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Identification of cullin family members as novel diagnostic and prognostic biomarkers in kidney renal clear cell carcinoma

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Abstract

Kidney renal clear cell carcinoma (KIRC) is a prevalent histological subtype of kidney cancer and one of the most invasive urinary tumors. Members of the cullin family play a pivotal role in driving the development and progression of neoplasms. Accumulating evidence suggests that cullins might play a significant part in the initiation and advancement of KIRC. In this study, the messenger ribonucleic acid expression profiles for eight members of the cullin family (*CUL1 – 9*) were noticeably upregulated in KIRC compared to normal tissue, whereas *CUL3*, *CUL5,* and *CUL7* were downregulated. Moreover, our analysis demonstrated that higher expression levels of *CUL1 – 3*, *CUL4A*, *CUL4B*, *CUL5*, and *CUL7* were significantly correlated with enhanced overall survival (OS) in KIRC patients. Co-expression gene analysis showed that the differential expression of cullins in KIRC was predominantly associated with 20 genes. Functional enrichment analysis indicated that cullins in KIRC primarily participated in ubiquitin-mediated protein degradation, facilitated protein polyubiquitylation, and regulated APC/C activators during the G1/S phase transition and early anaphase. Furthermore, cullin gene expression exhibited a positive correlation with the activity of tumor-infiltrating immune cells. These findings suggest that cullins may serve as diagnostic and prognostic biomarkers in KIRC patients.

Keywords: Kidney renal clear cell carcinoma; Cullin gene family; Bioinformatics analysis; Prognostic value

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1. Introduction

Renal cell carcinoma (RCC) is the most fatal tumor in the urinary system, accounting for 2 – 3% of adult cancers and 90% of renal malignancies.[1] Kidney renal clear cell carcinoma (KIRC), the most common histological subtype of RCC, accounts for approximately 80% of all RCC cases.[2] Since it is not sensitive to chemotherapy or conventional radiotherapy, and targeted therapies for metastatic RCC (mRCC) are costly, radical or partial nephrectomy remains the primary treatment for KIRC.[3] However, approximately 40% of patients diagnosed with KIRC and undergoing nephrectomy ultimately develop local

recurrence or metastatic disease.[4] Therefore, exploring effective biomarkers for KIRC diagnosis and prognosis is urgently needed.

Cancer development entails the gradual transformation of normal cells into malignant ones, stemming from abnormal responses to stimuli.[5] This process is regulated at multiple levels – including transcription, translation, and post-translational modifications – that are essential in maintaining cellular homeostasis.[6,7] The ubiquitin-proteasome system (UPS) facilitates rapid degradation of transient proteins and plays an indispensable role in diverse biological processes, including genome stability and cell cycle regulation.[8] As a result, aberrant UPS function and regulation disrupt protein homeostasis, leading to a variety of illnesses, including cancer.[9]

In humans, the cullin family forms cullin-RING ligases (CRLs) with a RING protein during the ubiquitination process. CRL dysfunction has been linked to kidney cancer in recent investigations.[10] For instance, RCC has been associated with the dysfunction of CRLs containing *CUL3*. In the inherited condition known as Von Hippel-Lindau (VHL) disease, mutations in the *CUL2*-associated substrate receptor VHL lead to the development of KIRC.[11] Furthermore, multiple investigations have found a link between *CUL3* and Kelch-like ECH-associated protein 1 (Keap1) malfunction and papillary renal cell cancer.[12]

Consequently, we aimed to investigate the biological roles and prognostic relevance of cullins in KIRC. In this study, we identified the transcriptional patterns of cullin family members using ONCOMINE. We then performed gene ontology (GO) functional and biological pathways analysis of cullins and their 20 related genes. Finally, we examined their clinical characteristics and prognostic values in KIRC. Our findings reveal the potential biological function and prognostic value of cullins, which may aid future diagnostic and therapeutic approaches for KIRC.

2. Materials and methods

2.1. ONCOMINE data analysis

Designed specifically for translational research, the ONCOMINE platform (http://oncomine.org) offers a vast suite of bioinformatics tools. These resources enable in-depth interrogation of gene expression profiles across the entire genome, empowering researchers to derive meaningful insights from complex genomic data.[13] Using the "gene overview" and "dataset exploration" functions, transcriptional profiles of cullin family members (*CUL1, CUL2, CUL3, CUL4A, CUL4B, CUL5, CUL7,* and *CUL9*) across multiple malignancies were retrieved. Statistical significance was calculated through Student's *t*-test. The following parameters were used: $p<0.05$, fold change >2, and genes ranked within the top 10%. Using the messenger ribonucleic acid (mRNA) expression from The Cancer Genome Atlas (TCGA, https://portal.gdc.cancer.gov/), cancer versus normal data were analyzed. For statistically significant data, the following information was collected: cancer types, genes, dataset reference, sample sizes, fold changes, *t*-test results, and *p*-values.

2.2. UALCAN data analysis

UALCAN (http://ualcan.path.uab.edu/analysis.html) is a robust web-based tool. It specializes in processing data from two prominent resources: The MET500 cohort and TCGA, enabling in-depth genomic analysis.[14] We analyzed the relative expression of *CUL1 – 3*, *CUL4A*, *CUL4B*, *CUL5*, *CUL7*, and CUL*9* based on cancer stage across various tumor subtypes. Student's *t*-test was employed to evaluate the disparity between the two groups, and statistical significance was defined as $p<0.05$.

2.3. Gene expression profiling interactive analysis (GEPIA)

The GEPIA tool (http://gepia.cancer-pku.cn/) is an intuitive web application integrating data from 8587 healthy and 9736 neoplastic samples from the TCGA and the Genotype-Tissue Expression initiatives.[15] We made use of the KIRC dataset within the TCGA database to explore how cullin family proteins were expressed in 523 patients. Patients were categorized into low- and high-expression groups based on individual gene transcript levels. The log-rank and Mantel-Cox tests were utilized for statistical comparisons.

