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Original Article

Expression and prognostic significance of CRKL in clear cell renal cell carcinoma

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Abstract: Clear cell renal cell carcinoma (ccRCC) is an aggressive kidney malignancy with limited prognostic biomarkers. CRKL has been implicated in cancer progression, but its prognostic value and immune associations in ccRCC remain unclear. Using TCGA, GEO, and UALCAN databases, we analyzed CRKL expression. Kaplan-Meier and multivariate Cox regression assessed survival; TIMER and TISIDB evaluated immune infiltration; LinkedOmics databases and GSEA identified enriched pathways. CRKL was significantly overexpressed in ccRCC tissues at both mRNA and protein levels. Surprisingly, high CRKL expression was associated with better overall survival (OS), disease-specific survival (DSS), progression-free interval (PFI), and disease free survival (DFS). Multivariate Cox analysis identified CRKL as an independent favorable prognostic factor (OS: HR = 0.469, 95% CI: 0.302-0.729, P < 0.001). CRKL positively correlated with immune cell infiltration and immune-related pathways. In conclusion, CRKL is overexpressed in ccRCC, paradoxically, higher expression predicts better prognosis and is associated with immune-active tumor microenvironment features. These findings suggest that the favorable prognosis associated with CRKL may be attributable to its immunomodulatory role within the tumor microenvironment, highlighting its potential as a novel prognostic biomarker for ccRCC.

Keywords: CRKL, clear cell renal cell carcinoma (ccRCC), prognosis, immune infiltration, bioinformatics

Introduction

Renal cell carcinoma (RCC) represents a prevalent malignant neoplasm that arises from renal tubular epithelial cells, constituting approximately 3% of all human malignant tumors and about 85% of all renal neoplasms [1]. This type of cancer is characterized by a significant rate of recurrence and a mortality rate that can reach as high as 40% [2]. Among the four main types of RCCs, clear cell renal cell carcinoma (ccRCC) is the most common and aggressive subtype, accounting for about 80% of RCC, with extremely high local invasion, malignant neoplasm, mortality and resistance to chemotherapy and radiotherapy [3]. Despite advances in targeted therapy and immunotherapy, the prognosis for patients with advanced ccRCC remains poor [4-7]. As a highly immune-infiltrated tumor [8], tumor-infiltrating immune cells (TICs) within the tumor microenvironment (TME) play a pivotal role in tumor assessment, assessing the responsiveness to clinical interventions, and affecting the survival rates of patients [9, 10]. Consequently, comprehensive investigations into the pathogenesis, novel molecular markers, and therapeutic targets for ccRCC hold substantial importance for enhancing its diagnosis and treatment strategies.

CRKL (v-crk sarcoma virus CT10 oncogene homologue (avian)-like), a member of CRK adapter protein family, is ubiquitously conserved across eukaryotic organisms [11]. It is composed by one N-terminal Src homology2 (SH2) domain, one N-terminal SH3 (SH3N) domain and one C-terminal SH3 (SH3C) domain [12, 13]. CRKL links to proline-rich proteins BCAR1, GAB, ABL-1, Pax, GEF, C3G, BCR-ABL and SOS to form timely and localized complexes critical

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to cell proliferation, adhesion and migration [14-16]. Research has highlighted the role of CRKL as a critical player in various malignancies, with its expression often correlating with poor prognosis [12, 17-21]. In our previous study, we found that CRKL was significantly overexpressed in ccRCC tissues compared with adjacent normal tissues, as confirmed by Western blot analysis of 28 clinical pairs. Moreover, functional assays demonstrated that CRKL overexpression promoted the migration and invasion of ccRCC 786-0 cells via the SOS1/MEK/ERK/MMP2/MMP9 pathway, while CRKL knockdown inhibited these abilities [22]. These findings suggested an oncogenic role of CRKL in ccRCC. However, the prognostic value of CRKL in ccRCC remained unclear, and the relationship between CRKL expression and the tumor immune microenvironment had not been explored.

In the present study, we aimed to systematically evaluate the expression, prognostic significance, immune correlations, and functional pathways of CRKL in ccRCC using multiple publicly available databases (TCGA, GTEx, GEO, UALCAN, TIMER, TISIDB, and LinkedOmics). Surprisingly, our analysis revealed a paradoxical finding: although CRKL is overexpressed and promotes migration/invasion in vitro, high CRKL expression correlated with better overall survival in ccRCC patients. This unexpected finding prompted us to further investigate the relationship between CRKL expression and tumor immune infiltration, as the tumor microenvironment (TME) plays a critical role in ccRCC progression and patient outcomes. Our findings provide new insights into the complex role of CRKL in ccRCC and highlights its potential as a novel prognostic biomarker.

Materials and methods

Acquisition and analysis of CRKL expression profiles in public databases

Utilizing the publicly accessible databases of TCGA (https://www.cancer.gov/) and GTEx (https://gtexportal.org/), a pan-cancer dataset was selected to analysis through the Xiantao Academic platform (https://www.xiantao.love/), which is an online bioinformatics analysis tool developed on the R programming framework. This dataset facilitated a comparative analysis of both unpaired and paired differential expression of CRKL across tumor and normal tissues from 33 distinct cancer types. Kidney renal clear cell carcinoma(KIRC) was selected as the target of this study. The differential expression levels of CRKL between tumor and normal tissues in KIRC were analyzed, including both paired and unpaired samples. To validate diagnostic performance, we additionally analyzed two independent GEO datasets (GSE53757 and GSE40435). The protein levels of CRKL in KIRC were then determined using UALCAN database (http://ualcan.path.uab.edu/) [23], an internet application that allows users to analyze TCGA and gene transcription data online. To provide independent experimental validation of these findings, we previously examined CRKL protein expression in 28 pairs of ccRCC and adjacent normal tissues using Western blot analysis, as detailed in our prior study [22]. Ethical approval was not required as the study used only secondary data from publicly available, de-identified sources.

Survival prognosis analysis of CRKL in ccRCC

First, we investigated how CRKL affected the overall survival (OS), disease-specific survival (DSS), Progress free interval (PFI) and disease free survival (DFS) of KIRC using online tools like Kaplan-Meier Plotter (http://kmplot.com/analysis/) and Xiantao Academic. We further looked into the relationships between CRKL expression and prognosis in distinct KIRC clinical subgroups. In this website, the median CRKL expression was used as a cutoff value to classify groups (low expression group: 0-50%; high expression group: 50-100%). *P* value less than 0.05 means statistical significance. Then, we used the GEPIA2.0 website (http://gepia2.cancerpku.cn/#index) to explore the association between CRKL expression and patient survival [24]. Next, we evaluated the diagnostic ability of CRKL for KIRC utilizing the receiver operating characteristic curve (ROC curve) of the Xiantao Academic web.

To rigorously assess the independent prognostic value of CRKL, we performed multivariate Cox regression analysis. The model included CRKL expression (dichotomized by median) and established clinical covariates: age (> 60 vs. ≤ 60), pathologic T stage (T1/T2 vs. T3/T4), N stage (N0 vs. N1), M stage (M0 vs. M1), over-

all pathologic stage, and histologic grade (G1/G2 vs. G3/G4). Hazard ratios (HRs) and 95% confidence intervals (CIs) were calculated. A two-sided *P*-value < 0.05 was considered statistically significant.

