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ORIGINAL ARTICLE

DOK1: A Promising Biomarker for Tumor Prognosis and Targeted Therapy in Renal Clear Cell Carcinoma

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Abstract

This study aimed to investigate the prognostic significance of Docking protein 1 (DOK1) in renal clear cell carcinoma (ccRCC) and its association with immune cell infiltration, and explicitly verified by in vitro assays. Differences in DOK1 expression in ccRCC tissues and normal tissues were assessed from The Cancer Genome Atlas (TCGA) and Gene Expression Omnibus (GEO) databases, and prognostic analyses were also performed. Lentiviral vectors were utilized to construct 786-O and 769-P cell lines knocking down DOK1. The function of DOK1 was further verified by a series of in vitro assays. Functional enrichment analysis was performed using the "Cluster Profiler" software package. Using the Tumor Immune Estimation Resource (TIMER) and the Tumor Immune System Interaction Database (TISIDB), the relationship between DOK1 and immune infiltration was investigated. This study elucidates that high expression of DOK1 predicts poor prognosis and is associated with demographic factors and clinicopathologic characteristics of ccRCC patients. In vitro functional experiments further confirmed it. Detailed bioinformatics analysis showed that DOK1 expression was significantly correlated with multiple immune cell infiltrations. High expression of DOK1 predicts poor prognosis in ccRCC patients. Its close association with the immune microenvironment and immunotherapeutic targets may provide new ideas for tumor therapy.

Keywords: Renal clear cell carcinoma; DOK1; prognostic biomarker; immune infiltration

Introduction

Renal cell carcinoma (RCC) has the highest yearly mortality rate among urologic malignancies, and its global frequency has been increasing in recent decades[1, 2]. RCC is a diverse malignancy, with renal clear cell carcinoma (ccRCC) accounting for 75-80% of all RCC[3]. Because of the cancer's insidious onset and quick progression, a lot of individuals with ccRCC get a late-stage diagnosis. Since ccRCC patients are resistant to chemotherapy and radiation, the present treatment remains unsatisfactory. Thus, it is imperative to find novel targets and investigate targeted therapies[4, 5]. The proliferative process of ccRCC is intricate and multifaceted, including a complex network of diverse genetic backgrounds and various oncogenes that result in oncogene alterations[6, 7]. Exploring the molecular pathways underlying the progression of ccRCC is extremely valuable for diagnosis and treatment.

Docking protein (DOK) is a tyrosine residue phosphorylated protein that belongs to the new regulatory protein family[8]. It has a significant function in the receptor tyrosine kinase signaling pathway[9, 10]. There are currently seven members of the DOK family (DOK1-DOK7) that

have been identified. Of these, DOK1-DOK3 are preferentially expressed in immune and hematopoietic cells. Recent research has revealed a strong correlation between aberrant DOK1 expression and leukemia, gastric cancer, colorectal cancer, lung cancer, and breast cancer[11, 12]. However, there is currently no systematic research on renal cell carcinoma[12, 13]. In this study, we used bioinformatics methods to investigate the relationship between the expression of this gene family and the overall survival rate of renal clear cell carcinoma. The potential function and unique prognostic significance of DOK1 was further revealed through its relationship with the tumor microenvironment.

The aim of this study was to investigate the role of DOK1 in the development of ccRCC and its correlation with immune infiltration using bioinformatics methods. On this basis, comprehensive in vitro experiments were performed to systematically explore the function of DOK1.

1. Materials and methods

2.1 Data collection and difference analysis

The Cancer Genome Atlas (TCGA) (https://genome-cancer.ucsc.edu/) comprises clinical data, genetic variations, mRNA expression levels, miRNA expression patterns, methylation status, and other relevant information for a wide range of human cancers, including tumor subtypes. The level of DOK1 gene expression in pan-cancer was analyzed using it. Based on the median expression of DOK1, patients were divided into groups with high and low expression. Using the R program "limma," DEGs between the two groups were found by setting thresholds of $P < 0.05$ and $|\text{Log2 (Fold Change)}| > 1$.

Focusing on KIRC, the expression levels of DOK1 gene in normal and tumor tissues were further analyzed by TCGA, GEO (https://www.ncbi.nlm.nih.gov/geo/), UALCAN Online Database (http://ualcan.path.uab.edu/index.html) [14] and Clinical Proteomic Tumor Analysis Consortium (CPTAC) databases. These databases are user-friendly, open repositories that can be used to analyze publicly available cancer information. Its correlation with various clinicopathologic parameters was further explored through the TCGA database.

The Human Protein Atlas Database (HPA) (https://www.proteinatlas.org/)[15] demonstrated the differential expression of DOK1 in normal and tumor tissues by immunohistochemistry. By immunohistochemistry, the high expression of DOK1 in CCRCC tissues was confirmed at the protein expression level. It provided a more comprehensive understanding of the gene expression. As a base, it further guided us to explore the role and clinical significance of DOK1 in CCRCC.

2.2 Prognostic correlation analysis

Based on the GEO, EGA and TCGA databases, using receiver operating characteristic curve (ROC) by demonstrating the relationship between true positive rate (TPR) and false positive rate (FPR), aimed to assess the predictive ability of DOK1 in distinguishing between two different categories of samples (ccRCC tumor tissues and normal tissues). Using the Kaplan Meier plotter to assess the relationship between DOK1 expression levels and overall survival (OS) of ccRCC patients[16].

