Translational Oncology 21 (2022) 101442

Contents lists available at ScienceDirect

Translational Oncology

journal homepage: www.elsevier.com/locate/tranon

Preliminary study on the role of the C5orf46 gene in renal cancer

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ARTICLE INFO

Keywords:
C5orf46
Prognostic
Biomarker
Immune cells
Renal cancer

ABSTRACT

Background: C5orf46 has been found to have antibacterial and anti-inflammatory effects via sequencing and microarray technologies, but its effects on cancer are unclear.
Methods: C5orf46 expression in renal cancer patients and cell lines was measured by quantitative polymerase chain reaction (qPCR). RNA sequencing data and clinicopathological information from renal cancer patients extracted from The Tumor Genome Atlas (TCGA) were analyzed to evaluate the prognostic value of C5orf46. The role of C5orf46 in vitro was verified by migration, proliferation and apoptosis experiments in renal cancer cell lines. Furthermore, the transcriptome of renal cancer cell lines with C5orf46 knocked down was sequenced to analyze potential signaling network pathways. Finally, the possible mechanisms of C5orf46 involvement in renal cancer development were analyzed by evaluating the immune microenvironment, mutation status and methylation levels.
Results: C5orf46 was highly expressed in renal cancer and was an independent prognostic factor. In vitro cell experiments showed that inhibition of C5orf46 expression could reduce renal cancer cell proliferation and migration and increase apoptosis. Transcriptomic sequencing after knockdown of C5orf46 in renal cancer cells revealed that it is involved in the malignant phenotype and immune microenvironment regulation of renal cancer. Finally, public databases suggest that C5orf46-related immune cell infiltration, mutational potential, and low methylation levels may contribute to poor prognosis in renal cancer.
Conclusion: These findings suggest that C5orf46 is associated with renal cancer progression and could be a potential target for improving renal cancer prognosis.

Background

Renal cancer, a common type of urinary system tumor, continues to increase in incidence throughout the human population [1] and is increasing the overall societal medical and financial burden [2]. The main histological subtypes of renal cancer include clear cell renal cell carcinoma (CcRCC), papillary renal cell carcinoma (PRCC) and chromophobe renal cell carcinoma (ChRCC), as well as other less common subtypes [3]. Despite our growing understanding of renal cancer and the available treatments, research questions remain for patients with locally advanced disease and distant metastases [4]. Therefore, new targets for the diagnosis and treatment of renal cancer have long been the focus of attention of urologists and oncology researchers [5].

C5orf46 is a protein-coding gene located in the open reading frame of chromosome 5. Few functional studies of the C5orf46 gene have been performed. Kunhong Zhong *et al.* [6] first reported in 2021 that C5orf46 can encode a small amphipathic secreted peptide. This peptide is an antimicrobial protein with a direct antibacterial effect on gram-negative bacteria. Zhong *et al.* referred to it as an antimicrobial peptide containing 64 amino acid residues (AP-64). Dylan J. Harney *et al.* [7] described a new enrichment assay (Small-protein Enrichment Assay, SPEA) for identifying proteins and found the previously undiscovered C5ORF46 protein in human plasma for the first time. Yanjie Zhao *et al* [8]. identified C5orf46 as a potential biomarker for poor prognosis in gastric cancer patients through bioinformatics analysis, but this finding was not validated experimentally.

The immune microenvironment is an important factor influencing cancer progression, and immunotherapy is now considered an effective adjuvant therapy for cancer treatment, especially for advanced metastatic cancers [9]. Renal cancer is considered an immune-responsive cancer [10]. C5orf46 can participate in the immune response, but its role in renal cancer is unknown. Therefore, our study aimed to preliminarily explore the role of C5orf46 in renal cancer, including its expression in renal cancer, its predictive value for clinical prognosis and

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https://doi.org/10.1016/j.tranon.2022.101442
Received 4 March 2022; Received in revised form 15 April 2022; Accepted 24 April 2022

its relationship with the tumor microenvironment.

Materials and methods

Tissue specimens and cell lines

Cancer tissues and paracancerous control tissues from patients with renal cancer were obtained from the First Affiliated Hospital of Nanchang University. The specimens were obtained with the informed consent of patients and the approval of the Ethics Committee of the First Affiliated Hospital of Nanchang University. Renal cancer cell lines (A498 and OSRC-2) and human renal tubular epithelial cells (HK-2) were purchased from the Chinese Academy of Sciences and cultured in Modified Eagle Medium (MEM), RPMI 1640 and DMEM/F12 medium supplemented with 10% fetal bovine serum (FBS), respectively.

Cell phenotyping assay

The Transwell migration assay was used to determine cell migration ability as follows: 4×10^4 treated cells were resuspended in 200 μl of serum-free medium and inoculated in the upper chamber of Transwell plates. The lower chamber was filled with 600 μl of medium containing 20% fetal bovine serum. After 24 hours of incubation, the cells in the lower chamber were collected, fixed and stained. Other cell function assays, such as plate colony formation [11] and Cell Counting Kit-8 (CCK-8) assays [12] and flow cytometry analyses of the cell cycle [13] and apoptosis [14], were conducted as previously described.

qPCR and sequencing

Cellular RNA was extracted with TRIzol and reverse-transcribed into cDNA by a Transgen (Beijing, China) kit for real-time quantitative polymerase chain reaction. Three pairs of renal cancer cell RNAs transfected with C5orf46-knockdown siRNA (si-1, si-NC) (HanBio, China) were extracted and sent to Novogene Co., Ltd. (Beijing, China) for transcriptome sequencing, as previously described [15].

Data mining

TIMER 2.0 [16](http://timer.cistrome.org/), TCGA(http://tcga-data.nci.nih.gov/tcga), Gene Expression Omnibus (GEO)(https://www.ncbi.nlm.nih.gov/gds/) and Human Protein Atlas (HPA) database [17] (https://www.proteinatlas.org/) were used to obtain C5orf46 expression at the transcriptional and protein levels in renal cancer. The clinical characteristics and prognostic value of C5orf46 in renal cancer patients in the TCGA database were analyzed using the R packages (http://www.r-project.org) ggplot [18], pROC [19], survminer [20] and rms [21]. C5orf46-related changes in the immune microenvironment were obtained from TIMER 2.0, the TISIDB website [22] (http://cis.hku.hk/TISIDB/) and Kaplan–Meier Plotter [23] (http://kmplot.com/analysis/). COSMIC [24](https://cancer.sanger.ac.uk/cosmic/), cBioPortal [25,26](http://www.cbioportal.org/), UALCAN [27](http://ualcan.path.uab.edu) and UCSC Xena [28](https://xena.ucsc.edu/) databases were used to mine the C5orf46 mutation and methylation information.