Relationships between CRKL expression and a variety of clinical traits in KIRC

We utilized the "Clinical" and "Subtype" modules of the Xiantao Academic website provided box plots for CRKL expression levels of individuals with various clinical features in KIRC. The RNA-seq data and linked clinical data were retrieved from the TCGA database. To detect two groups of data, the Shapiro-Wilk normality test, Kruskal-Wallis test, and Multiple hypothesis test (Dunn's test) with Bonferroni's method to adjust significant level were employed.

Immune infiltration analysis

TISIDB website (Tumor-Immune System Interactions and DrugBank, http://cis.hku.hk/TISIDB/) [25] and TIMER website (tumor immune estimation resource, http://timer.cistrome.org/) [26] were employed to elucidate the association between CRKL and immune infiltration in ccRCC. In TISIDB database, we selected the "Immunomodulator" module to analyze and evaluate the association between CRKL expression levels and the levels of immune checkpoint genes. TIMER estimates the abundance of eight tumor-infiltrating immune cell (TIIC) subsets, e.g., B cells, cancer associated fibroblast, $CD4^+$ T cells, $CD8^+$ T cells, dendritic cells, endothelial cell, macrophages, and neutrophils, in the ccRCC cohort (n = 533). It is important to note that these analyses are correlational and do not imply direct regulation.

LinkedOmics database analysis

The LinkedOmics database (http://www.linkedomics.org/admin.php) [27] serves as an online platform designed for the analysis of 32 multi-dimensional datasets associated with cancer from The Cancer Genome Atlas (TCGA). We selected the dataset "KIRC cohort", data type "RNAseq", and used Pearson's correlation test. The top 50 significant positively and negatively correlated genes with CRKL were shown in a heat map. Within LinkedOmics, the Function module conducts analyses of Gene Ontology (GO) biological processes (GO_BP), Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways, as well as Reactome pathway assessments, employing gene set enrichment analysis (GSEA).

Gene set enrichment analysis (GSEA)

To identify the biological pathways associated with CRKL expression, we performed GSEA comparing CRKL-high and CRKL-low groups. Patients were divided using the median expression level as the cutoff. The analysis utilized multiple gene set collections from the Molecular Signatures Database (MSigDB), including the Hallmark, KEGG, Reactome, PID, and WikiPathways (WP) gene sets. Pathways with a false discovery rate (FDR) < 0.05 were considered significantly enriched.

Statistical analysis

All statistical analyses were performed using R software and the Xiantao Academic platform. The Shapiro-Wilk test was used to assess normality. For comparisons between two groups, the Wilcoxon rank-sum test was applied. For multiple group comparisons, the Kruskal-Wallis test followed by Dunn's post-hoc test with Bonferroni correction was used. Kaplan-Meier survival curves were compared using the log-rank test. Multivariate Cox regression analysis was used to evaluate the independent prognostic value of CRKL, with results reported as hazard ratios (HR) and 95% confidence intervals (CI). Pearson correlation coefficients were used for co-expression analyses. All reported *P* values were two-sided, and a *P* value < 0.05 was considered statistically significant unless otherwise specified (e.g., FDR < 0.05 for GSEA).

Results

mRNA expression profiles of CRKL in tumor and normal tissues

The analysis of differential expression across multiple cancer types revealed that CRKL exhibited markedly elevated expression levels in tumor tissues compared to normal tissues, particularly evident in KIRC among various cancer classifications (**Figures 1A** and **2A**; *P*-value < 0.05). An examination of the KIRC dataset sourced from the TCGA database indicated

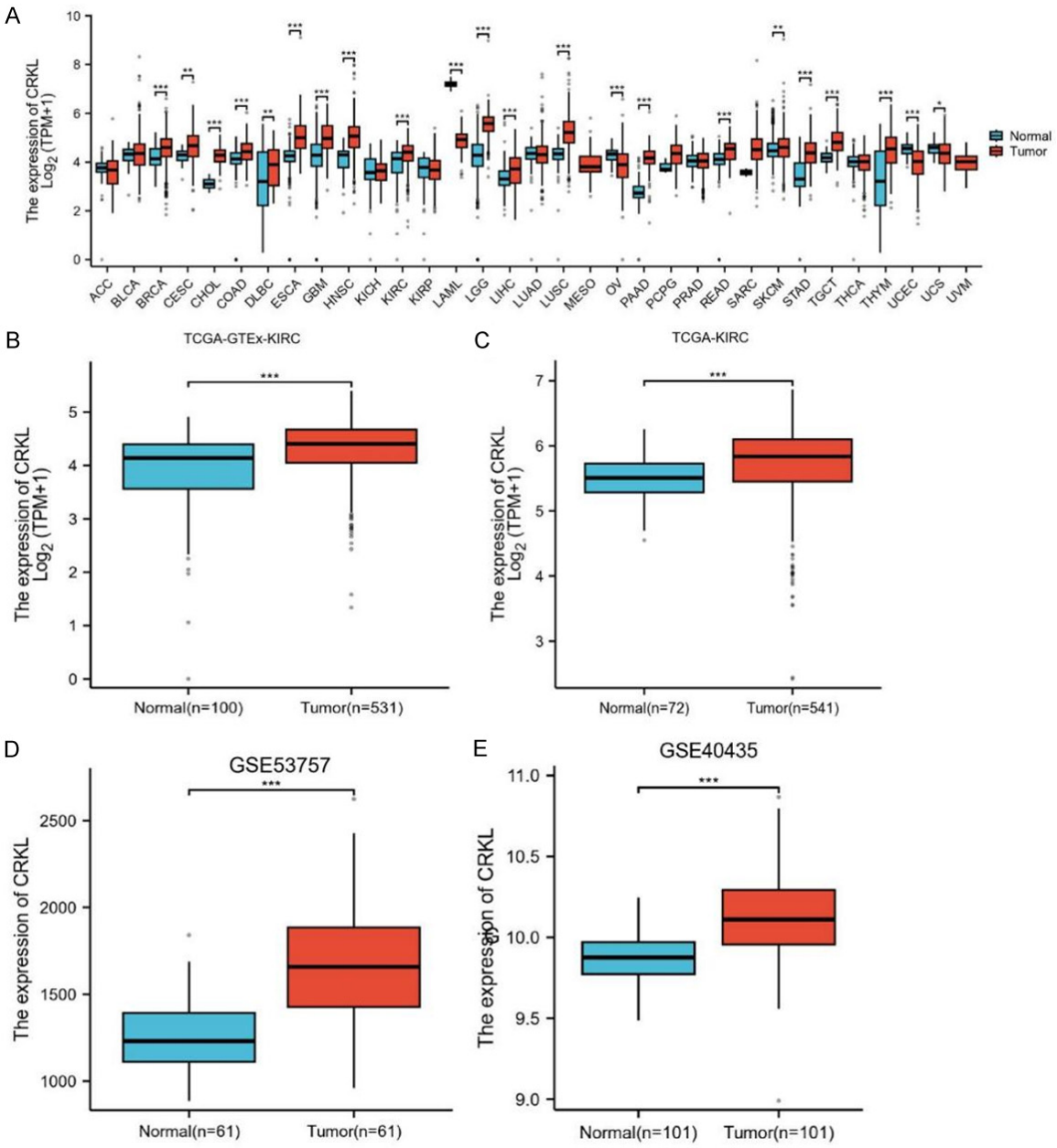

Figure 1. Analysis of CRKL mRNA expression in ccRCC. (A) Unpaired pan-cancer expression. (B, C) CRKL mRNA expression levels in ccRCC patients and matched adjacent normal samples in TCGA. (D, E) CRKL were upregulated in the ccRCC tissues compared to normal tissues in the GSE53757 (D) and GSE40435 (E) dataset. **P*-value < 0.05; ***P*-value < 0.01; ****P*-value < 0.001; ns: not significant.