To better understand the association between gene expression and patient prognosis, separate subgroup analyses based on various clinical indicators of the tumors (tumor grade I-IV, lymphatic metastasis N0, N1, and distant metastasis M0, M1) were performed using Kaplan Meier plotter.

2.3 Protein Interaction and Enrichment Analysis

The interactions between DOK1 and other proteins were analyzed using the STRING database to obtain a visualization of the PPI network. STRING (https://cn.string-db.org/) is an online tool for searching and constructing PPI interaction networks using interacting genes and proteins. The following main parameters were used to create the DOK1 co-expression network: 1) active sources of interactions: co-expression, co-occurrence, text-mining, experiments, databases; 2) meaning of the network edges: evidence; 3) maximum number of interactions: and 4) minimum interaction score required: highly corroborative (0.700).

Co-expressed genes of DOK1 were analyzed by

TCGA database. Gene Ontology (GO) enrichment of co-expressed genes and Kyoto KEGG pathway analysis encyclopedia were performed using the ClusterPro file package, and the results were visualized using "ggplot2".

2.4 Immune Infiltration Correlation Analysis

The Tumor Immune Estimation Resource (TIMER 2.0) (https:// timer.cistrome.org/) [17], the Gene Expression Profiling Interactive Analysis (GEPIA) online database (http://gepia.cancer-pku.cn/index.html) [18], TISIDB (http://cis. hku.hk/TISIDB/index.php) [19] are all available to systematically analyze the immune infiltration of various malignant tumors, and the data were processed and visualized using the R Project for Statistical Computing and the Practical Extraction and Report Language, etc.

These databases were used to analyze the infiltration of various immune cell populations. The association between DOK1 expression and various immune cell markers, chemokines, and immune targets in ccRCC was also analyzed.

2.5 In vitro assay

2.5.1 Cell lines and cell culture

Clear cell renal cell carcinoma cell lines 769-P and 786-O (Chinese Academy of Sciences) were cultured with RPMI1640 (BI, Israel) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin and streptomycin. Incubate in the humidified incubator at 37 °C with 5% CO2. This study did not involve human participants and animal participants.

2.5.2 Western blot assay

Cells were collected after trypsin digestion and centrifugation. Lysate was prepared by mixing RIPA lysate, protease inhibitor PMSF and phosphatase inhibitor NaF (NCM Biotech). Ensure that the final concentration of PMSF is 1 mM and the final concentration of NAF is 10 mM. collect the cells and add the lysate, 20-30min on ice. After centrifuging and sonicating the supernatant, use the BCA technique (Beijing Tiangen Biotechnology Co., Ltd.) to determine the protein content. The protein was transferred to a PVDF membrane (Millipore, Bedford, MA, USA) after SDS-PAGE separation. Use β-actin as a loading control. After leaving it overnight in the main antibody, let it sit in the secondary antibody for two hours. Using Image Quant LAS4000 (General Electric, Boston, Massachusetts, USA), protein bands were found, and ImageJ software was used to quantify them.

2.5.3 CCK8 assay

Cells were inoculated into 96-well plates with $3*10^3$ cells per well. After inoculation for 1 day, 10 μl of CCK8 was added to each well, and incubated at 37°C, 5% CO2 for 1 h. The OD450 absorbance was measured using the enzyme-labeled instrument (Thermo Multiskau FC). OD450 can measure the amount of formazan generated by intracellular dehydrogenase, which can be used to respond to the proliferation and viability of the cells. Following this procedure, measurements were made every 24 h for five consecutive days. Cell proliferation can be visualized by plotting the OD values for five consecutive times and comparing the growth curves of the experimental and control groups.

2.5.4 Evaluating cellular cycle by Flow cytometry

After treating cells for 48 hours with MPA (30 and 60 μM), gather the cells, stain, and incubate for 15 minutes. Cellular cycle was assessed using the cell cycle kit (BD Bioscience Pharmingen, San Diego, CA, USA). FACS flow cytometer was operated according to the instructions.

2.5.5 Evaluating cellular apoptosis by Flow cytometry

After treating cells for 48 hours with MPA (30 and 60 μM), gather the cells, stain, and incubate for 15 minutes. Cellular apoptosis was assessed using the FITC Membrane Associated Protein V Apoptosis Detection Kit (BD Bioscience Pharmingen, San Diego, CA, USA). Operated the FACS flow cytometer according to the instructions.

2.5.6 Wound healing assay

Cells were inoculated into 96-well plates with 20×10^4 cells per well. Incubate in 10% serum-containing medium until the cell density reaches 90%, and use a 1 ml pipette tip to scratch the wound. Change to serum-free medium and take pictures at 0, 24 and 48 hours.

2.5.7 Transwell assay

Cells were inoculated in 24-well plates. The upper chamber was serum-free medium and the lower chamber was medium containing 20% FBS.

Migration conditions were: 8×10^4 cells in the upper chamber and no Matrigel gel (BD Biosciences, USA) in the Transwell inserts. Invasion conditions were: 12×10^4 cells in the upper chamber and with Matrigel gel in the Transwell inserts. Incubate at 37°C, fixation with methanol and staining with crystal violet, and quantify under the optical microscope.

2.6 Statistical analysis

Expression variability analysis from database sources was performed using the Wilcoxon rank sum test. Cox regression analysis was performed to assess the correlation between clinicopathologic parameters and prognosis. 95% confidence intervals (CIs) and hazard ratios (HRs) were used to measure the risk of specific components. Every in vitro assay was conducted at least three times. GraphPad 7.0 was used to examine the data. The student's t-test, one-way and two-way analysis of variance (ANOVA) were used to establish statistical significance. P-values of less than 0.05 were deemed statistically significant in all two-sided tests.