Statistical analyses

Differential expression analysis was statistically calculated using the Wilcoxon signed-rank sum test in GraphPad Prism 9.2 (GraphPad Software, La Jolla, CA, USA). Sequencing data, mining of TCGA, GEO and other datasets, using statistical packages [29] in R language was conducted for automatic statistical analysis.

Results

C5orf46 is upregulated in renal cancer

The analysis of C5orf46 expression in different cancers and normal tissues was performed using TIMER 2.0. The results showed that C5orf46 was highly expressed in various cancers, including kidney, breast, lung and colorectal cancers (Fig. 1**A**). We used 539 kidney renal clear cell carcinoma (KIRC) tissues and 72 paired normal tissues from the TCGA database to further verify the expression of C5orf46 and found that C5orf46 was overexpressed in KIRC tissues (Fig. 1**B, C**). The same results were also validated using the GEO datasets GSE53000 [30] (56 renal cancer samples and 6 normal samples) and GSE40435 [31] (101 pairs of clear cell renal cell carcinoma and adjacent nontumour renal tissue) (Fig. 1**D, E**). The protein expression level of C5orf46 in renal cancer was obtained from immunohistochemical staining data from the HPA database (Atlas antibody, HPA079692). Compared with normal tissue (patient ID: 1859, 2184 and 2530), the expression of C5orf46 protein in renal cancer tissue (patient ID: 2261, 2477 and 3541) was significantly upregulated (Fig. 1**F**). Finally, we verified the high expression of C5orf46 in 14 pairs of renal cancer tissues collected from the First Affiliated Hospital of Nanchang University, and renal cancer cell lines A498, and OSRC-2 (Fig. 1**G, H**). Taken together, these results indicate that C5orf46 expression is upregulated in renal cancer.

Clinical value of C5orf46

As shown in Fig. 2**A**, the forest plot based on univariate Cox regression showed that C5orf46 is a risk factor for renal cancer, similar to tumor (T), node (N), metastasis (M) stage, histologic grade and pathologic stage. Similarly, we analyzed the expression and clinical data regarding C5orf46 in the TCGA-KIRC (539 KIRC tissues and 72 paired normal tissues) dataset and found that the transcription level of C5orf46 in renal cancer correlated with treatment outcome, tumor T, N, and M stage and pathological grade **(Supplementary material, S1 A-F)**. High expression of C5orf46 may predict poor prognosis in KIRC, and we used the Kaplan–Meier plotter to study the relationship between the expression of C5orf46 and the survival of KIRC patients. As shown in Fig. 2**B-D**, renal cancer patients with high C5orf46 levels had worse overall survival (OS: HR=1.55 (1.15~2.10), p=0.004), disease-specific survival (DSS: HR=2.22 (1.48~3.33), p<0.001) rates and progression-free intervals (PFI: HR=2.58 (1.84~3.62), p<0.001). Furthermore, we used the R package rms [21] & survival [20] to incorporate the histologic grade, primary therapy outcome and C5orf46 expression of renal cancer patients into a nomogram to predict patient survival outcomes at 1, 3 and 5 years (Fig. 2**E**). The nomogram showed good performance in predicting OS rates in renal cancer with a C-index of 0.817, and calibration plots for predicting model performance also showed good agreement (Fig. 2**F-H**). Finally, receiver operating characteristic (ROC) curves were used to evaluate the diagnostic value of C5orf46 in renal cancer. We found that C5orf46 showed good diagnostic performance in different groups (area under the ROC curve >0.94) (Fig. 2**I**). In summary, high expression of C5orf46 is associated with poor prognosis in renal cancer and may have high prognostic evaluation and diagnostic potential.

Silencing C5orf46 inhibits renal cancer progression

We performed phenotypic experiments in cell lines (A498 and OSRC-2) to verify that C5orf46 affects renal cancer progression. First, we determined that C5orf46 is predominantly located in the cytoplasm using nucleoplasmic isolation experiments, as previously described [32] (Fig. 3**A**). Next, we performed functional experiments by transfecting siRNAs to knock down the expression of C5orf46 in renal cancer cell lines (Fig. 3**B-C**). Transwell assay data showed that silencing C5orf46 inhibited the migration of A498 and OSRC-2 cells (Fig. 3**D, E**). Plate