that CRKL expression levels were markedly elevated in tumor tissues compared to normal tissues, as demonstrated by both unpaired and paired differential analyses (**Figures 1B**, **1C** and **2B**; *P*-value < 0.05). In order to enhance the validation of differential gene expression reliability, we downloaded the dataset GSE53757 and GSE40435 of ccRCC from the GEO database and analyzed the different level of CRKL in normal and tumor tissues, and the results showed that CRKL was highly expressed in tumor tissues compared with normal tissues (**Figures 1D**, **1E** and **2C**, **2D**; *P*-value < 0.05).

Protein expression profiles of CRKL in tumor and normal tissues

Then, we examined the protein-level expression pattern of CRKL in tumor and adjacent

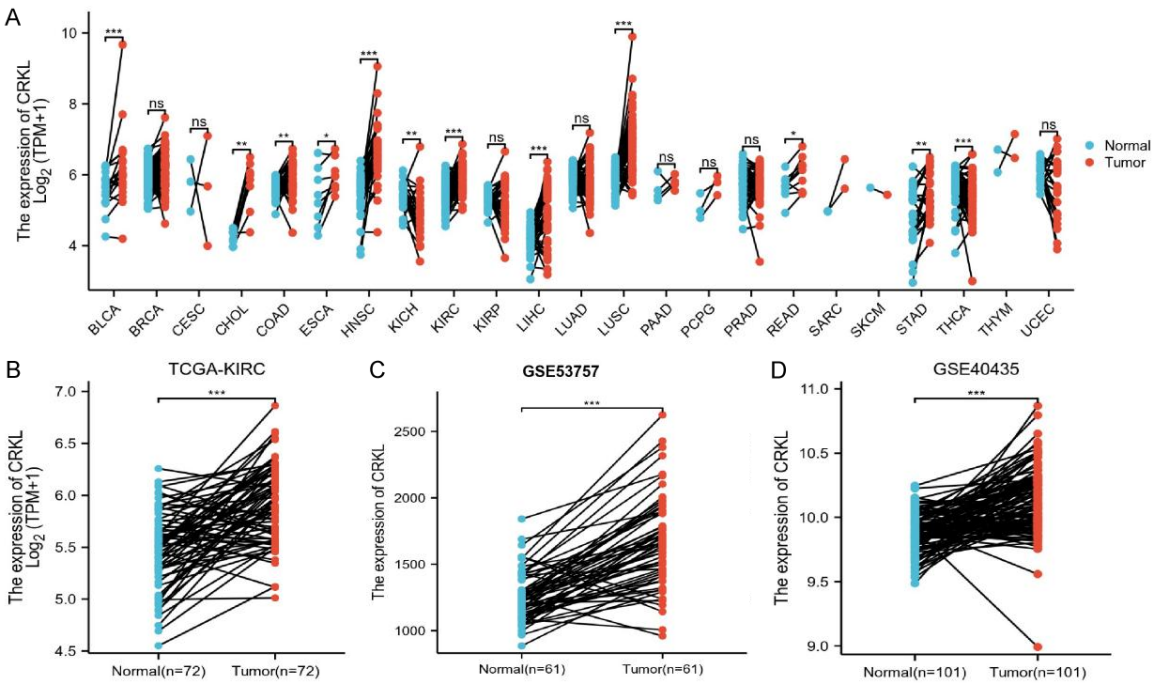

Figure 2. Analysis of CRKL mRNA expression in ccRCC. A. The image paired pan-cancer expression. B. The image paired CRKL mRNA expression levels in ccRCC patients in TCGA. C. The image paired CRKL mRNA expression levels in ccRCC patients in GSE53757. D. The image paired CRKL mRNA expression levels in ccRCC patients in GSE40435. **P*-value < 0.05; ***P*-value < 0.01; ****P*-value < 0.001; ns: not significant.

normal tissues at pan-cancer level. Results from the UALCAN database indicated that CRKL's protein expression was increased in ccRCC (**Figure 3A-C**), including two cluster samples, which was in line with the mRNA-level findings. It also was entirely consistent with our previous Western blot results in 28 clinical ccRCC pairs [22]. Analysis of CRKL expression with clinical parameters showed that its expression was significantly different across individual cancer stages and grades. Compared to normal controls, CRKL expression was significantly increased in cancer tissues regardless of gender, age, race, or weight (**Figure 4A-G**).

Association between CRKL expression and patient survival

The results of the survival analysis showed patients with high CRKL expression had better outcomes in terms of OS, DSS and PFI compared to the CRKL-low expression subgroup (**Figure 5A-C**). The results of the GEPIA2 survival analysis showed patients with high CRKL had a significantly longer survival time in terms of OS and DFS than those patients with low expression (**Figure 5D**, **5E**). In addition, the area under the curve (AUC) of CRKL from TCGA datasets was 0.75, demonstrating CRKL's excellent accuracy in predicting ccRCC and its positive diagnostic impact on this condition (**Figure 5F**).

To rigorously assess the independent prognostic value of CRKL, we performed multivariate Cox regression analysis incorporating established clinical factors, including pathologic T stage, N stage, M stage, overall pathologic stage, histologic grade, and age. The results demonstrated that CRKL expression remained an independent protective prognostic factor (HR = 0.469, 95% CI: 0.302-0.729, *P*-value < 0.001) (**Table 1**).

Subgroup K-M survival curves of different clinical variables in ccRCC also showed an overall trend of better prognosis with higher CRKL expression. Further comparison of clinical characteristics between CRKL-high and -low groups showed statistically significant differences in age, gender, N0 stage, M0 stage, and histologic grade G3&G4 (**Figure 6**). Those without statistical significance are not listed. This find-