3. Results

3.1 Expression level of DOK1 in ccRCC

The TIMER online database was initially used to investigate DOK1 mRNA expression in human cancers, and there was a significant variance in DOK1 expression across several tumor types (Fig. 1a). DOK1 showed differential expression between tumor tissues and normal tissues in various tumor types such as Cholangiocarcinoma (CHOL), Colon adenocarcinoma (COAD), Glioblastoma multiforme (GBM), and others. In this study we focused on its expression in the ccRCC. Using the TCGA and CPTAC databases, we discovered that DOK1 expression was greater in ccRCC tissues than in normal kidney tissues (Fig. 1b, 1c). Based on the TCGA database, DOK1 expression was considerably higher in the 56 paired ccRCC samples (Fig. 1d). Human Protein Atlas (HPA) immunohistochemical staining revealed that ccRCC tissue had higher levels of DOK1 protein than surrounding tissue (Fig. 1e, 1f).

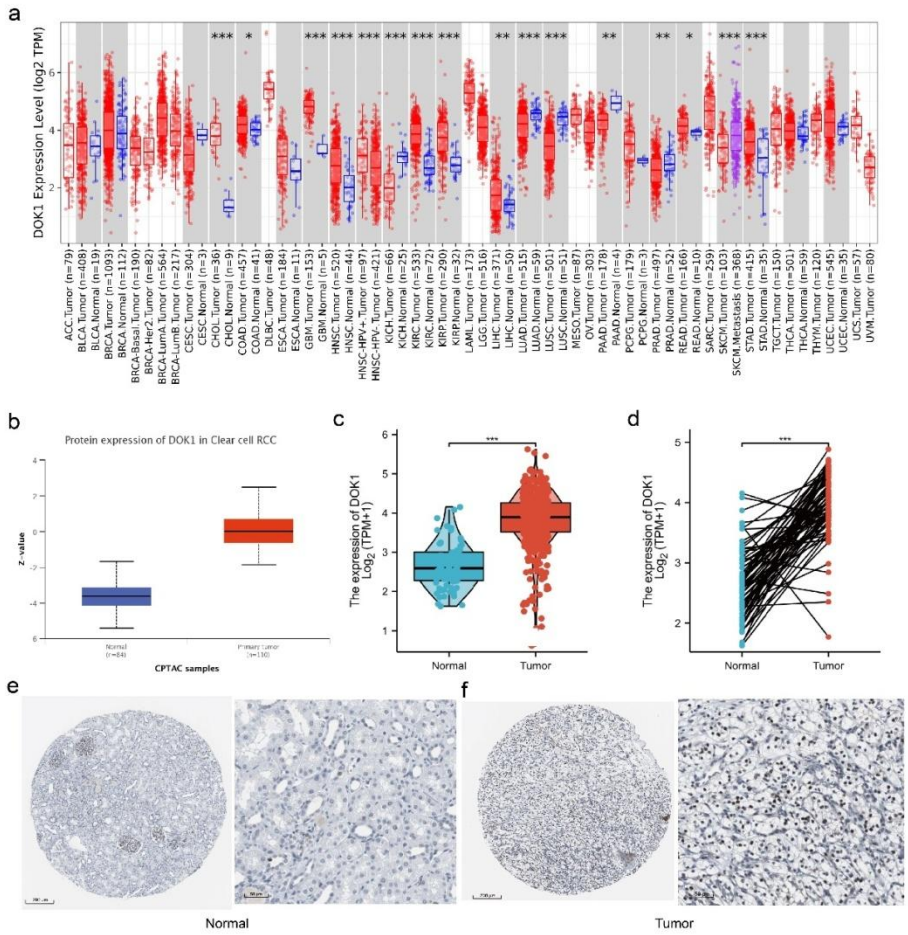


Figure1 (a) The expression of DOK1 in various cancer types based on the TCGA database. (b)