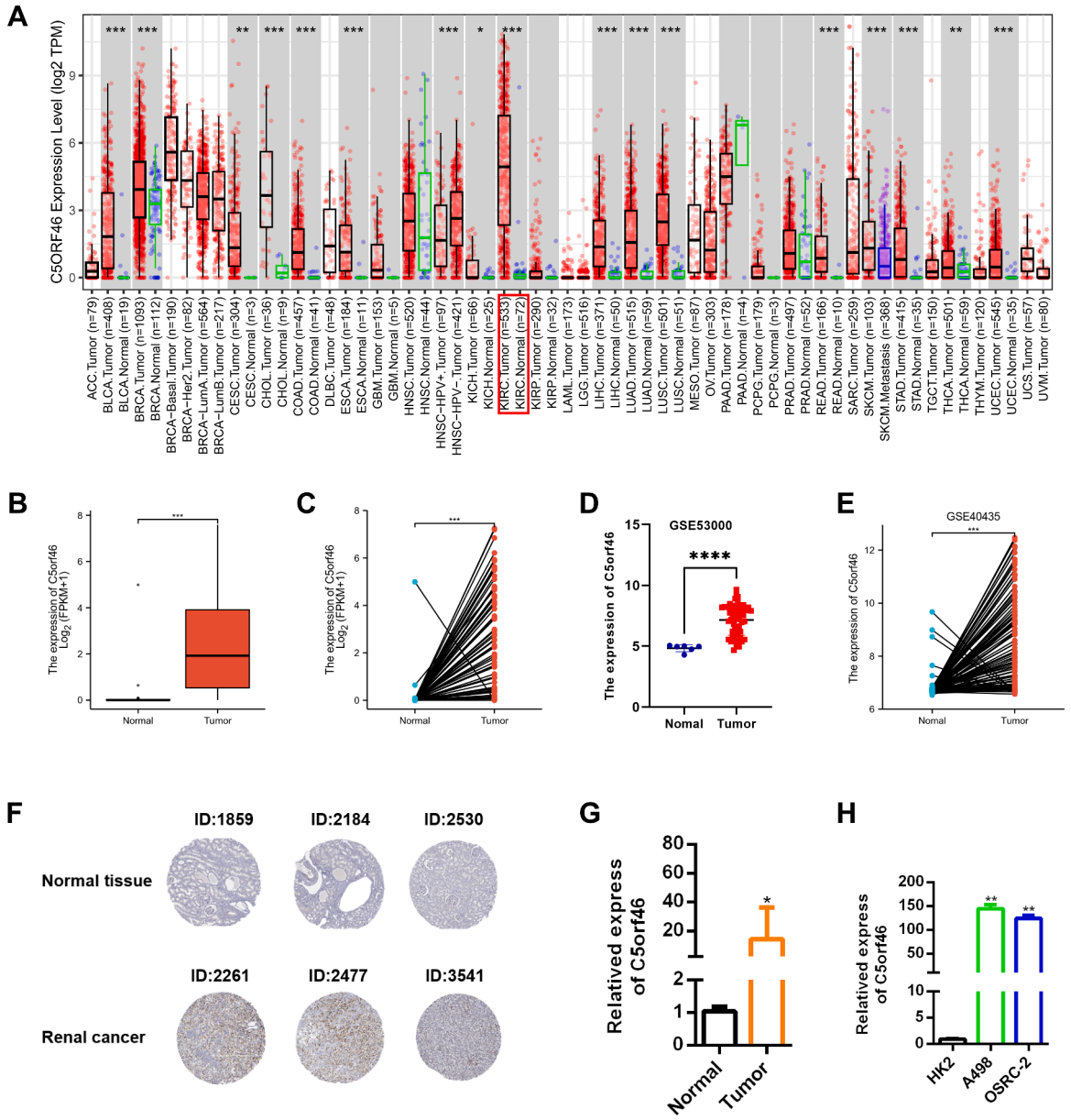

FIG. 1. C5orf46 expression is upregulated in renal cancer. **(A)** Expression analysis of C5orf46 using TIMER 2.0 for pancancer. **(B, C)** TCGA database analysis of C5orf46 expression in unpaired and paired renal cancer tissues. **(D, E)** C5orf46 expression levels in the GEO datasets GSE53000 and GSE40435. **(F)** Immunohistochemical map of C5orf46 antibody staining in renal cancer tissue and normal renal tissue. Positive staining = brown color. **(G, H)** Validation of C5orf46 expression in renal cancer tissues and cell lines. *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001.

colony formation and CCK-8 assays showed that inhibition of C5orf46 reduced renal cancer cell colony formation and inhibited cell growth compared with the transfected negative control (si-NC) group (Fig. 3**F-I**). Cell cycle progression is associated with proliferation; therefore, we used flow cytometry to examine the effect of C5orf46 knockout the renal cancer cell cycle. The results showed that knocking down C5orf46 arrested the renal cancer cell cycle in the G0/G1 phase (Fig. 3**J, K**). Proliferation, cell cycle progression and apoptosis can influence each other. We further analyzed apoptosis by flow cytometry using Annexin V-PI costaining. The results showed that apoptosis was increased in A498 and OSRC-2 cell lines with the C5orf46 gene silenced (Fig. 3**L, M**). The above results imply that C5orf46 can promote the migration and proliferation of renal cancer cells in vitro, consistent with the findings of the previous bioinformatics analyses.

Sequencing analysis after knockdown of C5orf46

We extracted total RNA from three sets of OSRC-2 cells with silenced C5orf46 (si1) and negative control (NC) groups and performed transcriptome sequencing analysis to comprehensively elucidate the biological function of C5orf46 in renal cancer. Supplementary material **S2 A** shows a cluster heatmap of the differentially expressed genes between the two groups. Genes upregulated in the knockdown C5orf46 group (si1) were expressed at low levels in the control group (NC), and conversely, genes downregulated in the si1 group were highly expressed in the NC group. A volcano map visualizing the distribution of differentially expressed genes between the two groups was constructed, as shown in **S2 B**. There were 1103 significantly differentially upregulated genes and 1233 downregulated genes in the C5orf46 knockdown group compared to the control group. A Venn diagram **(S2 C)** was generated to demonstrate the number of common and unique differential genes