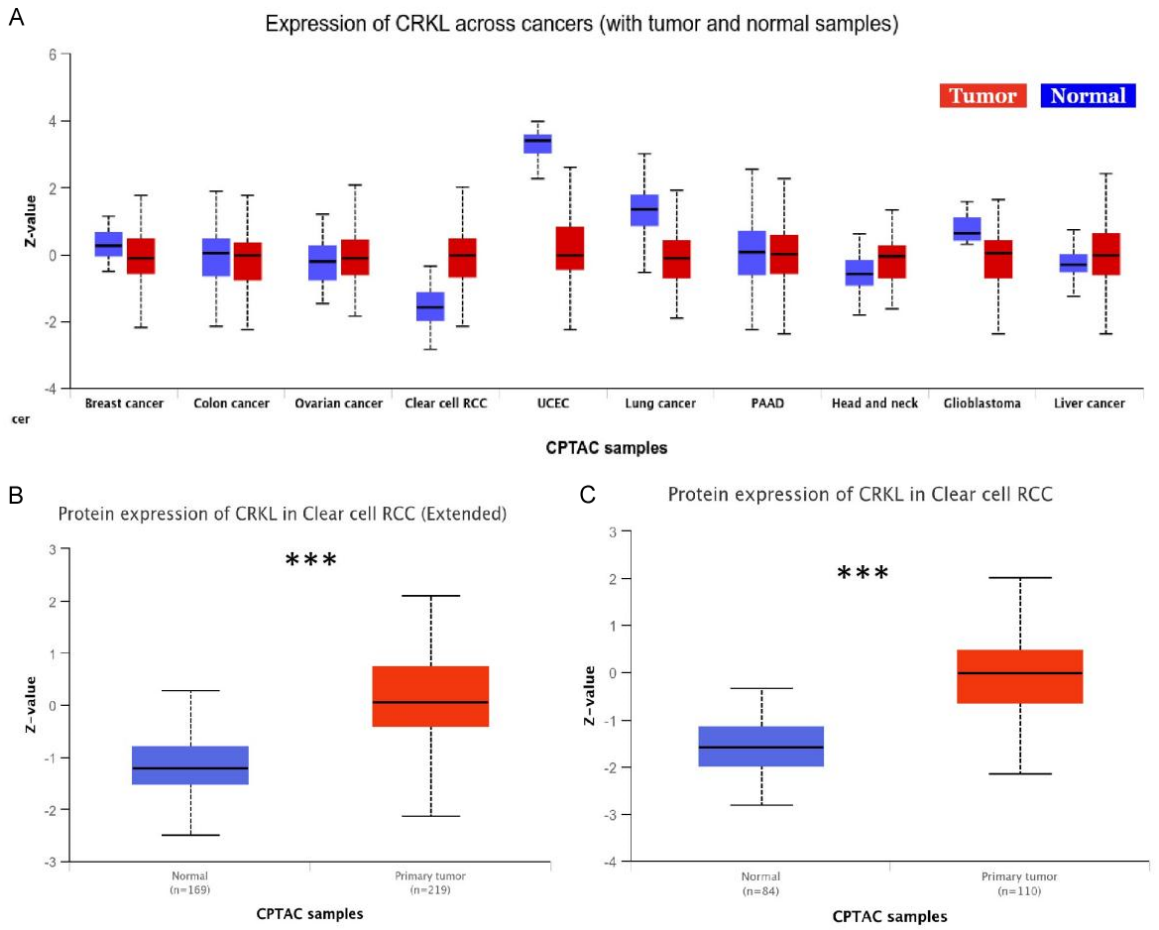

Figure 3. Analysis of CRKL protein in ccRCC. A. CRKL protein expression in pan-cancer cohorts by UALCAN. B, C. CRKL protein expression levels in ccRCC. All *P* values < 0.05.

ing robustly supports the potential of CRKL as a prognostic biomarker for ccRCC.

Relationship between CRKL expression and immune cell infiltration

Interestingly, while we previously reported that CRKL overexpression promotes the migration and invasion of ccRCC cells (786-0) in vitro [22], suggesting an oncogenic role, the current survival analysis paradoxically shows that high CRKL expression is associated with better patient outcomes. Given the paradoxical finding that CRKL overexpression is associated with improved survival, we hypothesized that this effect might be mediated through CRKL's involvement in the tumor immune microenvironment, potentially overriding its intrinsic pro-invasive function. We investigated the association between the expression levels of CRKL and different subtypes of immune cells in ccRCC. The expression levels of CRKL were observed to exhibit a significant positive correlation with the B cell, $CD4^+$ T cell, $CD8^+$ T cell, Eosinophils, Macrophages, Neutrophils and Dendritic cells using TIMER database (**Figure 7A**). We investigated the expression association between CRKL and these immune cell subtypes using the Xiantao Academic web, which further verified the results above (**Figure 7B**). Then, we conducted an in-depth examination the influence of CRKL on immune cells in TME. Immune cell and functional enrichment analysis exposed that the CRKL high-expression cohort exhibited a markedly higher enrichment compared to the low-expression cohort (**Figure 7C**). Furthermore, we used TISIDB database to investigate the correlation between CRKL expression and various immune signatures. We discovered that CRKL level was also positively correlated with immunostimulator, immu-

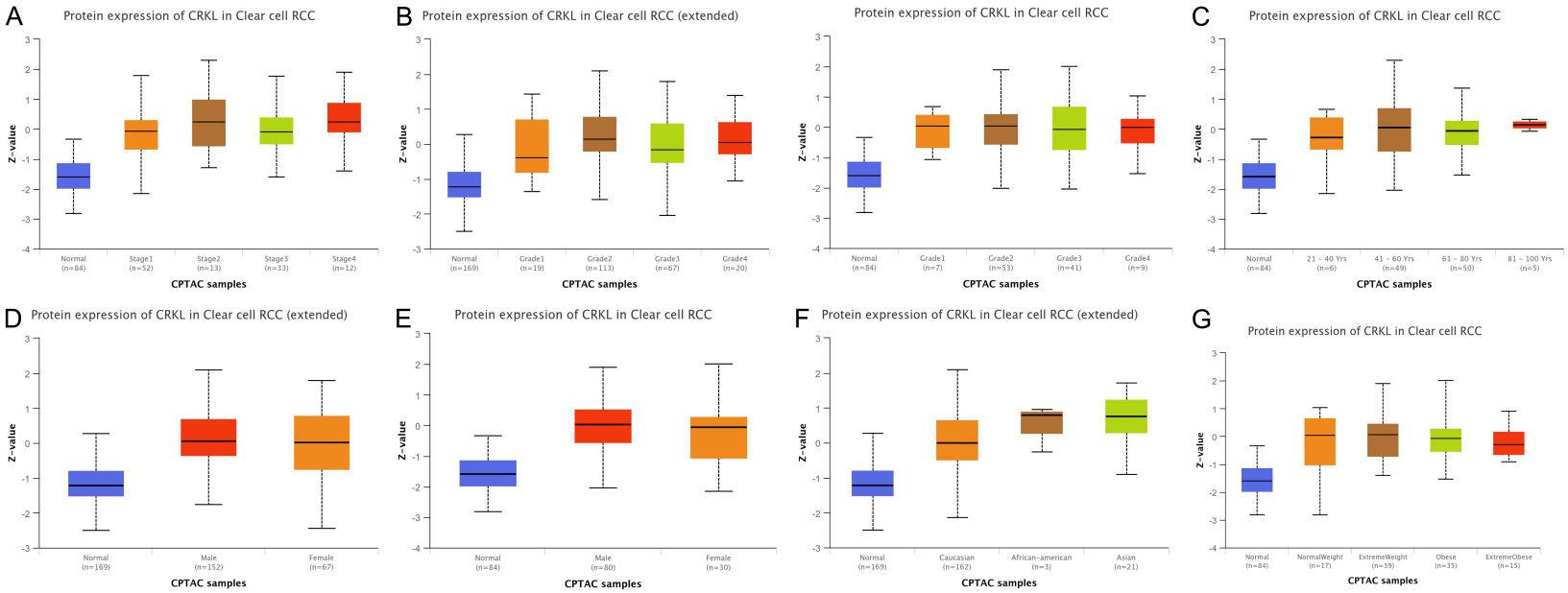

Figure 4. Analysis of CRKL protein in ccRCC in patient groups with different clinical parameters. A-G. From tumor stage, individual cancer stage, gender, age, race and weight are shown. All *P* values < 0.05.