In a separate cohort of 515 at-risk patients, we analyzed overall survival (OS) and disease-free survival (DFS). Where necessary, we calculated hazard ratios, 95% confidence intervals, and *p*-values to assess statistical significance.

2.4. GeneMANIA data analysis

GeneMANIA (http://www.genemania.org) serves as an online resource for comprehensive analysis of genetic and protein interactions, domain-protein similarities, co-localization, and biological pathways.[16] This tool was used to examine the link between cullins and their related genes in the current investigation.

2.5. Metascape analysis

Metascape (http://metascape.org) represents a software application designed for annotating genes and conducting enrichment analysis.[17] In this research, we used Metascape to explore the roles of cullin family proteins and their coexpressed genes. A significance threshold of $p=0.01$ was established, with enrichment factors >1.5 and a minimum count of 3 considered statistically significant.

2.6. TIMER database analysis

TIMER (https://cistrome.shinyapps.io/timer/) is a web-based tool aimed at enabling in-depth assessment of immune cell infiltration and its clinical significance.[18] We analyzed the expression levels of cullin proteins and their associations with tumor purity and immune cell infiltration in KIRC, including B cells, macrophages, neutrophils, $CD4^+$ T cells, $CD8^+$ T cells, and dendritic cells.

3. Results

3.1. Transcriptional expression of cullin genes in patients with kidney cancer

The ONCOMINE database was employed to probe the differential expression patterns of cullins in patients with kidney cancer. Initially, we assessed the transcriptional profiles in both malignant and healthy tissues. Our analysis revealed that the expression of all nine cullin family genes (*CUL1* – *9*) varied significantly in renal cancer (Figure 1). Specifically, ONCOMINE analysis showed elevated mRNA expression of *CUL1*, *CUL2*, *CUL4A*, *CUL4B*, and *CUL9*, whereas *CUL3*, *CUL5*, and *CUL7* were downregulated in kidney cancer. *CUL1* and *CUL2* mRNA levels were overexpressed 2.172-fold and 2.617-fold, respectively, in Wilms' tumor compared to healthy control tissues.[19] According to Jones *et al.*,[20] *CUL3* expression was reduced in clear cell RCC (CCRCC; relative change = −2.340) and renal pelvis urothelial carcinoma (relative change = −2.133). It was also downregulated in renal oncocytoma (relative change = −4.876) and chromophobe RCC (relative change = −5.238) in the Yusenko *et al.*[19] dataset. In the same dataset, *CUL4A* was also shown to be higher in Wilms' tumor (relative change = 4.757) compared to control samples.[19] When compared to healthy controls, Beroukhim *et al.*[21] discovered that in hereditary CCRCC, the expression of *CUL4B* mRNA was heightened (relative change = 2.192). *CUL5* expression was downregulated in renal oncocytoma (relative change = −3.055), CCRCC (relative change = −3.264), and renal pelvis urothelial carcinoma (relative change = 2.548) in a study by Jones *et al.*[20] *CUL5* expression was downregulated in Wilms tumor (relative change = −2.094), according to Cutcliffe *et al.*[22]

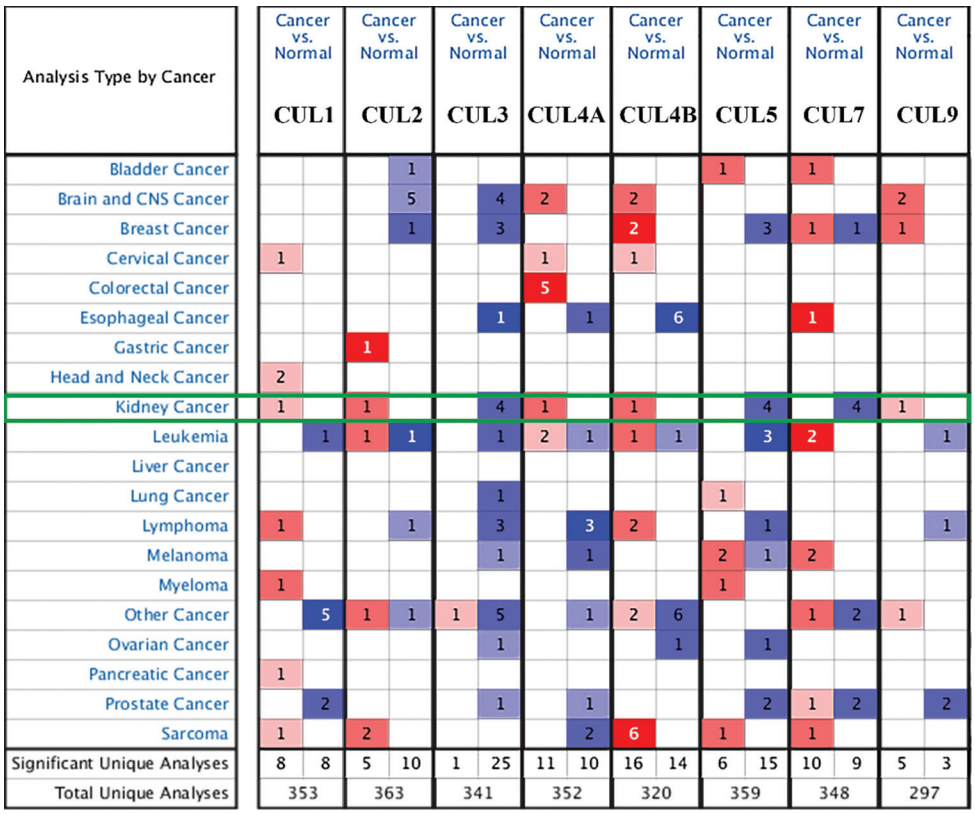


Figure 1. Differences in the transcription levels of cullin genes in cancer versus normal tissues across various cancer types. The threshold is using the following stringent criteria: an absolute $\log_2$ fold change ($|\log_2(\text{fold change})|$) of ≥1 and $p<0.05$. These criteria were employed to ensure that only data exhibiting both substantial and statistically significant alterations were considered. The numerical values within each cell denote the number of datasets that fulfill these criteria, indicating instances where the biological or experimental differences were both quantitatively pronounced and statistically robust. In the heatmap, the intensity of the red coloration corresponds to the significance level of upregulation, whereas the intensity of the blue coloration reflects the significance level of downregulation.