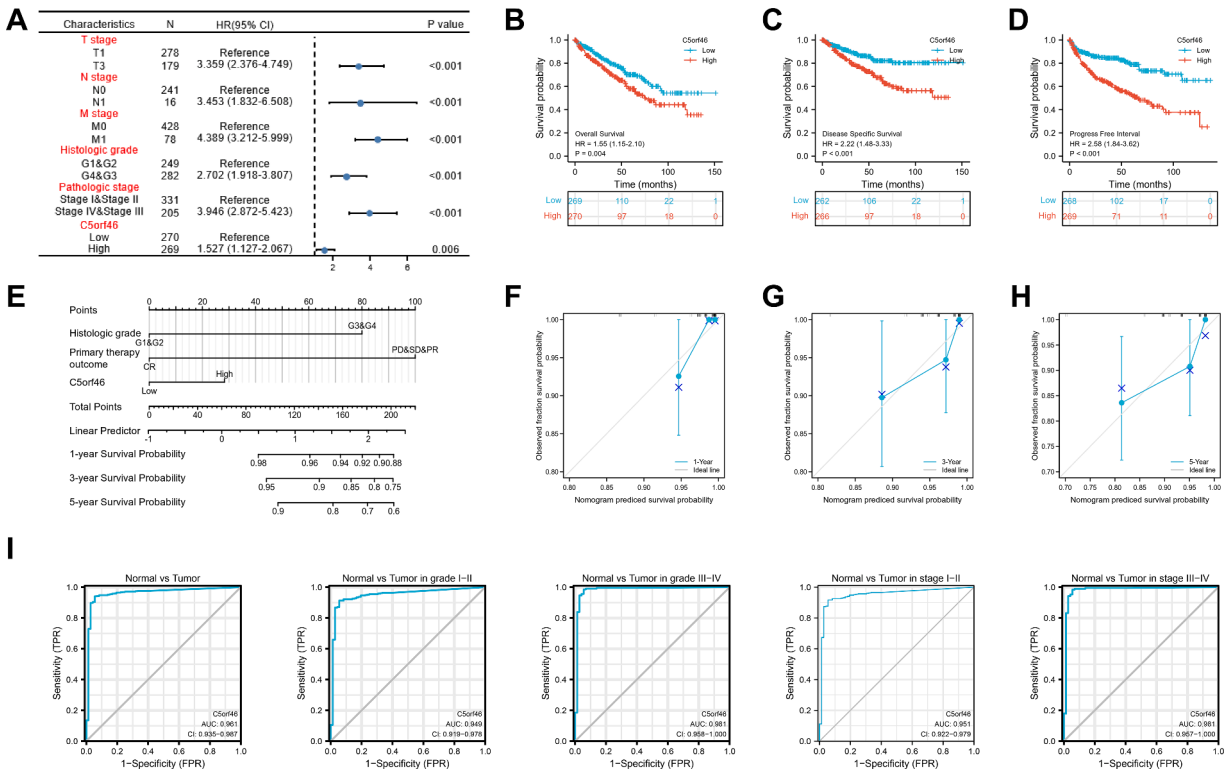

FIG. 2. The clinical value of C5orf46 in renal cancer. **(A)** Univariate Cox regression forest plots of risk factors for renal cancer. **(B-D)** Kaplan–Meier curves of C5orf46 expression and OS, DSS, and PFI in KIRC patients. **(E-H)** Nomogram of C5orf46 expression in renal cancer patients and performance calibration graph. **(I)** ROC curves demonstrating the diagnostic value of C5orf46 in renal cancer.

between the two groups. We screened for 11,563 common differential genes; 328 unique genes were found in the si1 group, and 381 in the NC group. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genome (KEGG) analyses were performed on the differentially expressed genes, as shown in Fig. 4**A, B**. The C5orf46 gene may be involved in immune responses in multiple pathways and is associated with biological processes such as cell adhesion and proliferation. Fig. 4**C, D** shows the protein–protein interaction (PPI) network analysis of differential gene expression between the two groups based on sequencing data and the string database [33] (http://string-db.org/). We obtained potential target genes with a significant increase in known interactions among them. Gene set enrichment analysis (GSEA), as shown in Fig. 4**E**, showed that the genes in the NC group were mainly enriched in tumor pathways, immune infiltration, cell adhesion and other related signaling pathways, suggesting that C5orf46 may be a potential indicator of the state of the immune microenvironment in renal cancer.

Correlation of C5orf46 with immunomarkers in renal cancer

The R package GSVA [34] was used to analyze the correlation between C5orf46 and immune infiltration in renal cancer (TCGA-KIRC, 539 KIRC tissues). The results suggested that C5orf46 was positively correlated with the degree of infiltration of Tregs, B cells, NK cells and macrophages, and negatively correlated with Th17 cells (Fig. 5**A**). In the TISIDB database (534 KIRC tissues), we also found three immune factors positively associated with C5orf46 gene expression in renal cancer, chemokine receptor CXCR4 (C-X-C Motif Chemokine Receptor 4), inflammatory amplification factor TMEM173 (Stimulator Of Interferon Response CGAMP Interactor 1) and oncogenic factor TGFB1 (Transforming Growth Factor Beta 1) (Fig. 5**B-D**). In addition, the relationship between C5orf46 expression in renal cancer and immune subtypes was analyzed by the TISIDB database. As shown in Fig. 5**E**, C5orf46 was most highly expressed in the INF-gamma dominant and inflammatory subtypes and least expressed in the immunologically quiet subtype. This result suggests that C5orf46 expression is directly related to immunity and is involved in renal cancer progression. We further compared the C5orf46 gene at different immune cell copy numbers in renal cancer using the TIMER database SCNA module. As shown in Fig. 5**F**, there were no statistically significant differences in infiltration changes per copy number status for each immune subset in renal cancer. This result suggests that the copy number of C5orf46 does not affect immune cell infiltration. Finally, we plotted the survival curves of C5orf46 gene expression against different immune cell-infiltrated renal cancers (288 KIRP, kidney renal papillary cell carcinoma) on the Kaplan–Meier plotter website. Patients with high C5orf46 expression and abundant immune cell subtypes had shorter survival times (Fig. 5**G**). Overall, these data suggest that C5orf46 may be involved in immune infiltration in renal cancer directly or indirectly and ultimately affects patient prognosis.

Mutation and DNA methylation status of C5orf46 in renal cancer

The COSMIC website shows the distribution of different types of mutations in C5orf46 in cancer (Fig. 6**A**). Missense substitution and synonymous substitution were the most common mutation types, and G>A and C>T were the most common substitution mutations. Mutations in the C5orf46 gene in renal cancer (TCGA, Firehose Legacy; Nature 2013; PanCancer Atlas) were analyzed by the cBioPortal database. The results revealed significantly altered C5orf46 copy number in 9% of renal cancer patients (Fig. 6**B**). Alterations in DNA methylation affect gene expression and are involved in tumor progression. We explored the differences in the DNA methylation of C5orf46 between normal control tissues and renal cancer tissues (TCGA-KIRC: 160 normal, 324 primary tumor) based on the UALCAN and UCSC Xena (Illumina human methylation 450 (n=483)) platforms. We found that promoter