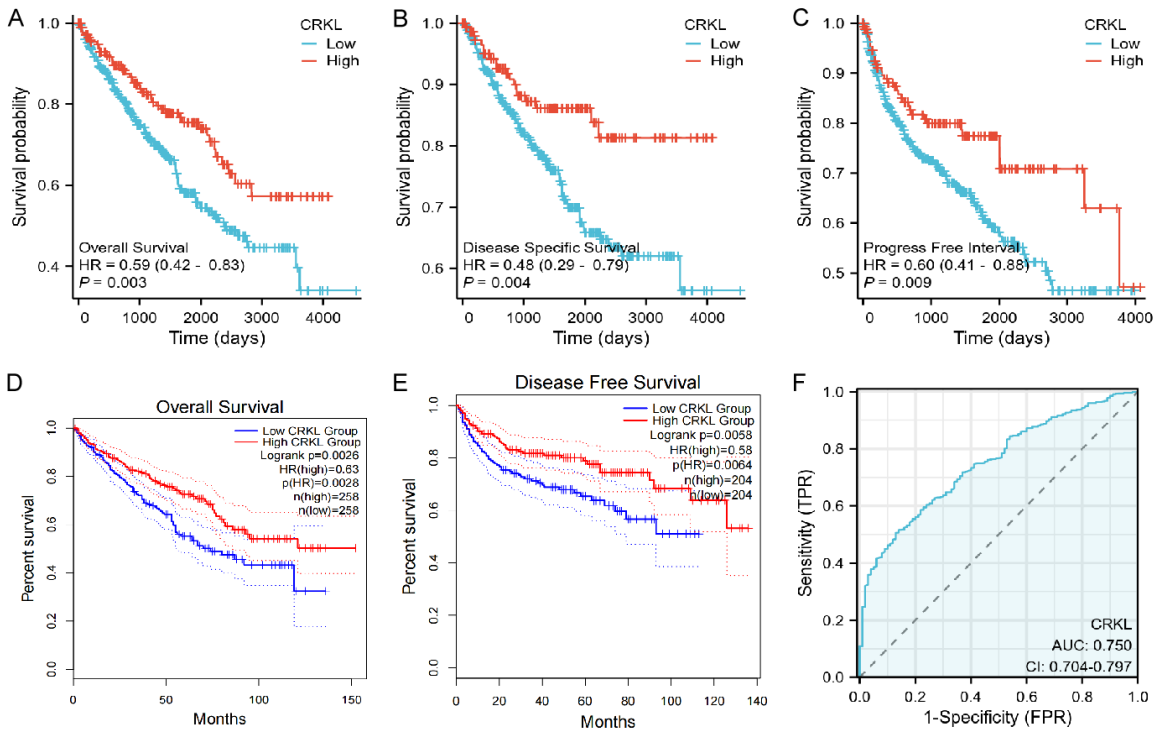

Figure 5. Prognostic value of CRKL in ccRCC. (A-C) Kaplan-Meier curves for OS (A), DSS (B), and PFI (C) from Xiantao platform. (D, E) OS and DFS curves from GEPIA2. (F) The receiver operating characteristic (ROC) curve reflected the CRKL's diagnostic ability for ccRCC (AUC = 0.75). All *P* values < 0.05.

noinhibitor and MHC molecule in ccRCC, comparing with other cancers (**Figure 7D-F**). These findings suggest that CRKL is linked to antitumor immunity in ccRCC.

CRKL co-expressed networks and gene enrichment analysis in ccRCC

The LinkedOmics database identified genes co-expressed with CRKL in KIRC. The top 50 significantly positively and negatively correlated genes were shown in a heat map (**Figure 8A-C**). GO analysis indicated CRKL and its co-expressed genes participated in the regulation of angiogenesis, T cell activation, and response to type I interferon (**Figure 8D**). KEGG pathway analysis showed enrichment in Th1 and Th2 cell differentiation, Th17 cell differentiation, and Cell adhesion molecules (**Figure 8E**). Reactome analysis showed enrichment in Interleukin-4 and -13 signaling, and Integrin cell surface interactions (**Figure 8F**). These results suggested that CRKL expression might play an essential role in kidney cancers by regulating the immune response of the TME.

Gene set enrichment analysis (GSEA) reveals immune-related pathway enrichment in CRKL-high ccRCC

To further characterize the functional implications of CRKL expression, we performed GSEA comparing CRKL-high versus CRKL-low groups. The bar plot and enrichment map (EMAP) revealed that CRKL-high tumors were significantly enriched in multiple immune-related pathways (**Figure 9**). Notably, pathways such as Reactome PD-1 signaling (NES = 3.97), Reactome CD22-mediated BCR regulation (NES = 5.35), Reactome FCGR activation (NES = 5.55), PID TCR pathway (NES = 3.41), PID CD8 TCR pathway (NES = 3.24), BIOCARTA CTLA4 pathway (NES = 3.22), and WP cancer immunotherapy by PD1 blockade (NES = 3.41) were among the top enriched gene sets (FDR < 0.001 for all). Additional pathways, including Th17 cell differentiation (NES = 3.06) and graft versus host disease (NES = 3.02), were also significantly enriched. These results further support the notion that CRKL is closely linked to the regulation of the tumor immune microenvironment.

Table 1. Multivariate Cox regression analysis of CRKL expression and clinical factors for overall survival in ccRCC

Characteristics	Total (N)	HR (95% CI) Univariate analysis	*P* value Univariate analysis	HR (95% CI) Multivariate analysis	*P* value Multivariate analysis
Pathologic T stage	541				
T1 & T2	350	Reference		Reference	
T3 & T4	191	3.210 (2.373-4.342)	< 0.001	1.861 (0.809-4.279)	0.144
Pathologic N stage	258				
N0	242	Reference		Reference	
N1	16	3.422 (1.817-6.446)	< 0.001	1.848 (0.915-3.733)	0.087
Pathologic M stage	508				
M0	429	Reference		Reference	
M1	79	4.401 (3.226-6.002)	< 0.001	0.602 (0.077-4.722)	0.629
Pathologic stage	538				
Stage I & Stage II	332	Reference		Reference	
Stage III & Stage IV	206	3.910 (2.852-5.360)	< 0.001	1.038 (0.405-2.659)	0.937
Histologic grade	533				
G1 & G2	250	Reference		Reference	
G3 & G4	283	2.665 (1.898-3.743)	< 0.001	1.674 (1.019-2.748)	0.042
Age	541				
≤ 60	269	Reference		Reference	
> 60	272	1.791 (1.319-2.432)	< 0.001	1.845 (1.192-2.855)	0.006
CRKL	541				
Low	270	Reference		Reference	
High	271	0.723 (0.536-0.974)	0.033	0.469 (0.302-0.729)	< 0.001

Abbreviations: HR, hazard ratio; CI, confidence interval; ccRCC, clear cell renal cell carcinoma.