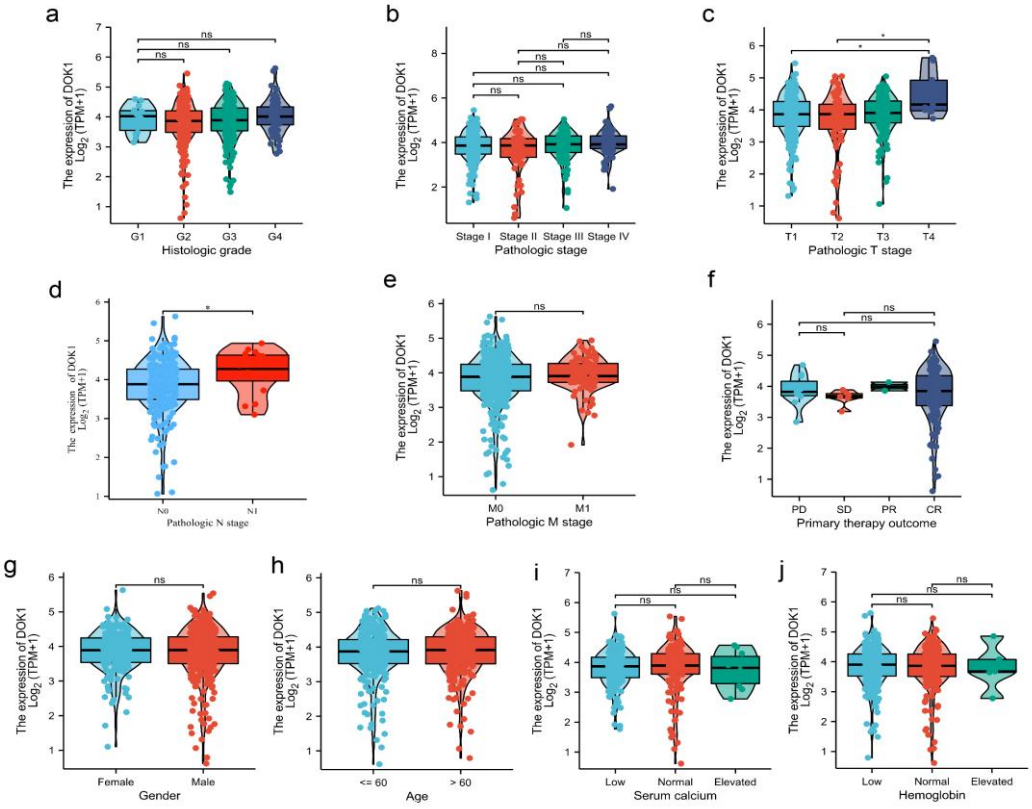
Examination of DOK1 expression in ccRCC based on UALCAN. (c) DOK1 expression in ccRCC and adjacent normal tissues based on TCGA. (d) The statistical examination of DOK1 expression in 56 pairs of ccRCC tissues and nearby normal tissues by the TCGA database. (e) Normal tissues: DOK1 protein level by the Human Protein Atlas. (f) Tumor tissues: DOK1 protein level by the Human Protein Atlas.
Note: *P < 0.05, **P < 0.01, ***P < 0.001 and ****P < 0.0001.

Given that DOK1 expression is increased in ccRCC, not only suggested its possible potential rol

e in tumor development, but also led us to explore its role in tumor diagnosis, prognostic assessment, and treatment.

3.2 Correlation with clinical features and prognosis

The connection between DOK1 mRNA expression and clinical pathological characteristics in ccRCC samples was assessed using the Dunn and Kruskal Wallis tests. Table 1 compares the clinical features of ccRCC patients in the high and low DOK1 expression groups. We also examined the connection between clinical characteristics and DOK1 expression (S1 Fig.).



S1 Figure Relationships between DOK1 mRNA levels and clinical pathological characteristics. Higher expression levels of DOK1 were observed in patients with a high T stage (C) and N stage (D). No statistically significant correlation was found between the expression levels of DOK1 and histologic grade (A), pathologic grade (B), M stage (E), primary therapy outcome (F), gender (G), age (H), serum calcium (I), hemoglobin (J).
Note: ns: no significance; *P < 0.05, **P < 0.01, ***P < 0.001 and ****P < 0.0001.

S2 Fig. After knocking down DOK1 in 786-O, 769-P cells using shRNA, the expression of DOK1 was detected by Western Blot after 72 hours

Patients with high T stage (Fig. 2a) and high N stage (Fig. 2b) had higher expression levels of DOK1. T stage was used to indicate the size and degree of local invasion of the tumor, while N

stage was used to indicate whether the tumor had lymph node metastasis. The high expression of DOK1 was positively correlated with both T and N stages, suggesting that it may be associated with enhanced invasive and migratory ability of the tumor. Since higher T and N stages usually indicated poorer prognosis, high expression of DOK1 could also be a potential prognostic indicator for patients with ccRCC.

Table 1. DOK1 expression differences in clinical features of ccRCC patients.

characteristics	Low expression of DOK1	High expression of DOK1	P
n	270	271	
T stage, n (%)			**0.046**
T1	143 (26.4%)	136 (25.1%)	
T2	38 (7%)	33 (6.1%)	
T3	88 (16.3%)	92 (17%)	
T4	1 (0.2%)	10 (1.8%)	
N stage, n (%)			**0.048**
N0	122 (47.3%)	120 (46.5%)	
N1	4 (1.6%)	12 (4.7%)	
M stage, n (%)			0.842
M0	217 (42.7%)	212 (41.7%)	
M1	39 (7.7%)	40 (7.9%)	
Pathologic stage, n (%)			0.669
Stage I	142 (26.4%)	131 (24.3%)	
Stage II	31 (5.8%)	28 (5.2%)	
Stage III	57 (10.6%)	66 (12.3%)	
Stage IV	39 (7.2%)	44 (8.2%)	
Primary therapy outcome, n (%)			0.163
PD	6 (4.1%)	5 (3.4%)	
SD	6 (4.1%)	0 (0%)	
PR	1 (0.7%)	1 (0.7%)	
CR	68 (46.3%)	60 (40.8%)	
Gender, n (%)			0.952
Female	93 (17.2%)	94 (17.4%)	
Male	177 (32.7%)	177 (32.7%)	
Race, n (%)			**0.016**
Asian	0 (0%)	8 (1.5%)	
Black or African American	28 (5.2%)	29 (5.4%)	
White	240 (44.9%)	229 (42.9%)	
Age, n (%)			0.414
≤ 60	139 (25.7%)	130 (24%)	
> 60	131 (24.2%)	141 (26.1%)	
Histologic grade, n (%)			0.231
G1	6 (1.1%)	8 (1.5%)	
G2	124 (23.3%)	112 (21%)	
G3	105 (19.7%)	102 (19.1%)	
G4	30 (5.6%)	46 (8.6%)	
Serum calcium, n (%)			0.951
Low	106 (28.9%)	98 (26.7%)	

Normal	77 (21%)	76 (20.7%)	
Elevated	5 (1.4%)	5 (1.4%)	
Hemoglobin, n (%)			0.763
Low	130 (28.2%)	134 (29.1%)	
Normal	100 (21.7%)	92 (20%)	
Elevated	3 (0.7%)	2 (0.4%)	
Laterality, n (%)			0.601
Left	123 (22.8%)	130 (24.1%)	
Right	146 (27%)	141 (26.1%)	
OS event, n (%)			0.728
Alive	191 (35.3%)	188 (34.8%)	
Dead	79 (14.6%)	83 (15.3%)	

Note. ccRCC: renal clear cell carcinoma.