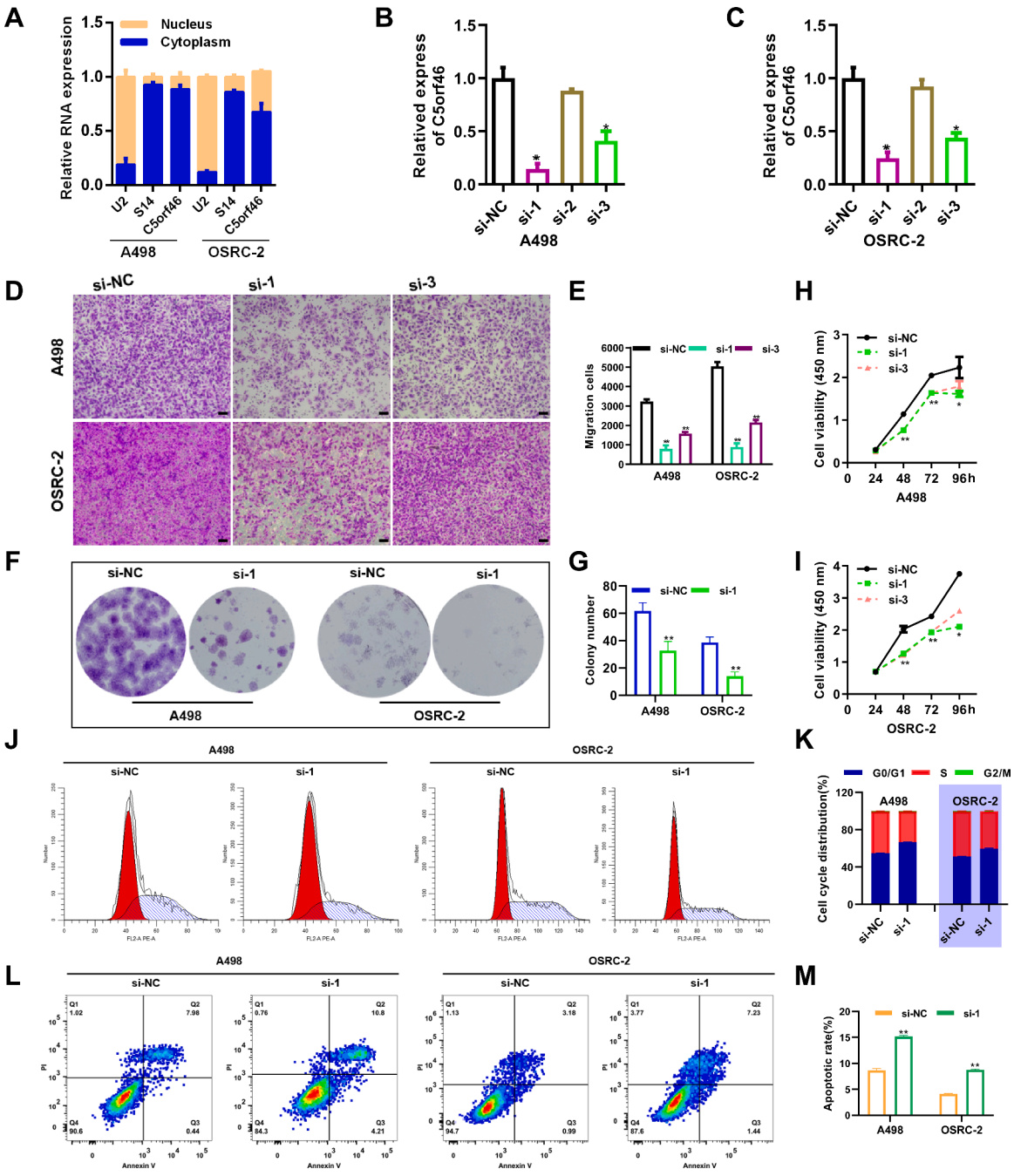

FIG. 3. Knockdown of C5orf46 inhibits the tumorigenicity of renal cancer. **(A)** Subcellular localization of C5orf46 in renal cancer cell lines. **(B, C)** Knockdown efficiency of three siRNAs of C5orf46 in the renal cancer cell lines A498 and OSRC-2. NC, negative control. **(D, E)** Changes in the migratory capacity of A498 and OSRC-2 cells in Transwell assays after knockdown of C5orf46. Scale bars, 50 μm. **(F-K)** Changes in cell proliferation capacity after knockdown of C5orf46 on plate clones, CCK-8, and cell cycle assays. **(L, M)** Effect of C5orf46 knockdown on apoptosis levels in A498 and OSRC-2 cells. *p<0.05; **p<0.01.

methylation of C5orf46 was lower in renal cancer than in the normal group and more significant in higher stages and grades **(**Fig. 6**C-F)**. The correlation between the degree of methylation of C5orf46-DNA methylation sites in renal cancer (TCGA-KIRC and Illumina human methylation 450, 485 KIRC) was analyzed using the R package ggplot2 [18]. The results revealed that C5orf46 molecular expression was negatively correlated with cg18818210 and cg24686270 probes, which may represent their specific methylation sites in renal cancer **(**Fig. 6**G, H)**. In conclusion, C5orf46 gene mutation and methylation may affect C5orf46 expression levels; however, more specific experiments are needed to verify this hypothesis.

Discussion

Renal cell carcinoma is the most common kidney malignancy, with over 25% of renal cancer patients presenting with locally advanced or metastatic disease at diagnosis and 20-40% of patients with localized primary tumors developing metastatic disease [35]. As radiotherapy and chemotherapy are ineffective for patients with metastatic renal cell carcinoma, these patients are not treated adequately and effectively and have poor prognoses. In the last decade, small molecule inhibitors, RNA-targeting strategies, immunomodulatory drugs and targeted therapeutics based on the initiation/editing of the immune system have provided hope to patients with metastatic renal cancer. However, the