Discussion

Clear cell renal cell carcinoma (ccRCC) is a prevalent malignancy of the kidney, recognized for its poor prognosis and increasing incidence globally. Cancer biomarkers refer to substances or molecules that can be reliably identified in cells, bodily fluids, or tissues, serving as indicators of cancer presence or as predictors of patient prognosis [19]. Consequently, it is crucial to recognize biomarkers that may reflect the prognosis of patients. In this study, we illustrated that the expression levels of CRKL were associated with various clinicopathological characteristics and the immunological regulation in individuals diagnosed with ccRCC. Furthermore, our findings indicated that elevated CRKL expression serves as a protective factor in ccRCC.

CRKL has been previously reported as a high-risk factor that promotes tumor growth in several cancers, including lung cancer, gastric cancer, hepatocellular carcinoma, and melanoma, where higher CRKL expression correlated with poor prognosis [28-31]. In contrast to most other cancer types, our present study reveals a paradoxical finding: CRKL overexpression in ccRCC is associated with better patient survival. Notably, this paradox is not an artifact of computational bias, as our previous study has already validated CRKL protein expression by Western blot in 28 pairs of ccRCC tissues and matched adjacent normal tissues, demonstrating significant upregulation in tumor tissues, consistent with the analysis results from public databases such as TCGA. This strongly confirms that the aberrant expression of CRKL in ccRCC is a bona fide biological event, rather than a computational prediction artifact.

Furthermore, we performed multivariate Cox regression analysis incorporating established clinical factors. The results demonstrated that

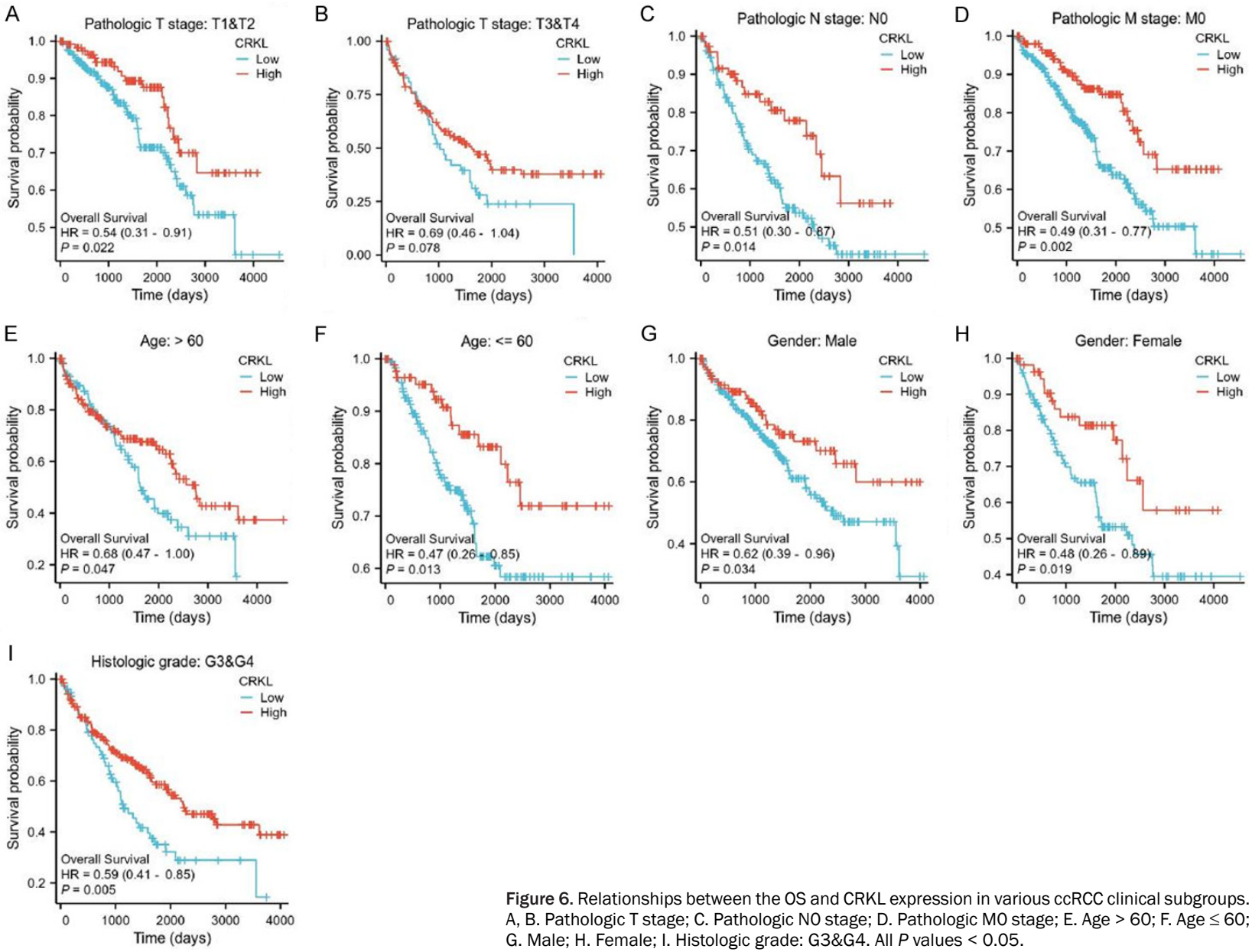

Figure 6. Relationships between the OS and CRKL expression in various ccRCC clinical subgroups. A, B. Pathologic T stage; C. Pathologic N0 stage; D. Pathologic M0 stage; E. Age > 60; F. Age ≤ 60; G. Male; H. Female; I. Histologic grade: G3&G4. All *P* values < 0.05.