We conducted in-depth research on the prognostic value of DOK1. The ROC curve analysis in Fig. 2c showed a correlation between DOK1 and Area Under Curve (AUC) values of 0.907 (95% CI: 0.875-0.940). In univariate analysis, DOK1 expression was associated with T stage, N stage, M stage, pathological stage, age, histological grade, and OS. In multivariate analysis, M stage, age, histologic grade and DOK1 expression were independent prognostic factors for ccRCC (Table 2).

Table 2. Univariate and multivariate Cox proportional hazards analysis of DOK1 expression and OS of ccRCC patients.

Characteristics	Total (*N*)	Univariate analysis		Multivariate analysis	
		Hazard ratio (95% CI)	P value	Hazard ratio (95% CI)	P value
Pathologic T stage	541				
T1&T2	350	Reference		Reference	
T3&T4	191	3.210 (2.373 - 4.342)	< **0.001**	1.601 (0.706 - 3.630)	0.260
Pathologic N stage	258				
N0	242	Reference		Reference	
N1	16	3.422 (1.817 - 6.446)	< **0.001**	1.790 (0.896 - 3.573)	0.099
Pathologic M stage	508				
M0	429	Reference		Reference	
M1	79	4.401 (3.226 - 6.002)	< **0.001**	2.732 (1.613 - 4.628)	< **0.001**
Age	541				
<= 60	269	Reference		Reference	
> 60	272	1.791 (1.319 - 2.432)	< **0.001**	1.715 (1.119 - 2.629)	**0.013**
Histologic grade	533				
G1&G2	250	Reference		Reference	
G3&G4	283	2.665 (1.898 - 3.743)	< **0.001**	1.646 (1.008 - 2.686)	**0.046**
Pathologic stage	538				
Stage I&Stage II	332	Reference		Reference	

Stage III&Stage IV	206	3.910 (2.852 - 5.360)	< **0.001**	1.209 (0.477 - 3.065)	0.689
Gender	541				
Female	187	Reference			
Male	354	0.924 (0.679 - 1.257)	0.613		
DOK1					
Low	270	Reference		Reference	
High	269	1.957 (1.424 – 2.671)	< **0.001**	2.059 (1.303 – 3.255)	**0.002**

Note. ccRCC: renal clear cell carcinoma; OS: overall survival.

This might indicate that the expression level of DOK1 is associated with tumor aggressiveness and metastatic ability, and plays a potential role in tumor progression and metastasis. High DOK1 expression was significantly correlated with poorer OS in ccRCC patients (Fig. 2d). In addition, high expression of DOK1 was associated with OS in ccRCC patients of pathological stage IV (Fig. 2h), N0 stage (Fig. 2i), M1 stage (Fig. 2l). In pathological stage I-III (Figure 2e, f, g), pathological N1 stage (Figure 2j) and pathological M0 stage (Figure 2k), no association between DOK1 expression and OS was observed. The Kaplan Meier survival analysis results showed that ccRCC patients with high expression of DOK1 had a poorer prognosis in the following categories: M1, age>60, histologic grade G3 and G4 (Fig. 2m). These findings emphasized the prognostic significance of DOK1 expression in ccRCC tissues.

In order to provide a helpful quantitative model to help clinicians determine the correct prognosis of ccRCC patients, we created a nomogram that combines patient-independent and survival-related clinical features (TNM stage, Pathological stage, Histologic grade, gender, age, DOK1 expression) to find the patients' survival after 1, 3, and 5 years (Fig. 2n). There was a significant degree of consistency between the observed and projected values, as indicated by the deviation correction line in the calibration chart's proximity to the ideal curve, also called the 45-degree line (Fig. 2o). In conclusion, the findings showed that the nomogram is a better model than a single prognostic indicator and can predict the patients' long-term survival (one, three, and five years).