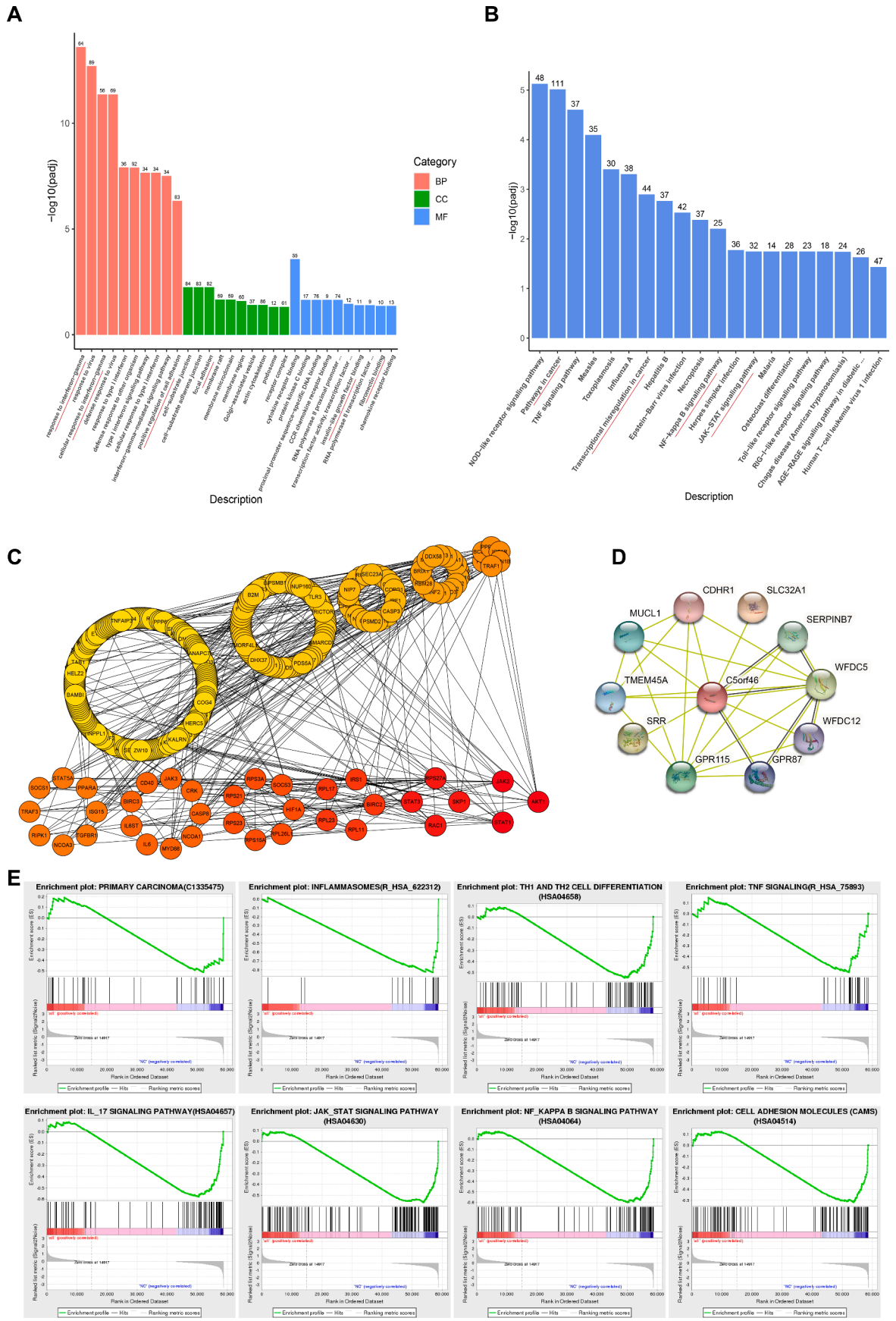

FIG. 4. Analysis of the sequencing results of C5orf46 knockdown. **(A, B)** GO and KEGG enrichment analysis plots for genes that differ between the two groups. BP, biological process; CC, cellular component; MF, molecular function. **(C, D)** PPI network analysis from sequencing data and the String database. **(E)** Pathway enrichment analysis of C5orf46 knockout versus control cells. NC, negative control.