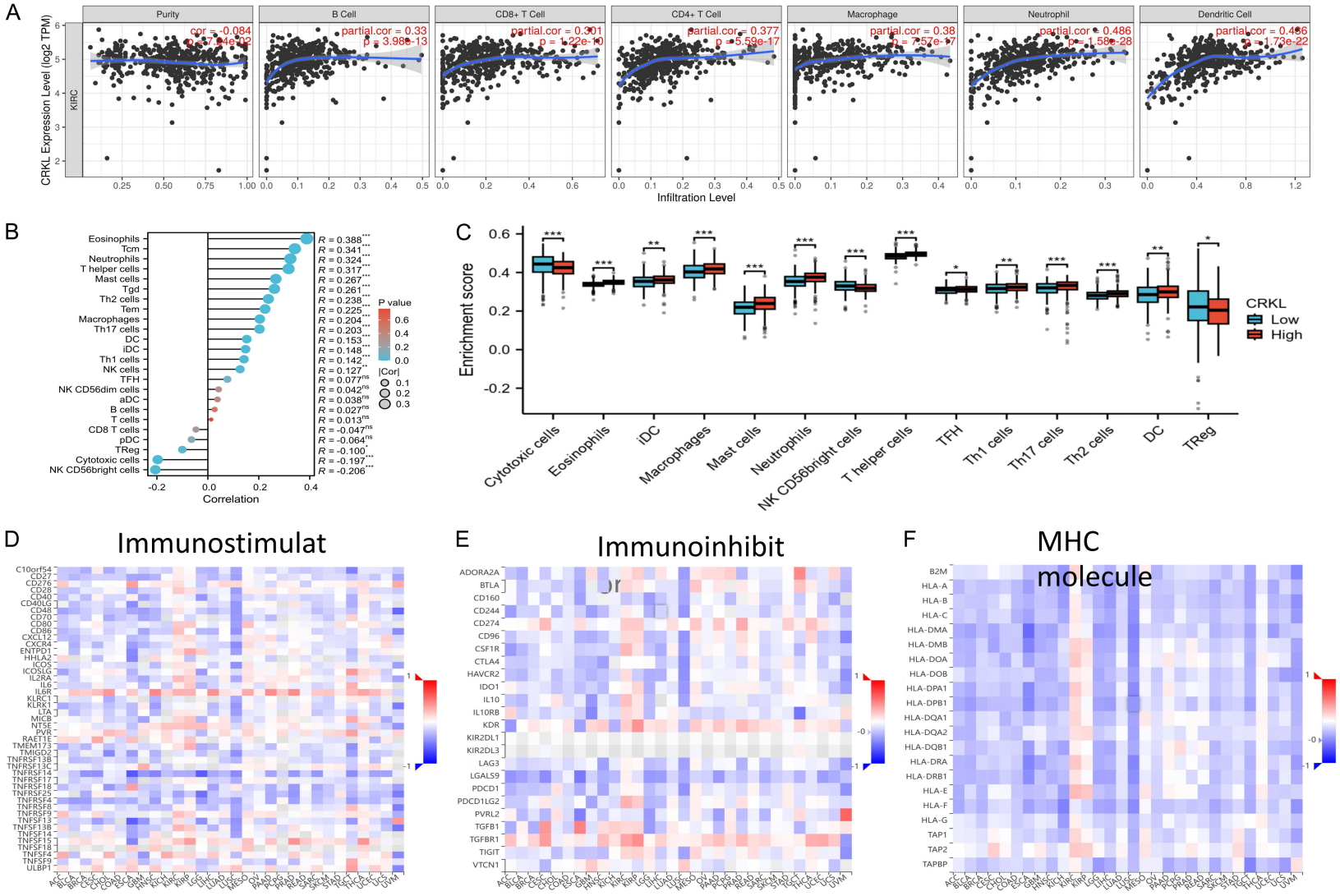

Figure 7. Correlation of CRKL expression with immune infiltration in ccRCC. (A) TIMER analysis showing correlation between CRKL and immune cell abundance. (B) Bar graph of correlations with immune cell subtypes. (C) Enrichment scores of 12 immune cell subtypes in CRKL-high vs. CRKL-low groups. (D-F) Correlations of CRKL with immunostimulators (D), immunoinhibitors (E), and MHC molecules (F) from TISIDB. **P*-value < 0.05; ***P*-value < 0.01; ****P*-value < 0.001; ns: not significant.

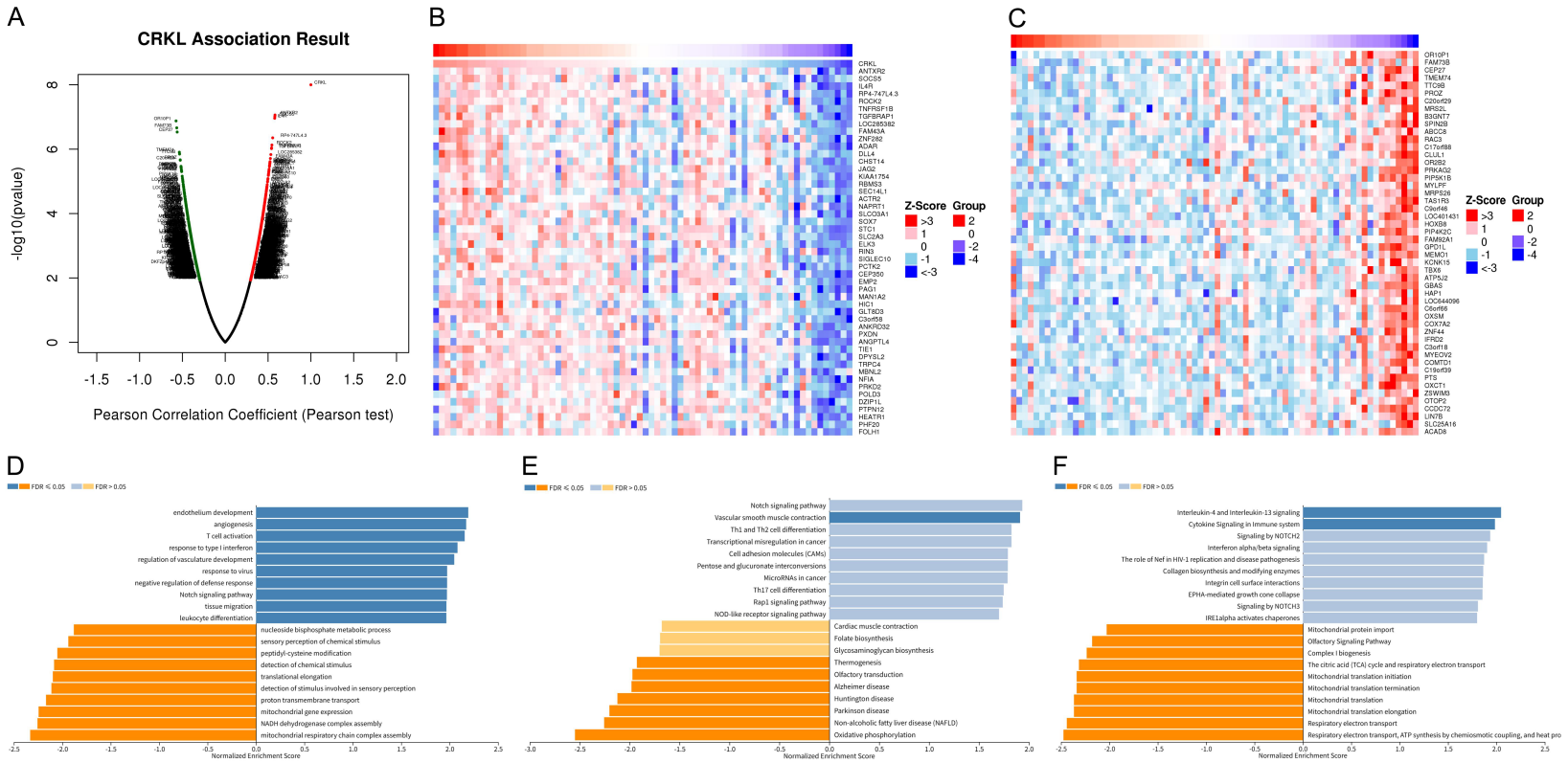

Figure 8. CRKL co-expression network and functional enrichment in ccRCC. (A) Volcano plot of CRKL co-expressed genes (LinkedOmics, Pearson test). (B, C) Heat maps of top 50 positively (B) and negatively (C) co-expressed genes. (D) GO biological process enrichment. (E) KEGG pathway enrichment. (F) Reactome pathway enrichment. FDR < 0.05 was considered significant.

