROC curve of 6 genes; C) Three-year prognostic ROC curve of 6 genes; D) Five-year prognostic ROC curve of 6 genes; E) The AUC curve of TRPM3, TRPV3 and TRPM7; F) The AUC curve of TRPA1, TRPV4 and TRPM2.



Correlation between TRPV3 expression and clinicopathology of KIRC

Based on the above discussion, TRPV3 was considered to have the most clinical value, so further study was undertaken. The correlation between TRPV3 expression and clinicopathology of KIRC were analyzed through the R package "stats". A total of 7 clinically relevant indicators were explored, including Pathologic T/N/M stage, pathologic stage I, II, III, IV, histologic grade 1, 2, 3, 4, and primary therapy outcome Progressive Disease (PD), Stable Disease (SD), Partial Response (PR), Complete Response (CR) and OS event (alive or dead). Expression data and clinical data of KIRC patients were downloaded from the TCGA database. Sort 541 patients by TRPV3 expression level, and divide them into two groups based on the median value-low and high TRPV3 expression groups. In addition, there were some patients with incomplete clinical information, which was excluded during the analysis. Finally, the clinicopathological correlation analysis Table 2 was obtained. The findings proved that except for the primary therapy outcome (P=0.457) and N stage(P=0.218), other indicators had statistical significance (P <0.05). Compared with the T1 and T2 group, TRPV3 expression level was higher in T3 and T4 group (P<0.001).

Similarly, compared with the control group, TRPV3 expression level was higher in N1, M1, Stage III and Stage IV, G3-G4, Dead. These results suggested the higher level of clinical correlation index, the higher expression of TRPV3 in contrast with the lower grade group, and TRPV3 could be associated with clinical progression and metastasis of tumors.

Table 2. Correlation between TRPV3 expression and clinicopathology of KIRC.

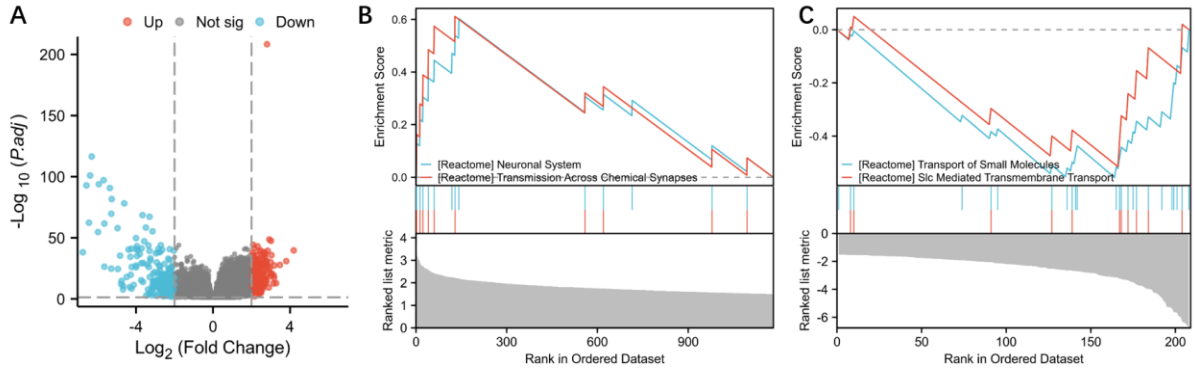
Characteristics	Low expression of TRPV3	High expression of TRPV3	P value
n	270	271	
Pathologic T stage, n (%)			0.003
T1 and T2	191 (35.3%)	159 (29.4%)	
T3 and T4	79 (14.6%)	112 (20.7%)	
Pathologic N stage, n (%)			0.218
N0	114 (44.2%)	128 (49.6%)	
N1	5 (1.9%)	11 (4.3%)	
Pathologic M stage, n (%)			0.027
M0	221 (43.5%)	208 (40.9%)	
M1	30 (5.9%)	49 (9.6%)	
Pathologic stage, n (%)			0.003
Stage I and Stage II	182 (33.8%)	150 (27.9%)	
Stage IV and Stage III	86 (16%)	120 (22.3%)	
Histologic grade, n (%)			0.043
G1 and G2	135 (25.3%)	115 (21.6%)	
G3 and G4	128 (24%)	155 (29.1%)	
Primary therapy outcome, n (%)			0.218
PD	4 (2.7%)	7 (4.8%)	
SD and PR and CR	82 (55.8%)	54 (36.7%)	
OS event, n (%)			<0.001
Alive	210 (38.8%)	156 (28.8%)	
Dead	60 (11.1%)	115 (21.3%)	