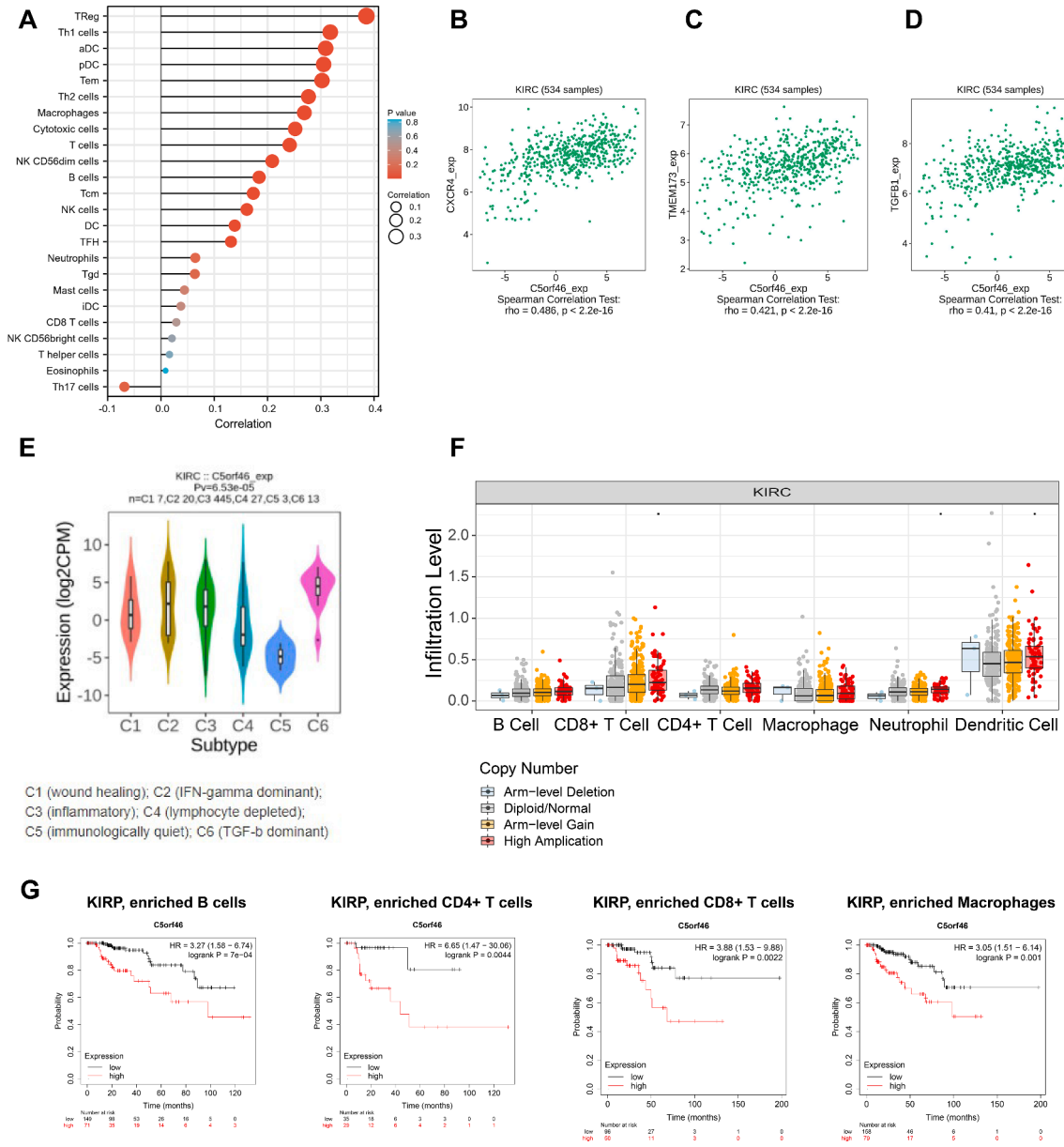

FIG. 5. C5orf46 is associated with the immune microenvironment. **(A)** Immune cell subtypes associated with C5orf46 in renal cancer. **(B-D)** Correlation of C5orf46 gene expression with CXCR4, TMEM173 and TGFB1 in renal cancer. **(E)** Relationship between C5orf46 expression and immune subtypes in renal cancer. **(F)** Changes in the infiltration of immune subpopulations at each copy number status of C5orf46. **(G)** KM profile of C5orf46 gene expression in renal cancer with different immune cell infiltration levels. KIRP, kidney renal papillary cell carcinoma.

mortality rate of patients with advanced metastatic renal cancer has not changed substantially, and treatment remains a serious challenge [36].

C5orf46 is a new gene molecule recently identified as a prognostic predictor in gastric cancer, and its product has bactericidal and anti-inflammatory properties. However, the additional functions of C5orf46 and its mechanisms in other cancers have not been reported. We found aberrant expression of C5orf46 in renal cancer using data obtained from several website and datasets and from our own collection of tissue specimens and cell lines. Combined with clinicopathological parameters, it was found that high C5orf46 expression was associated with poor prognosis in renal cancer patients, and C5orf46 expression levels had high diagnostic value. To further validate the results of our bioinformatics analysis, we used siRNA knockdown of C5orf46 to experimentally verify its effects on proliferation, migration and apoptosis in renal cancer cell lines. We also analyzed the differentially expressed genes and associated signaling pathway networks associated with the immune microenvironment in the knockdown C5orf46 group and the control group by transcriptome sequencing.

Tumor immunotherapy has emerged as an effective treatment for tumors and has demonstrated significant therapeutic benefits in melanoma, non-small cell lung cancer, renal cancer and prostate cancer [37]. The tumor microenvironment comprises the tissue surrounding the tumor, including immune cells, stromal cells and the extracellular matrix [38]. The tumor microenvironment, especially the immune microenvironment, has been intensively studied in recent years. Various molecules and cells in the tumor immune microenvironment can influence tumor development and treatment outcomes [39]. Therefore, exploring more effective tumor immune-related biologic factors based on high-throughput sequencing and microarray technology can help prolong patient survival. Renal cancer is considered an immunogenic tumor, uniquely characterized by the extent of leukocyte infiltration and a luxurious vascular system driven by vascular endothelial growth factor

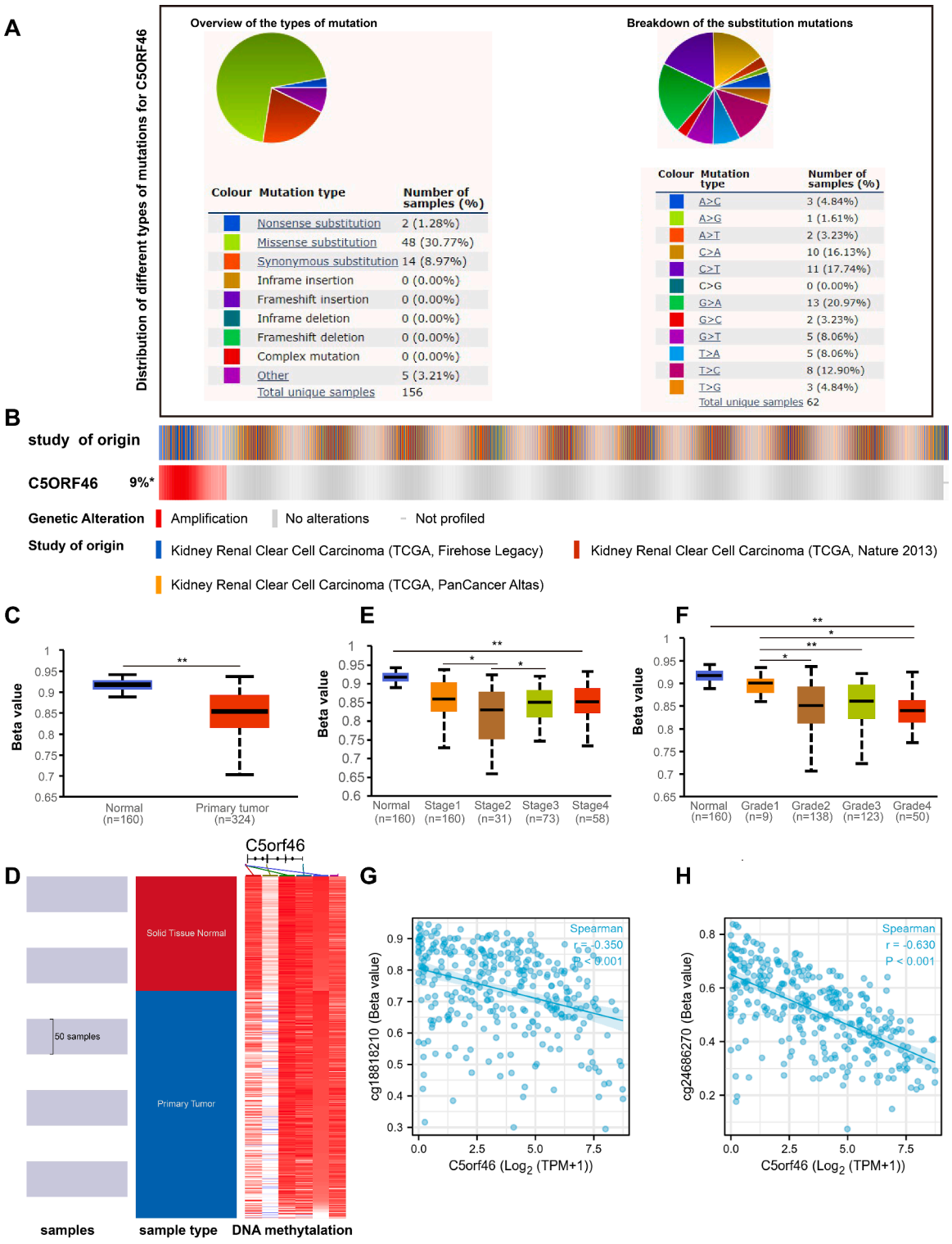

FIG. 6. The mutational and methylation status of C5orf46 in renal cancer patients. **(A)** The distribution of different types of C5orf46 mutations in cancer. **(B)** C5orf46 mutation status analyzed in the cBioPortal database. **(C-F)** Differences in the DNA methylation of C5orf46 between different renal cancer tissue sample types. **(G, H)** Correlation of C5orf46 molecular expression with methylation sites cg18818210 and cg24686270 probes. *p<0.05; **p<0.01.