CUL7 expression was reduced in multiple renal cancers in the Jones *et al.*[20] dataset: CCRCC (relative change = −2.639), renal pelvis urothelial carcinoma (relative change = −2.909); chromophobe RCC (relative change = −2.489); papillary RCC (relative change = −3.151).[20] *CUL9* expression was found to be enhanced in renal oncocytoma (relative change = 2.118)[19] (Table 1).

3.2. Prognostic value of cullin family members in patients with KIRC

We employed UALCAN to investigate the connection between differential expression levels of cullin genes and tumor grades in KIRC, aiming to find cullin members involved in carcinogenesis, progression, and prognosis. The expression of *CUL1*, *CUL2*, *CUL3*, *CUL4A*, *CUL4B*, *CUL5*, *CUL7*, and *CUL9* (p<0.001) was significantly associated with tumor grade (Figure 2). The expression levels of these genes demonstrated a strong correlation with tumor progression, and survival curves stratified by gene expression and tumor grade showed notable differences in outcomes, suggesting that cullin proteins may serve as potential prognostic biomarkers in KIRC. To further explore these associations, we utilized the GEPIA database to explore the OS and DFS in KIRC prognosis (Figure 3). For KIRC patients, elevated transcription of *CUL1* (p=0.0067), *CUL2* (p=0.0012), *CUL3* (p=0.0041), *CUL4A* (p=0.0001), *CUL4B* (p=0.0031), *CUL5* (p<0.0001), and *CUL7* (p=0.023) correlated with extended OS. Conversely, overexpression of CUL9 (p=0.0001) was linked to a different survival trend. Moreover, for DFS in KIRC patients, elevated transcription levels of *CUL1* (p=0.04), *CUL4A* (p=0.0002), and *CUL5* (p=0.007) were significantly associated with prolonged DFS.

3.3. Co-expression and functional enrichment analysis of cullin family genes in KIRC patients

Leveraging GeneMANIA, we constructed an interaction network encompassing cullin family components and their functionally associated genes, aiming to explore the core biological functions of the cullin family in KIRC. The analysis identified *CACUL1*, *ANAPC2*, *ANAPC10*, *EXOC7*, *RP11-343C2.9*, *COG8*, *VPS51*, *HSF1*, *PRR5*, *ZZEF1*, *HSF2*, *HECTD3*, *EXOC8*, *HERC2*, *RNF217*, *ARIH1*, *ARIH2*, *RNF144B*, *RNF19B*, and *RNF19A* as key interactors, highlighting their roles in the regulatory functions of differentially expressed cullin genes (Figure 4).

Subsequently, we employed Metascape to determine how the cullin family genes and the 20 co-expressed genes mentioned above (Figure 5). The most significantly biological processes included "ubiquitin-dependent protein catabolic process," "ubiquitin-mediated proteolysis," "protein polyubiquitination," "regulation of mitotic cell cycle," "cell division," "positive regulation of mitotic cell cycle," and

Table 1. Differential expression of cullin genes between cancer and normal tissues in the ONCOMINE database

Gene	Type of kidney cancer	*p*-value	t-statistic	Fold change	References
CUL1	Wilms tumor	0.008	3.267	2.172	Yusenko *et al.*[19]
CUL2	Wilms tumor	<0.001	5.268	2.617	Yusenko *et al.*[19]
CUL3	Renal oncocytoma	<0.001	−5.452	-4.876	Yusenko *et al.*[19]
	Chromophobe RCC	0.004	−4.014	-5.238	Yusenko *et al.*[19]
	CCRCC	<0.001	−14.229	-2.340	Jones *et al.*[20]
	Renal pelvis urothelial carcinoma	<0.001	−11.715	-2.133	Jones *et al.*[20]
CUL4A	Wilms tumor	0.002	4.499	4.757	Yusenko *et al.*[19]
CUL4B	Hereditary CCRCC	<0.001	11.583	2.192	Beroukhim *et al.*[21]
CUL5	Renal oncocytoma	<0.001	−12.826	−3.055	Jones *et al.*[20]
	CCRCC	<0.001	−14.018	−3.264	Jones *et al.*[20]
	Renal pelvis urothelial carcinoma	<0.001	−7.377	−2.548	Jones *et al.*[20]
	Wilms tumor	0.006	−2.973	−2.094	Cutcliffe *et al.*[22]
CUL7	CCRCC	<0.001	−21.724	−2.639	Jones *et al.*[20]
	Renal pelvis urothelial carcinoma	<0.001	−21.319	−2.909	Jones *et al.*[20]
	Chromophobe RCC	<0.001	−20.148	−2.489	Jones *et al.*[20]
	Papillary RCC	<0.001	−24.359	−3.151	Jones *et al.*[20]
CUL9	Renal oncocytoma	0.001	5.157	2.118	Yusenko *et al.*[19]

Abbreviations: CCRCC: Clear cell renal cell carcinoma; RCC: Renal cell carcinoma.