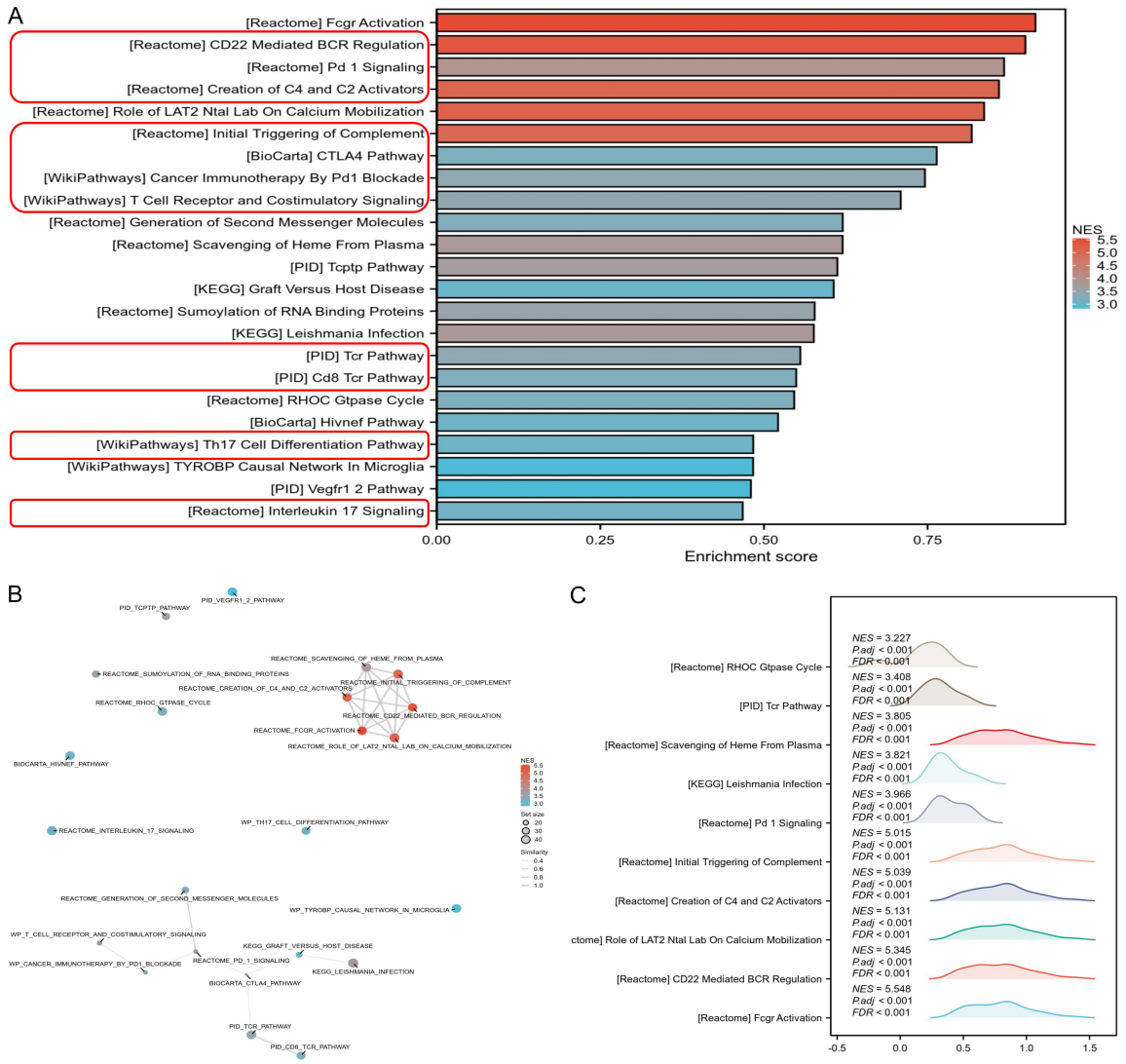

Figure 9. GSEA of significantly enriched functional pathways in CRKL-high vs. CRKL-low ccRCC. A. Bar plot of all enriched pathways ranked by NES. B. Network interaction diagram of enriched pathways. C. Representative GSEA enrichment curves with NES, P, and FDR values annotated. All FDR < 0.05.

after adjusting for pathologic T stage, N stage, M stage, overall pathologic stage, histologic grade, and age, CRKL expression remained an independent protective prognostic factor (HR = 0.469, 95% CI: 0.302-0.729, P < 0.001). This analysis provides robust statistical support for the potential of CRKL as a prognostic biomarker in ccRCC.

Interestingly, this finding contradicts the conventional oncogenic role of CRKL reported in other cancers and our own in vitro migration/invasion data. Based on this foundation, we propose several possible explanations for this survival paradox. First, the conflicting prognostic role of CRKL may be context-dependent, shaped by the unique composition of the ccRCC tumor microenvironment (TME). Our immune infiltration analysis shows that CRKL expression is positively correlated with the infiltration of multiple anti-tumor immune cells, such as $CD8^+$ T cells, $CD4^+$ T cells, and dendritic cells (**Figure 7**). More importantly, GSEA revealed that multiple immune-related pathways, such as PD-1 signaling, TCR pathway, CD8 TCR pathway, CTLA4 pathway, and cancer immunotherapy by PD1 blockade, are significantly enriched in CRKL-high tumors (**Figure 9**). It is plausible that in ccRCC, the immune-activating effect of CRKL outweighs its intrinsic

pro-invasive function, leading to prolonged survival in patients with high CRKL expression. Second, tumor heterogeneity may contribute to this paradox: CRKL could function as a tumor suppressor in early-stage or specific ccRCC subtypes, while its oncogenic role becomes dominant only in advanced stages. Third, the discrepancy may stem from different endpoints: in vitro assays measure cellular behaviors (migration/invasion), whereas survival reflects the complex in vivo immune landscape and host defense, which are not captured by simple functional assays. Although these findings suggest that CRKL may participate in immune-related pathways, they should be interpreted cautiously as they are derived solely from computational predictions. Further experiments, such as co-culture assays or immune cell-specific expression analysis, are needed to confirm the functional role of CRKL in modulating the ccRCC immune microenvironment. Collectively, these results indicate that CRKL may have a unique mechanism of action in renal cancer that distinguishes it from other cancer types, partially explaining its association with a better prognosis.

This study has several limitations. First, although we validated the upregulation of CRKL expression in 28 ccRCC tissues by Western blot, the sample size is relatively small and the validation is retrospective. Future studies should incorporate multicenter validation in large-scale, prospective cohorts and supplement with immunohistochemistry (IHC) analysis to comprehensively confirm the expression pattern of CRKL. Second, all functional enrichment and immune infiltration analyses are based on public databases and lack direct wet-lab validation. Future research should include more comprehensive functional experiments (e.g., CRKL knockdown/overexpression in ccRCC cells, immune cell co-culture models and specific analysis of immune cells) to verify the proposed mechanisms. Third, whether the dysregulation of CRKL occurs primarily in immune cells or in cancer cells, and how it influences the TME, remains to be determined. These limitations highlight important directions for our subsequent research.

In conclusion, this study investigated the prognostic significance and immune features of CRKL in ccRCC. Through both public database analysis and independent Western blot validation in tissue samples, we confirmed the aberrant upregulation of CRKL in ccRCC. Multivariate Cox regression further established CRKL as an independent protective prognostic factor for ccRCC. We also found that CRKL expression is correlated with the infiltration of multiple immune cells and is associated with favorable overall survival. These results suggest that CRKL plays an important role in tumor immunity and may serve as a novel prognostic biomarker for ccRCC. However, its unique biological function and clinical translational potential require further experimental and clinical investigation.

Disclosure of conflict of interest

None.

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