Analysis of volcano plot and GSEA curve for TRPV3 related differential genes

Results from the AUC and ROC analyses enabled the selection of TRPV3 for subsequent investigation. The volcano plot in Figure 6A depicted the differential expression of genes in two groups, one with high expression of TRPV3 and the other with low expression. The x-axis represented the log2 fold change between the two groups, while the y-axis meant the statistical significance of the -log10 p-value. Genes with higher log2 fold change and lower P-value were considered to be more differentially expressed. In our study, we found that TRPV3 itself showed significantly differential expression, with absolute value a log2 fold change of 1.5 and a P-value of 0.05. Furthermore, GSEA plot were selected to enrich these differential genes. We applied the GSEA tool to identify pathways or processes that were enriched in the high TRPV3 expression group in comparison with the low TRPV3 expression group (Figures 6B and 6C). The y-axis represented the enrichment score, reflecting the degree to a gene set, which was overrepresented at the top or bottom of the ranked list of genes. The x-axis indicated the position of the gene set in the ranked list. In our analysis, we found that gene sets related to neuronal system and transmission across chemical-synapses were significantly enriched in the high TRPV3 expression group, meanwhile, the gene sets involved in small molecules transport and SLC mediated trans membrane transport were linked with the low TRPV3

expression group. Based on the outcomes, TRPV3 could potentially be implicated in pathways related to neurotransmitter transmission, neuronal development, or neuroprotection, given its role in the nervous system and the enrichment of neuronal gene sets in the high TRPV3 expression group. Furthermore, the predominance of gene sets pertaining to transmembrane transport and small molecule transport, mediated by SLC, in the group with low TRPV3 expression reinforced the possibility that TRPV3 plays a role in controlling intracellular substance transport and metabolism.

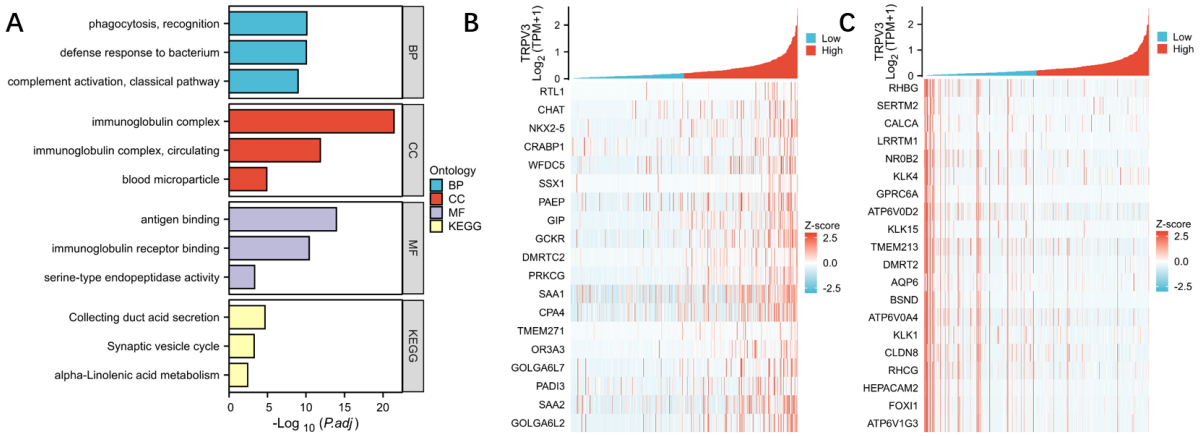
Figure 6. GSEA enrichment analysis of TRPV3 differential genes. A) The volcanic map of TRPV3 differential gene; B) GSEA analysis of TRPV3 differentially expressed up-regulated genes; C) GSEA analysis of TRPV3 differentially expressed down-regulated genes.



Functional enrichment analysis of TRPV3 related differential genes

The differentially expressed genes of TRPV3 were subjected to GO and KEGG pathway enrichment analysis. The results revealed enriched biological functions and pathways associated with the differentially expressed genes, providing insights into their potential roles in TRPV3 function. The KEGG enrichment analysis of the dataset indicates that genes involved in duct acid secretion, synaptic vesicle cycle, and alpha-linolenic acid metabolism are over-represented (Figure 7A). Kidney acid-base balance and fluid regulation would be affected by collecting duct acid secretion, whereas synaptic vesicle cycle was essential for information transmission between neurons, and alpha-linolenic acid metabolism was one of the essential fatty acids in the human body, with multiple physiological functions. Consequently, the TRPV3 gene could be responsible for a wide variety of biological activities, as suggested by its KEGG enrichment values, including kidney function, neuronal communication, and fatty acid metabolism. Furthermore, we selected the top 20 genes with the highest absolute values among differentially expressed genes in TRPV3 and conducted a heatmap analysis for each of them. Figure 7B provided the top 20 genes with a positive relationship with TRPV3, and Figure 7C highlighted the top 20 genes with a negative correlation with TRPV3. TRPV3 was tightly connected to these genes, implying that studying TRPV3's mechanism in depth might throw light on these genes.

Figure 7. RNF7-related gene enrichment analyses. A) GO and KEGG analyses of TRPV3 -differential genes in renal cancer; B) The heat map of the top 20 genes positively associated with TRPV3; C) The heat map of the top 20 genes negatively associated with TRPV3.