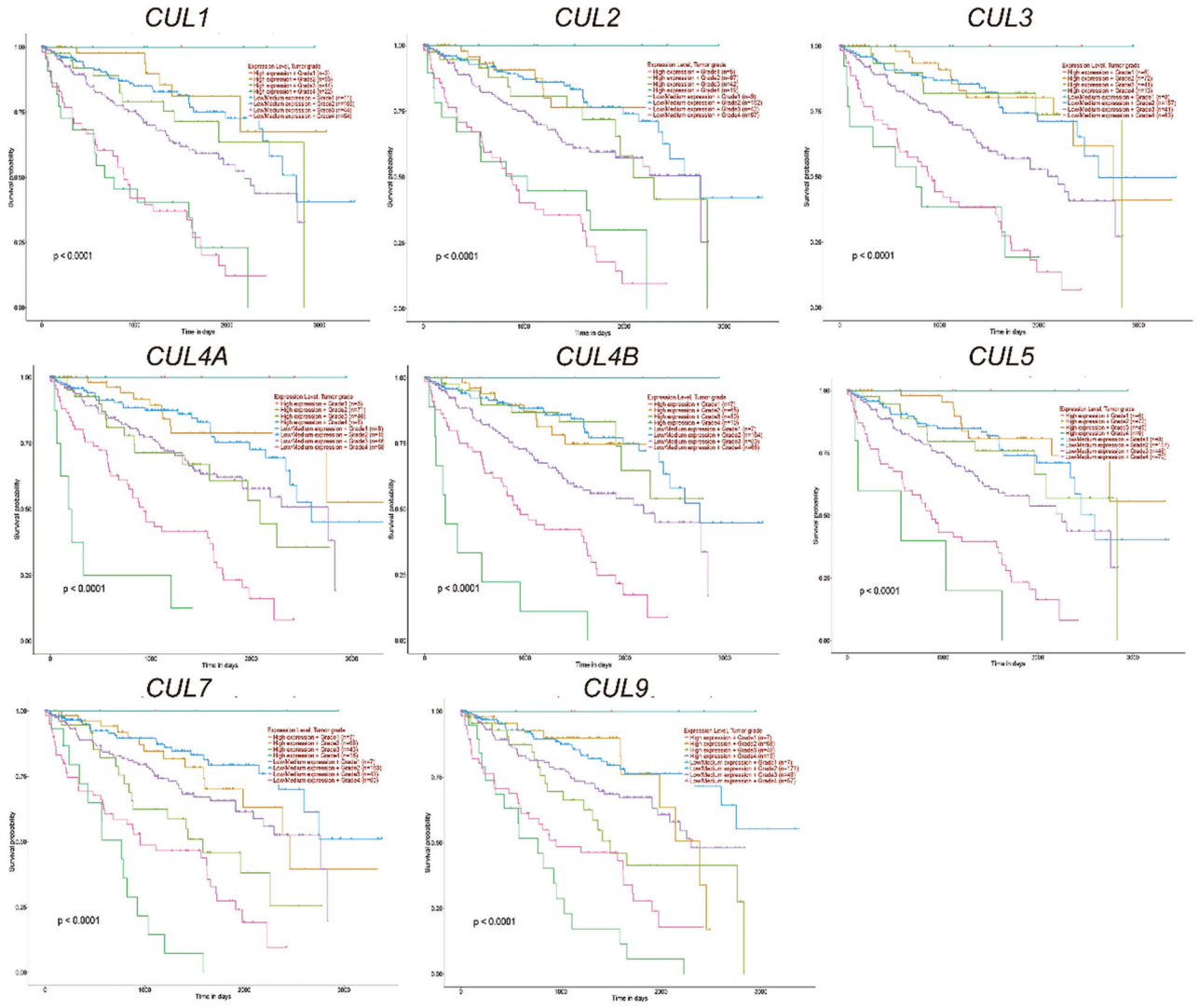


Figure 2. The impact of cullin expression levels on KIRC patient survival by tumor grade (UALCAN). The results demonstrated statistically significant associations for all eight cullin genes shown (p<0.001). These findings suggest that the expression of cullin family members may be involved in the oncogenesis and development of KIRC.
Abbreviation: KIRC: Kidney renal clear cell carcinoma.

"mGolgi vesicle transport" (Figure 5A). A network of these enriched terms was constructed and visualized based on cluster and identifier (Figures 5B and C). Furthermore, a protein-protein interaction (PPI) network was generated and analyzed using the molecular complex detection (MCODE) algorithm within Metascape (Figure 5D-F). The results identified a prominent MCODE component consisting of ubiquitin E3 ligase complex members (CSN1, CSN8, HRT1, SKP1, SKP2, CUL1, CUL2, and CUL3). These proteins were predominantly associated with key biological processes, including proteasome-mediated, ubiquitin-dependent protein catabolic processes and proteasomal protein degradation pathways.

3.4. Immune cell infiltration related to cullin expression in KIRC

The tumor microenvironment (TME), comprising fluids, immune cells, stromal cells, extracellular matrix components, and a variety of cytokines and chemokines, plays a crucial role in tumor growth and recurrence.[23] Using TIMER, we investigated the relationship between cullin family genes and immune cell infiltration in KIRC (Figure 6). The results revealed that *CUL1* and *CUL2* mRNA expressions were adversely associated with tumor purity. Moreover, we observed significant positive associations between the expression of multiple cullin family members and the infiltration of various immune