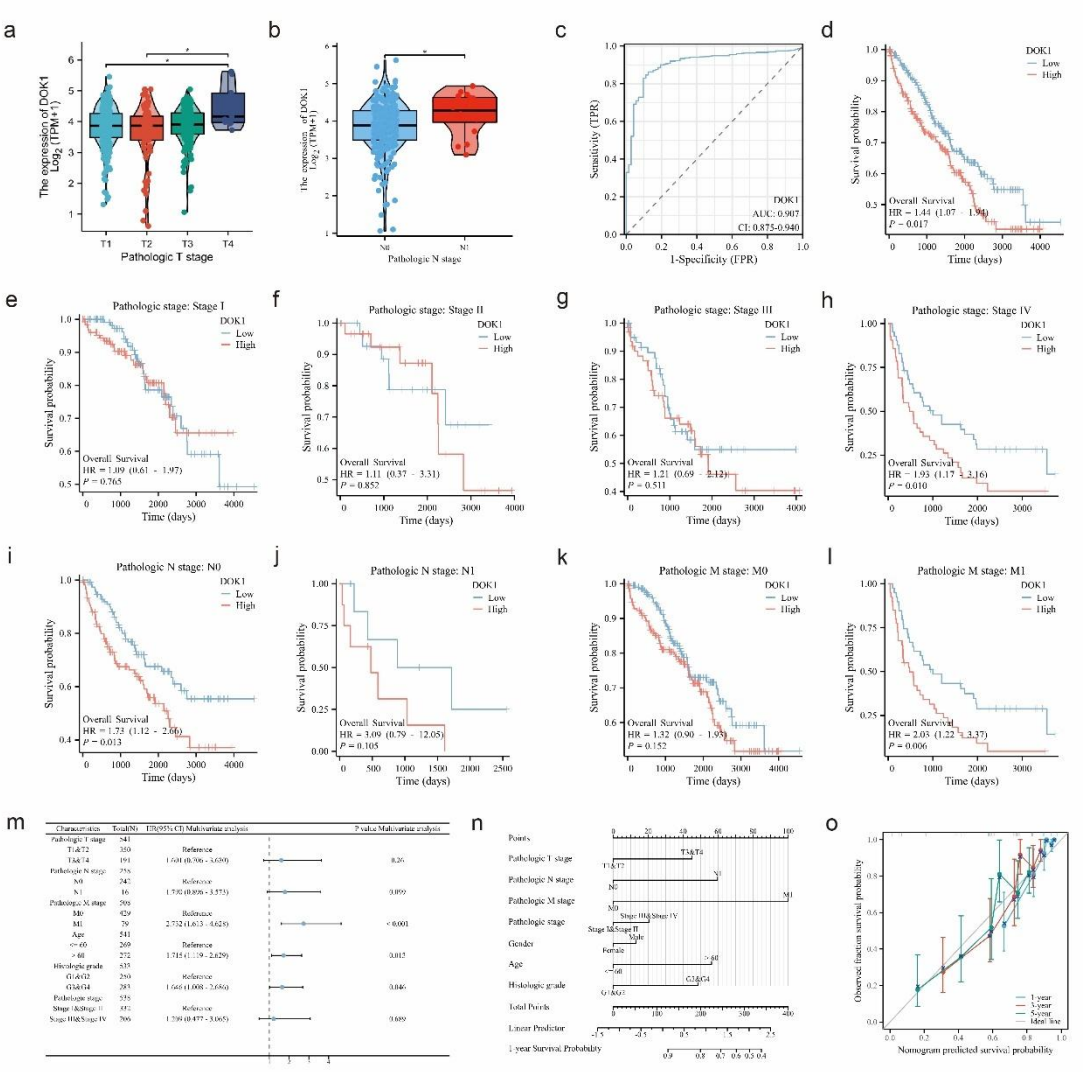


Figure 2 Higher expression levels of DOK1 were observed in patients with a high T stage (a) and N stage (b). (c) DOK1 distinguished between ccRCC tissue and normal control tissue in the ROC curve (AUC = 0.907). (d) Patients with high expression of DOK1 had shorter OS in the Kaplan-Meier survival curves. Subgroup analyses were performed respectively to correlate DOK1 expression with T-stage (e-h), N-stage (i-j), and M-stage (k-l). (m) Multivariate survival analysis examining the relationship between DOK1 expression and overall survival in patients with different stages of cancer. (n) Nomogram to forecast the likelihood of OS for patients with colorectal cancer at1,3, and 5 years. (o) Calibration plots of nomograms for OS probability prediction.
Note: OS: overall survival

3.3 In vitro assays to explore the function of DOK1

To further confirm the biological function of DOK1, we conducted a series of in vitro assays. After knocking down DOK1 in 786-O, 769-P cells using shRNA, the expression of DOK1 was detected by Western Blot (WB) after 72 hours (Fig. 3a). The proliferative capacity of the cells was examined by CCK-8 assay. The results showed that the OD values (450 nm) of both 786-O and 769-P cell lines of shDOK1 were significantly lower than the shControl groups ($P < 0.05$, Fig. 3b). Compared to the shControl group, the ratio of the G1/G0 phase increased ($P < 0.01$) and the ratio of the S phase decreased ($P < 0.05$) in the 769-P-shDOK1 cell line. Essentially consistent was that the ratio of the G1/G0 phase increased in the 786-O-shDOK1 cell line ($P < 0.05$, Fig. 3c). Indicating that DOK1 mainly functioned in the cell cycle from the G1 phase to the S phase, further influencing the cell proliferation capacity. Flow cytometry examined the effect of DOK1 expression on apoptosis in ccRCC cells. The percentage of apoptosis was elevated in both 786-O and 769-P cells with

knockdown of DOK1 (Fig. 3d).

We tested the effect of down-regulation of DOK1 on the migration ability of renal cancer cell lines by wound assay. The results showed that the 36h migration distance of the 769-P cell line and 786-O cell line were significantly decreased after knocking down DOK1 compared to control groups (Fig. 3e). The effect of knocking down DOK1 on the migratory motility of ccRCC cells was detected by migration assay. The results showed that the migration ability of 786-O and 769-P cells after knockdown of DOK1 was significantly lower than control groups ($P < 0.01$, Fig. 3f). Finally, we tested the effect of down-regulation of DOK1 on the invasion ability of ccRCC cells by Transwell invasion assay. The results showed that the invasion ability of 786-O and 769-P cells after knocking down DOK1 was significantly lower than that of the shControl group ($P < 0.001$, Fig. 3g). The above experimental results indicated that down-regulation of DOK1 could significantly inhibit the proliferation, migration and invasion ability of ccRCC cells in vitro.