DISCUSSION

Researching biomarkers for early diagnosis and targeted treatment of cancer could be greatly aided by multiomics bioinformatics analysis, shedding light on the underlying molecular abnormalities of different cancers [38,39]. Therefore, in this study, we focused on the role of the TRP gene family in KIRC. The results showed that TRPM3, TRPV3, TRPA1, TRPV4, TRPM7, TRPM2 had significant statistical significance in univariate cox regression. Among them, TRPV3, TRPM7 and TRPM2 could be regarded as prognostic risk factors. Next, Lasso regression results indicated that the survival time of the high-risk group was shorter than that of the low-risk group. Furthermore, TRPV3 and TRPM2 would be the key molecules for poor prognosis of KIRC. In mRNA expression concept, it was found that TRPV4, TRPA1, TRPV4 and TRPM2 were highly expressed in tumor tissues, while TRPM3 was low expressed in tumor tissues. In addition, we also found that high expression of TRPM2 and TRPV3 were significantly correlated with poor OS of KIRC. However, the high expression of TRPA1, TRPV4, TRPM3 and TRPM7 was significantly correlated with better OS of KIRC. Taken together, these findings suggested that the TRP gene family could be a useful predictor of cancer prognosis, especially TRPM2 and TRPV3, which have practical applications. However, no studies have been conducted on mutations in the TRP gene family in human cancers. Using the cBioPortal database, we found that amplification is the most common alteration of TRP gene family in KIRC. In TRP gene family variant group, we firstly presented evidence of a potential association between the expression of TRP gene family and grade and nodal metastasis. The results revealed that the high expression of TRPV3 and TRPM2 in KIRC was closely related to the stage of tumor. The expressions of TRPM3, TRPM7 and TPRV4 were negatively correlated with tumor staging. Further analysis showed that the expression of TRPV3 and TRPM2 had positive correlation with nodal metastasis. Based on these results, we suggested the expression of TRPV3 and TRPM2 could influence tumor clinical progression in cancer patients, which would contribute to a better understanding of the mechanisms of tumor metastasis therapy. Depending on the preceding evaluation, TRPV3 has been chosen for further investigation because it had the highest potential for therapeutic consequences. After the clinic pathological correlation between TRPV3 and KIRIC being analyzed. We observed that the expression of TRPV3 was associated with pathologic T/M stage, Stage I, II, III, IV, Histologic grade I, II, III, IV, OS event. Furthermore, TRPV3 expression was increased in the high-grade than the low-grade cohort, suggesting that TRPV3 could be involved in the clinical

progression of KIRC tumors. Moreover, we realized that genes positively related to TRPV3 are highly represented in the nervous system and chemical-synapse transmission, while TRPV3 negatively associated gene enrichment in transport of small molecules and SLC mediated trans membrane transport.

TRP channel is a non-selective cationic channel with high permeability to calcium previously, the TRP family has been extensively studied in the nervous system and is involved in a variety of physiological processes including pain, temperature perception, intracellular Ca^{2+} homeostasis and signal transduction [40,41]. In recent years, more and more studies have reported that TRP gene family contributed to the development, proliferation, migration of tumors and was closely profoundly linked to the poor prognosis of tumors, including Colorectal Cancer cells, cervical squamous cell carcinoma, etc [13,42-45]. This is consistent with our results. However, there is no further in-depth study on the molecular mechanism in this study and no animal experiment to support it. Further experiments will be conducted in the future to clarify the molecular mechanism.

CONCLUSION

Our results revealed TRP gene family could be used as an essential prognostic marker in tumor clinical practice, as its abnormal expression is closely linked to the occurrence, development, and prognosis of KIRC. Additionally, an abnormally high expression of TRPV3 has a strong connection with a poor prognosis and could shorten the survival time of patients and lead to the cancer progression. These results suggested that the function and regulatory mechanisms of TRPV3 in the nervous system are complex and may be associated with various biological processes.

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AUTHOR CONTRIBUTIONS

Conceptualization: J.W. (Jiaxin Wang) and X.Z.; Methodology: X.Z. and R.X.; Software: Q.Z. and Z.L.; Writing—original draft: J.W. (Jiaxin Wang) and X.Z. (Xin Zhang); Writing—review & editing: R.X., L.N. (Lei Niu); Visualization: G.H.; Supervision: G.H. and N.L. All authors have read and agreed to the published version of the manuscript.

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INSTITUTIONAL REVIEW BOARD STATEMENT

Not applicable.

INFORMED CONSENT STATEMENT

Not applicable.

DATA AVAILABILITY STATEMENT

The data which support the findings of our study are available from the UALCAN (http://ualcan.path.uab.edu/analysisprot.html), GEPIA2 (http://gepia2.cancer-pku.cn/), CPTAC (http://ualcan.path.uab.edu/analysisprot.html), cBioPortal (http://www.cbioportal.org/), GeneMANIA website (https://genemania.org/), Kaplan–Meier Plotter (http://kmplot.com) and WebGestalt (http://www.webgestalt.org/), TCGA website (https://portal.gdc.cancer.gov).

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

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