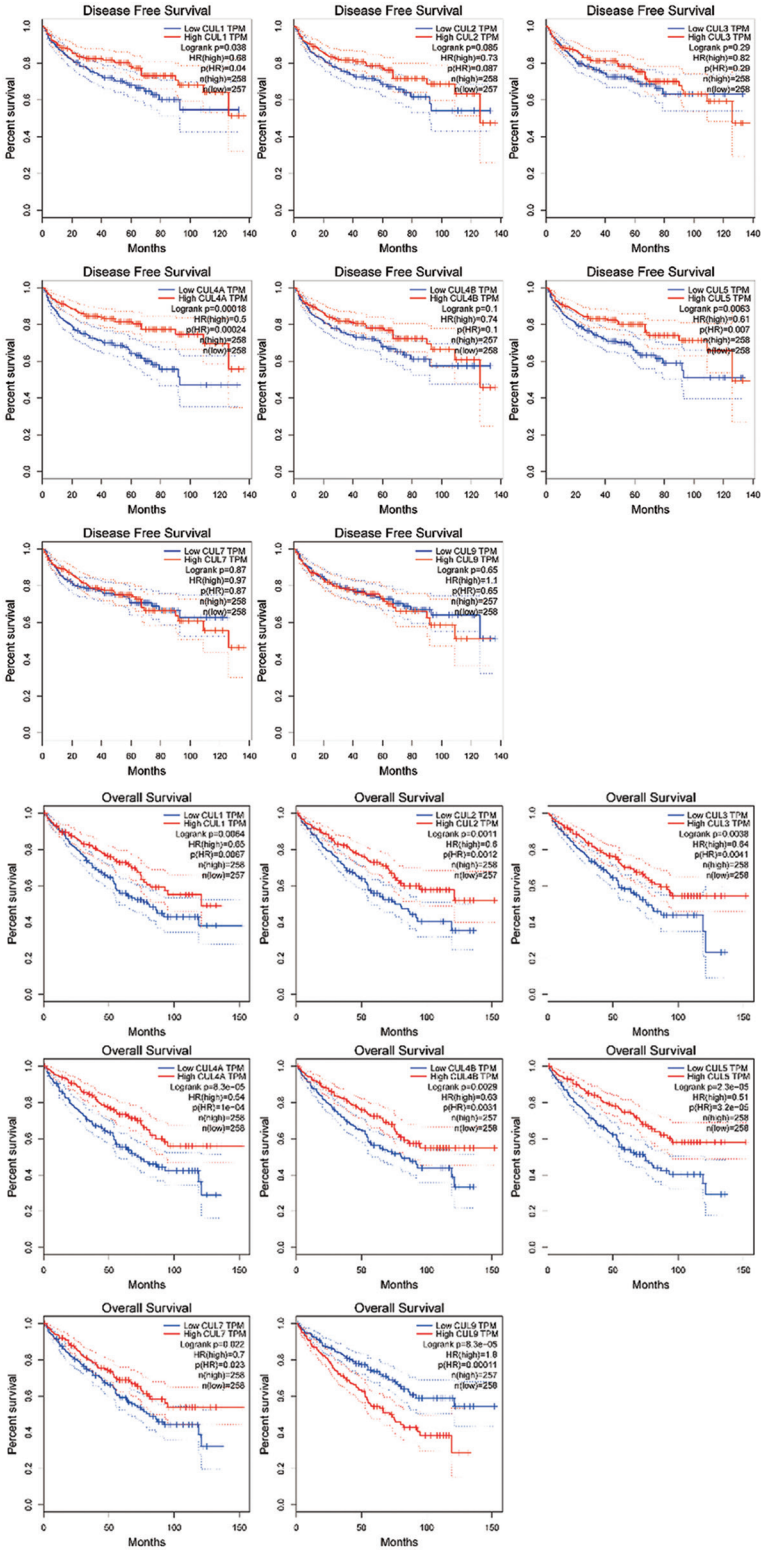


Figure 3. Kaplan-Meier survival analysis of cullin family members in KIRC using GEPIA. Association between cullin family members with OS and DFS, respectively.

Abbreviations: DFS: Disease-free survival; KIRC: Kidney renal clear cell carcinoma; OS: Overall survival; GEPIA: Gene expression profiling interactive analysis.

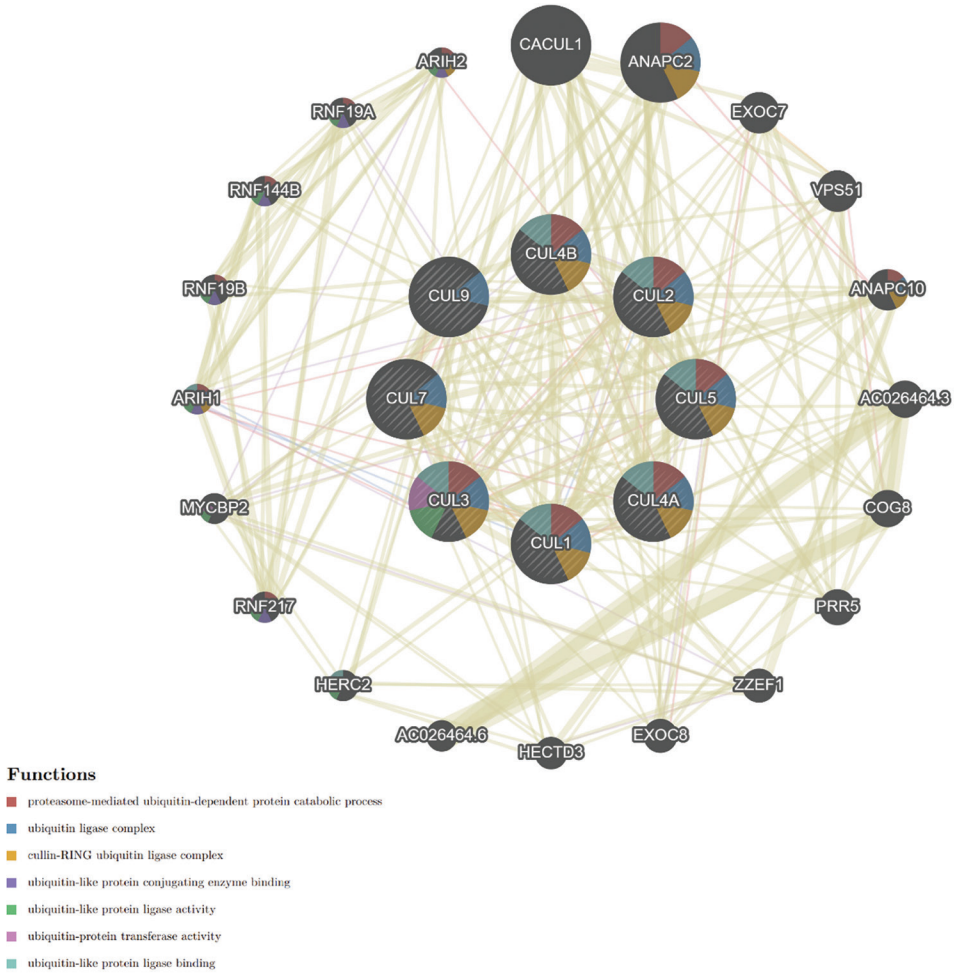
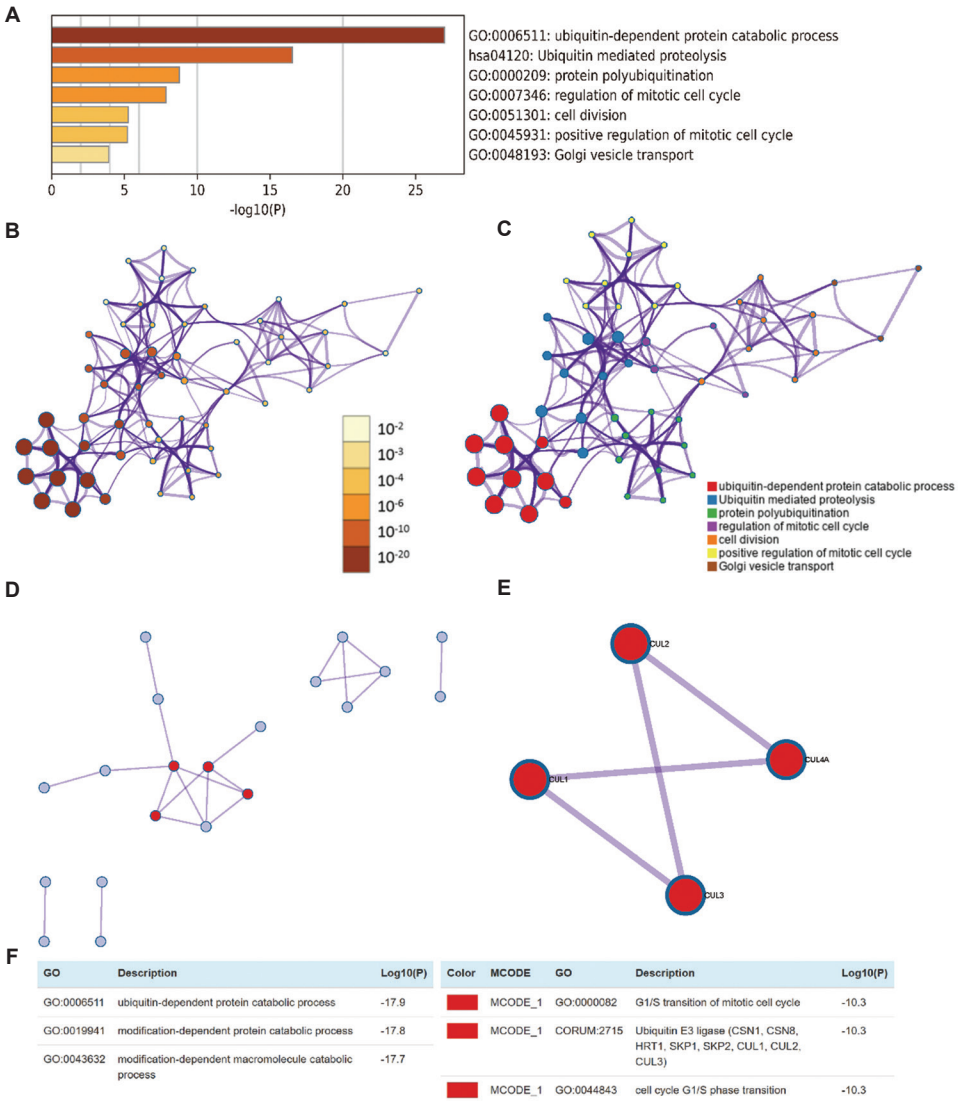