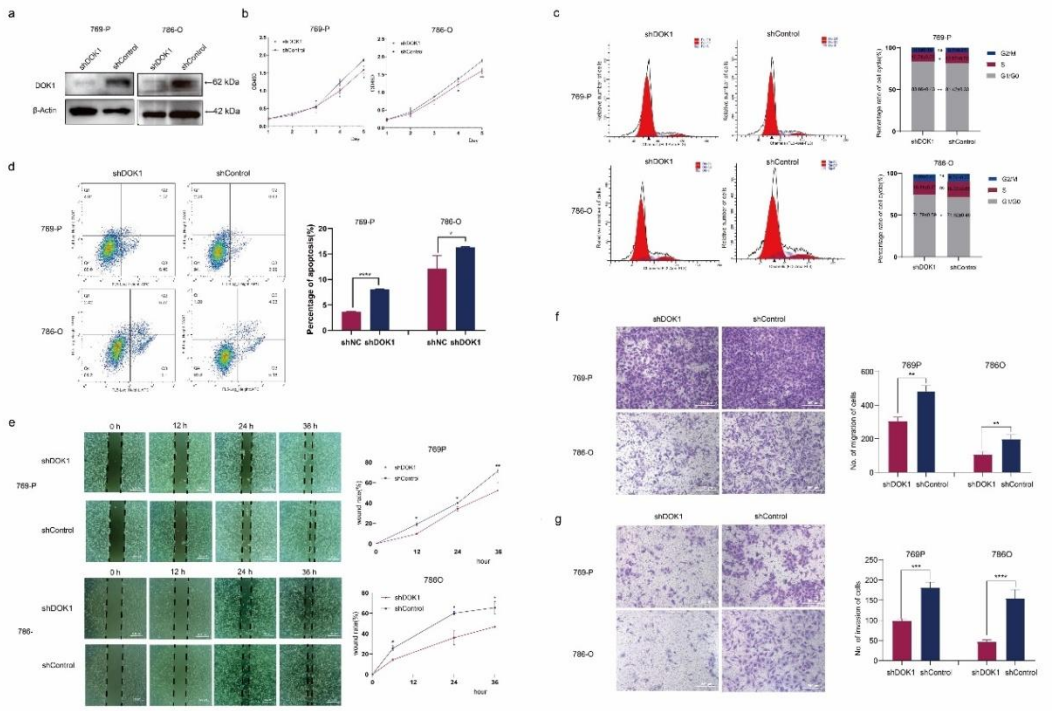


Figure 3 (a) DOK1 protein levels in control and DOK1 knockdown 786-O and 769-P cells. (b) DOK1 knockdown cells growth curves in comparison to control cells. (c) Using flow cytometry to assess how DOK1 inhibition affects the cycle of cell proliferation. (d) The proportion of apoptotic cells after knockdown of DOK1 by apoptosis assay. (e) The ability of migration after deletion of DOK1 in 786-O and 769-P cells by wound healing assay. (f) The capacity of migration after deletion of DOK1 in 786-O and 769-P cells by transwell assay. (g) The capacity of invasion after deletion of DOK1 in 786-O and 769-P cells by transwell assay.

Note: All experiments were independently repeated at least three times. *P < 0.05, **P < 0.01, ***P < 0.001 and ****P < 0.0001 indicate statistical significance.

3.4 Enrichment analysis of DOK1

Based on the TCGA database, and using the DSEeq2 program in R for analysis (|logFC| >2, modified P value <0.05), 398 differentially expressed genes (DEGs) were identified. In the high expression group, 290 had downregulation and 108 had upregulation (Fig. 4a). The 18 most significant DEGs in the high-level and low-level DOK1 expression groups were heatmapped in Fig. 4b. The molecular mechanism by which DOK1 affects ccRCC was further explored through GSEA enrichment analysis, and the results were consistent with our previous cell cycle experiments. The projects in the first 20 related signaling pathways showed enrichment of Cell Cycle Checkpoints and Mitotic G1 Phase and G1-S Transition (Fig. 4c).

We conducted STRING database, KEGG analysis to build a PPI network and annotate functions. The network of DOK1 and its ten co-expressed genes were displayed in Fig. 4d. According to the results of the KEGG pathway analysis, the top 10 associated genes found in the PPI network were related to interleukin 10 signaling, programmed death 1 (PD-1) signaling, Chemokine Receptors Bind Chemokines, Cd8 T cell receptor signaling (Tcr) Pathway, Generation of Second Messenger Molecules, Graft Versus Host Disease, Syndecan 1 Pathway, Intestinal Immune Network For Iga Production, Cytotoxic T lymphocyte associate protein-4 (CTLA4) Pathway and Tcr Pathway (Fig. 4e). Figure 4f demonstrated a significant enrichment of multiple biological processes associated with the immune response, with the classical pathway of complement activation being the most significant process. In addition, humoral immune response mediated by circulating immunoglobulin, complement activation, immunoglobulin mediated immune response, B cell mediated immunity, phagocytosis, recognition, adaptive immune response based on somatic recombination of immune receptors built from immunoglobulin superfamily domains, phagocytosis, phagocytosis, engulfment, B cell receptor signaling pathway also showed high enrichment scores and significant p-values. Figures 4g and 4h illustrated the composition of cells and their molecular functions.