(VEGF) in the immune microenvironment. Extensive infiltration of Tregs, myeloid cells and other cell types inhibits tumor clearance by cytotoxic T cells and NK cells. These cells limit the effectiveness of immune surveillance and the activity of immune checkpoint inhibitors [37, 40, 41]. Therefore, combining other immunological agents may compensate for the current deficiencies in the treatment of renal cancer. We are the first to propose that C5orf46 may be involved in the tumor immune microenvironment (Fig. 4**A, B and** Fig. 5**A**). We also found that, together with immune cells, C5orf46 may influence the prognosis of renal cancer patients (Fig. 5**G**).

The accumulation of mutations can lead to cancer and aging. Genome sequencing has led to a deeper understanding of cancer somatic mutations, providing a detailed view of the mutational processes and genes that drive cancer [42]. Currently reported mutations in clear renal

cell carcinoma include BAP1 (BRCA1 Associated Protein 1), PBRM1 (Polybromo 1), SETD2 (SET Domain Containing 2, Histone Lysine Methyltransferase) and PIK3CA (Phosphatidylinositol-4,5-Bisphosphate 3-Kinase Catalytic Subunit Alpha). Papillary renal cell carcinoma exhibits high levels of mutations in MET (MET Proto-Oncogene, Receptor Tyrosine Kinase), and sarcomatoid renal tumors may be associated with mutations in TP53 (Tumor Protein P53) and NF2 (Moesin-Ezrin-Radixin Like Tumor Suppressor) [43]. Exploring the mechanism by which mutations affect the prognosis and treatment response of renal cancer patients can bring more benefits to patients. In our study, missense substitution was the most common mutation type in C5orf46 among 156 cancer samples, present in 48 (30.77%); synonymous substitution was present in 14 (8.97%). In terms of substitution mutations, among the 62 cancer samples, G>A was the most common in 13 (20.97%), followed by C>T 11 (17.74%), C>A 10 (16.13%), and T>C 8 (12.9%) **(**Fig. 6**A)**. The coding sequence (CDS) mutation substitutions were c.-13C>A and c.71-916T>A in two renal cancer samples from the COSMIC platform, while the amino acid mutation was unknown.

DNA methylation is a common and heritable epigenetic modification in eukaryotic cells and plays an important role in regulating cell proliferation and differentiation [44]. In recent years, abnormal DNA methylation levels have also been closely associated with tumor development [45]. Studies have found that the overall level of DNA methylation in tumor cells is low, and high levels may occur locally [46]. Current DNA demethylation drugs are poorly specific; thus, drugs with higher specificity are being investigated for cancer treatment [47]. Unlike somatic mutations in renal cancer, altered DNA methylation has often been associated with clinicopathological features and patient survival. Several prognostic methylation markers, such as BNC1 (Basonuclin 1), SCUBE3 (Signal Peptide, CUB Domain And EGF Like Domain Containing 3) and GATA5 (GATA Binding Protein 5), have also been reported in renal cancer, but none have led to changes in patient treatments [48]. We found that the hypomethylation status of C5orf46 in renal cancer may correlate with tumor stage and grade. **(**Fig. 6**C-F)**. Since the upstream and downstream methylation conditions corresponding to the initial position of C5orf46 may affect its expression, we further analyzed the possible DNA methylation sites of C5orf46 **(**Fig. 6**G, H)**. However, the expression of C5orf46 is affected by a variety of factors. In addition to DNA methylation, the transcription level is also affected by transcriptional regulatory processes, such as histone modifications and the actions of transcription factors. The causal relationship between C5orf46 expression and methylation needs further experimental verification. In summary, C5orf46 mutation and methylation may be mechanisms by which C5orf46 is involved in the development of renal cancer, but stronger evidence is needed.

Although we conducted a systematic analysis of C5orf46 in renal cancer, our study still has limitations. First, the transcriptomic data and clinicopathological information of the patients in this study were obtained from public databases. Data heterogeneity, platform differences, and incomplete information on relevant clinical treatments were present. Second, the immune-related mechanisms and the methylation of C5orf46 in renal cancer are based on bioinformatic predictions and lack further experimental validation. In the future, we will remedy these deficiencies and further validate the bioinformatics predictions. In summary, we conducted a preliminary study on the role of C5orf46 in renal cancer for the first time through public database analyses and experiments.

Conclusion

In this study, we found that C5orf46 was highly expressed at the transcriptome and protein levels in renal cancer and was associated with poor patient prognosis. C5orf46 may contribute to the development of renal cancer by affecting cell proliferation, migration and the immune microenvironment. Our study identified a pathogenic role for C5orf46 and provides a potential therapeutic target for renal cancer patients.

Ethics statement

All research involving human participants was approved by the Institutional Review Board of the First Affiliated Hospital of Nanchang University, and informed consent was obtained from participants.

Author contributions

Siyuan Wang designed the experiments. Ming Ma and Zhicheng Zhang analyzed the data. Yifu Liu, Zhilong Li, Shengqiang Fu and Qiang Cheng interpreted the results. Siyuan Wang and Ming Ma wrote the paper. All authors contributed to the article and approved the submitted version.

Consent

All research involving human participants was approved by the Institutional Review Board of the First Affiliated Hospital of Nanchang University, and informed consent was obtained from participants.

FUNDING

This work was supported by the National Natural Science Foundation of China (82060144)

S1 | **(A-F)** Expression levels of C5orf46 in various subgroups of KIRC. *p<0.05; **p<0.01; ***p<0.001.

S2 | **(A-C)** Heatmap, volcano map and Venn diagram of six sequenced samples are shown. NC, negative control.

Declaration of Competing Interest

We declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.tranon.2022.101442.

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