Figure 4. Gene-gene interaction network of *CUL1 – 9* in KIRC (GeneMANIA). This network was constructed to elucidate the fundamental mechanisms underlying cullin family gene functionality. The analysis uncovered numerous genes involved in the regulatory functions of differentially expressed cullin family genes.
Abbreviation: KIRC: Kidney renal clear cell carcinoma.

cell types: (1) *CUL1* expression was positively associated with B cells, $CD8^+$ T cells, $CD4^+$ T cells, macrophages, and neutrophils. (2) *CUL2* expression was positively correlated with B cells, $CD8^+$ T cells, $CD4^+$ T cells, macrophages, neutrophils, and dendritic cells. (3) *CUL3* showed associations with infiltration by B cells, $CD8^+$ T cells, $CD4^+$ T cells, macrophages, neutrophils, and dendritic cells. (4) *CUL4A* expression was positively linked to B cells, $CD4^+$ T cells, macrophages, neutrophils, and dendritic cells. (5) *CUL4B* showed positive correlations with B cells, $CD8^+$ T cells, $CD4^+$ T cells, macrophages, neutrophils, and dendritic cells. (6) *CUL5* expression was also associated with infiltration by B cells, $CD8^+$ T cells, $CD4^+$ T cells, macrophages, neutrophils, and dendritic cells. (7) *CUL7* and *CUL9* exhibited significant associations with $CD4^+$ T cells and neutrophils.

4. Discussion

In this study, the mRNA expression levels of *CUL1, CUL2, CUL4A, CUL4B*, and CUL9 were found to be significantly higher in kidney cancer tissues compared to normal tissues. Furthermore, elevated expression levels of *CUL1 – 3*, *CUL4A*, *CUL4B*, *CUL5*, and *CUL7* were notably correlated with improved OS in patients with KIRC, whereas high *CUL9* expression was markedly associated with poorer OS outcomes. CRLs, composed of a RING protein and cullin protein, are involved in the degradation of several oncogenic proteins and tumor suppressors, and their dysregulation is associated with increased tumor growth.[24] There is significant evidence that CRL malfunction contributes to kidney tumorigenesis.[10] KIRC is caused by mutations in the *CUL2* substrate receptor VHL in the inherited VHL syndrome. RCC has also been linked to *CUL3*-containing



GO	Description	Log10(P)
GO:0006511	ubiquitin-dependent protein catabolic process	-17.9
GO:0019941	modification-dependent protein catabolic process	-17.8
GO:0043632	modification-dependent macromolecule catabolic process	-17.7

Color	MCODE	GO	Description	Log10(P)
	MCODE_1	GO:0000082	G1/S transition of mitotic cell cycle	-10.3
	MCODE_1	CORUM:2715	Ubiquitin E3 ligase (CSN1, CSN8, HRT1, SKP1, SKP2, CUL1, CUL2, CUL3)	-10.3
	MCODE_1	GO:0044843	cell cycle G1/S phase transition	-10.3

Figure 5. Metascape-based enrichment analysis of cullins and their top 20 coexpressed genes in KIRC patients. (A) Heatmap visualizing GO and KEGG enrichment results, with color intensity indicating statistical significance. (B-C) Interactive network of enriched GO and KEGG terms, with node coloring based on statistical significance. (D-F) PPI network and MCODE components identified.
Abbreviations: GO: Gene ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes; KIRC: Kidney renal clear cell carcinoma; MCODE: Molecular complex detection; PPI: Protein-protein interaction.

CRL dysfunction. A link between malfunctioning mutations in *CUL3* and Keap1 and papillary RCC has been established in several investigations.[12] In addition, the present study has identified a reduction in *CUL3*, in conjunction with the substrate adaptors RhoBTB1 and RhoBTB3, within the context of RCC.[25] Recent developments in genomic technologies have facilitated precise molecular characterization of cullin expression patterns throughout the nephron,[26] and transcriptome analysis has demonstrated abundant expression of various cullins along the nephron segments.[9]

Cullins represent a family of proteins that serve as a scaffold for E3 ubiquitin ligase and are linked to tumor development. *CUL1* has been identified as a key player in several types of cancer, including lung cancer,[27] glioma,[28] hepatocellular carcinoma,[29] melanoma,[30] gastric cancer,[31] and breast cancer.[32] Studies have indicated that its expression levels are associated with poor prognosis. Previous research has shown that *CUL2* serves primarily as a scaffolding protein for the E3 ubiquitin ligase during viral infection.[33,34] Xu *et al.*[30] observed significantly elevated *CUL2* expression in cervical cancer cells and

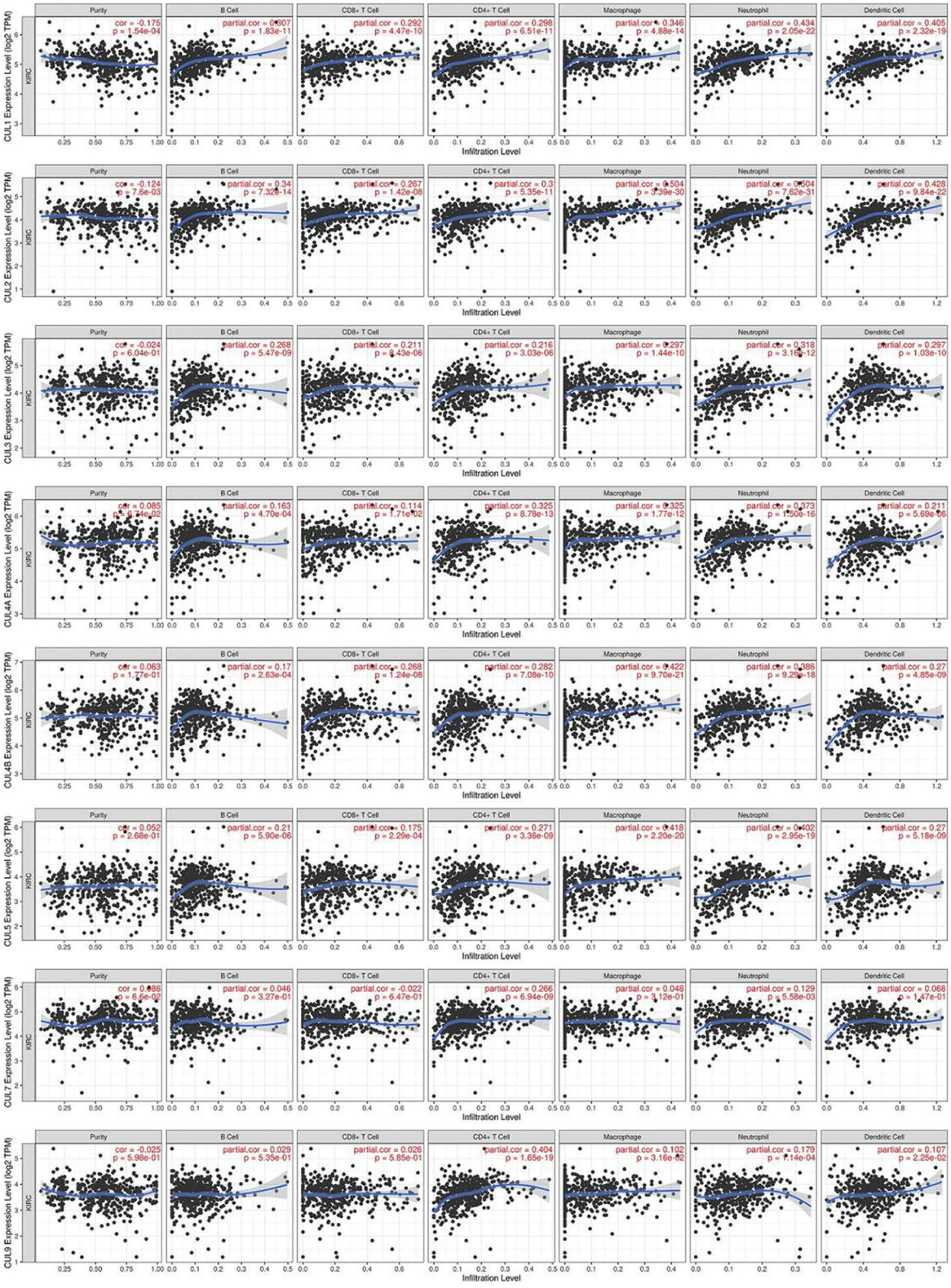


Figure 6. Correlations between cullin expression and immune cell infiltration in KIRC patients (TIMER). The results showed that *CUL1* and *CUL2* mRNA expressions were inversely associated with tumor purity.
Abbreviations: KIRC: Kidney renal clear cell carcinoma; mRNA: Messenger ribonucleic acid.

tissues harboring HPV16, in contrast to those positive for other high-risk genotypes. *CUL4A* and *CUL4B*, two CRL4 proteins, have been reported to have increased expression or amplification in a wide range of cancers.[6,32,35-39] *CUL7* (also known as KIAA0076, p185, p193) is an oncogene that enhances cell growth, migration, proliferation, and invasion in hepatocellular carcinoma.[40,41] By increasing p27, p53, and p21 protein expression, *CUL7* knockdown decreased lung cancer cell proliferation and increased survival.[41,42] The downregulation of *CUL7* also enhanced microtubule re-establishment and induced migratory and invasive capabilities in breast cancer cell lines, such as MDAMB-231 and BT549.[43,44] *CUL7*, associated with Rabl3, is implicated in adverse prognostic outcomes for hepatocellular carcinoma and in paclitaxel responsiveness in breast cancer cellular models.[32]

In our study, we conducted GO and KEGG enrichment analyses to investigate the interplay between the cullin genes and their associated genes. The biological processes identified include ubiquitin-dependent protein degradation, ubiquitin-mediated proteolysis, and protein polyubiquitination. In addition, the activation of the APC/C complex between the G1/S phase and early anaphase, Golgi vesicle trafficking, and mitotic nuclear division were also enriched.

The TME plays a pivotal role in tumorigenesis and therapy response.[23] Immune cells residing within TME can either promote or inhibit tumor growth, thereby contributing to varied clinical outcomes.[45] The profile of tumor-infiltrating immune cells is increasingly acknowledged as a prognostic biomarker for customized treatment selection and better patient management.[46] Using the TIMER database, we found that cullin expression is closely linked to the infiltration of six immune cell types in KIRC: dendritic cells, B cells, $CD4^+$ T cells, macrophages, neutrophils, and $CD8^+$ T cells. This suggests that cullins may be associated with both immunological status and disease prognosis. Our study aims to provide insights into the immune landscape of KIRC, facilitating the advancement of novel immunotherapeutic strategies. The immune response has been identified as a key aspect of carcinogenesis and treatment efficacy in KIRC.[23] In view of the considerable morbidity and mortality associated with KIRC, identifying prognostic molecular markers that alter immune response is essential for improving patient outcomes.

In the context of cancer, alterations in the cullin protein expression are of significant clinical significance. As high expression of some cullin genes is associated with tumorigenesis and progression, they can serve as potential diagnostic markers. For instance, *CUL1* is expressed at high levels in a variety of cancers and is associated with a poor prognosis. Such alterations in expression can aid medical professionals in identifying potential tumors in the early stages of the disease. This, in turn, can enhance the efficacy of treatment and patient survival rates. Furthermore, in KIRC, high expression of *CUL1 – 3, CUL4A, CUL4B, CUL5,* and *CUL7* correlates with favorable OS, whereas *CUL9* is linked to poor prognosis. This differential prognostic value highlights the potential for personalized treatment planning based on cullin expression profiles, allowing clinicians to adopt more aggressive therapies or intensive monitoring for high-risk patients.

Nevertheless, our research has certain limitations. First, all data were gathered and evaluated through online sources; hence, more research involving *in vitro* tests and clinical samples is needed to validate our findings. Second, the underlying mechanisms, molecular connections, and clinical applications of individual cullin family members in KIRC remain incompletely understood and warrant further investigation. Finally, as a retrospective investigation, prospective outcomes clinical validation is essential to confirm the observed associations.

Future research should focus on elucidating the molecular mechanisms through which cullin proteins regulate tumor and immune-related processes in KIRC. Integration of advanced genomic and proteomic techniques may offer deeper insights into their functional roles. Furthermore, clinical trials are needed to validate cullins as reliable biomarkers and therapeutic targets. The development of small-molecule inhibitors or immunomodulators specifically targeting cullin pathways may open new avenues for precision oncology.

5. Conclusion

The present study investigated the potential value of cullin family members as diagnostic and prognostic biomarkers for KIRC through a systematic bioinformatic analysis. First, the mRNA expression patterns of cullin family members in renal cancer patients were analyzed using the ONCOMINE database. The results demonstrated that *CUL1*, *CUL2*, *CUL4A*, *CUL4B,* and *CUL9* exhibited significant up-regulation in renal cancer tissues, whereas *CUL3*, *CUL5,* and *CUL7* demonstrated significant downregulation. Subsequently, the prognostic value of cullin family members in patients with KIRC was further evaluated, revealing that the genes may be involved in the development of KIRC. To further investigate their biological mechanisms, we constructed an interaction network comprising cullin family members and their functionally associated genes using GeneMANIA, identifying 20 coexpressed genes in the process. Functional

enrichment analysis for differentially expressed cullin genes and their coexpressed counterparts was conducted using the Metascape platform. Furthermore, the PPI network was constructed and analyzed by MCODE. *CUL1*, *CUL2*, and *CUL3* were found to be predominantly involved in a variety of biological processes, particularly those related to proteasome-mediated, ubiquitin-dependent proteolytic metabolism. Finally, leveraging the TIMER database, we systematically explored the relationship between cullin gene expression and immune cell infiltration within the TME. We found that the expression levels correlated significantly and positively with the infiltration of diverse immune cell populations. In conclusion, this study demonstrates that cullin family members may serve not only as potential diagnostic and prognostic biomarkers for KIRC but also as promising targets for the development of immunotherapeutic strategies. These findings provide a theoretical basis and new direction for future research into the pathogenesis and clinical treatment of KIRC.

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Conflicts of interest

Shaoping Ji is an Associate Editor of this journal, but was not in any way involved in the editorial and peer-review process conducted for this paper, directly or indirectly. Separately, other authors declared that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

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Investigation: Shaohao Liu, Yange Wang, Dandan Wang
Methodology: Shaohao Liu, Yange Wang, Dandan Wang
Writing – original draft: Shaohao Liu, Yange Wang, Dandan Wang
Writing – review & editing: Shaohao Liu, Fei Wang

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data

Data are available from the corresponding author upon reasonable request.

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