Further enrichment analysis of co-expressed genes was performed according to the KEGG database (Fig. 4i). The correlation between DOK1 and co-expressed genes was further detailed as shown in Fig. 4j-q. The expression level of DOK1 was positively correlated with CRKL (CT10 regulator of kinase-like) ($r = 0.145$, $P < 0.001$), INPP5D (Inositol polyphosphate-5-phosphatase) ($r = 0.496$, $P < 0.001$), PTK6 (Protein tyrosine kinase 6) ($r = 0.386$, $P < 0.001$), and others.

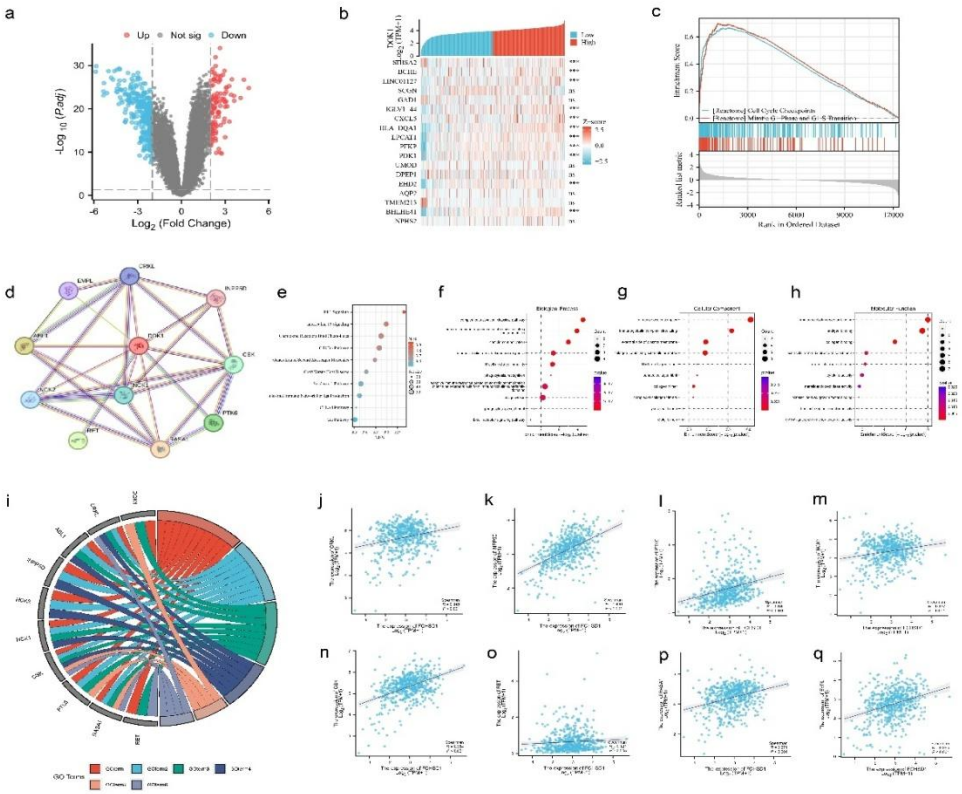


Figure 4 (a) Genes that differ in the DOK1 high and low expression groups were plotted in a volcano. (b) Heatmap showed the genes that were substantially varied in the DOK1 high and low expression groups. (c) GSEA pathway enrichment analysis demonstrated the role of DOK1 in the cell cycle. (d) The PPI network of DOK1 and its coexpression genes. KEGG (e), BP (f), CC (g), MF (h) enrichment analysis of 10 involved genes. (i) KEGG enrichment analysis of DOK1 and co-expressed genes. (j–q) Analysis of the relationship between co-expressed genes in ccRCC and DOK1 expression.
Note: BP, Biological Process; CC, Celluar Components; MF, Molecular Function; KEGG, Kyoto Encylopedia of Genes and Genomes; ccRCC, clear cell renal cell carcinoma.

3.5 Correlation analysis between DOK1 expression and immune cell infiltration in ccRCC.

Using the TIMER database, we examined the possible correlation between DOK1 expression and six distinct kinds of immune cells that infiltrate tumors. B cells (r = 0.35, P = 1.18e-14), CD8+ T cells (r = 0.346, P = 9.17e-14), CD4+ T cells (r = 0.494, P = 1.13e-29), macrophages (r = 0.493, P = 8.81e-29), neutrophils and dendritic cells were all linked to DOK1 expression, according to Fig. 5a.

Additionally, we evaluated the relationship between DOK1 expression and 28 different TILs using the TISIDB database. The association between DOK1 expression and 28 distinct TIL types in human cancer was displayed in Fig. 5b. Figure 5c-j demonstrated a significant positive correlation between DOK1 expression levels and the degree of enrichment of multiple immune cell types: B cells, CD8 T cells, Eosinophils, dendritic cells (DC), macrophage, mast cells, natural killer cells (NK cells), regulatory T cells (TREG). Examine the relationship between co-expressed genes and DOK1 expression in ccRCC by the GEPIA database. In ccRCC, we also examined the relationship between DOK1 and immunological regulatory variables. The association between DOK1 expression and the degree of immune cell infiltration in ccRCC as determined by TCGA, GEPIA, and TIMER databases was shown in Tables 3 and 4, respectively.

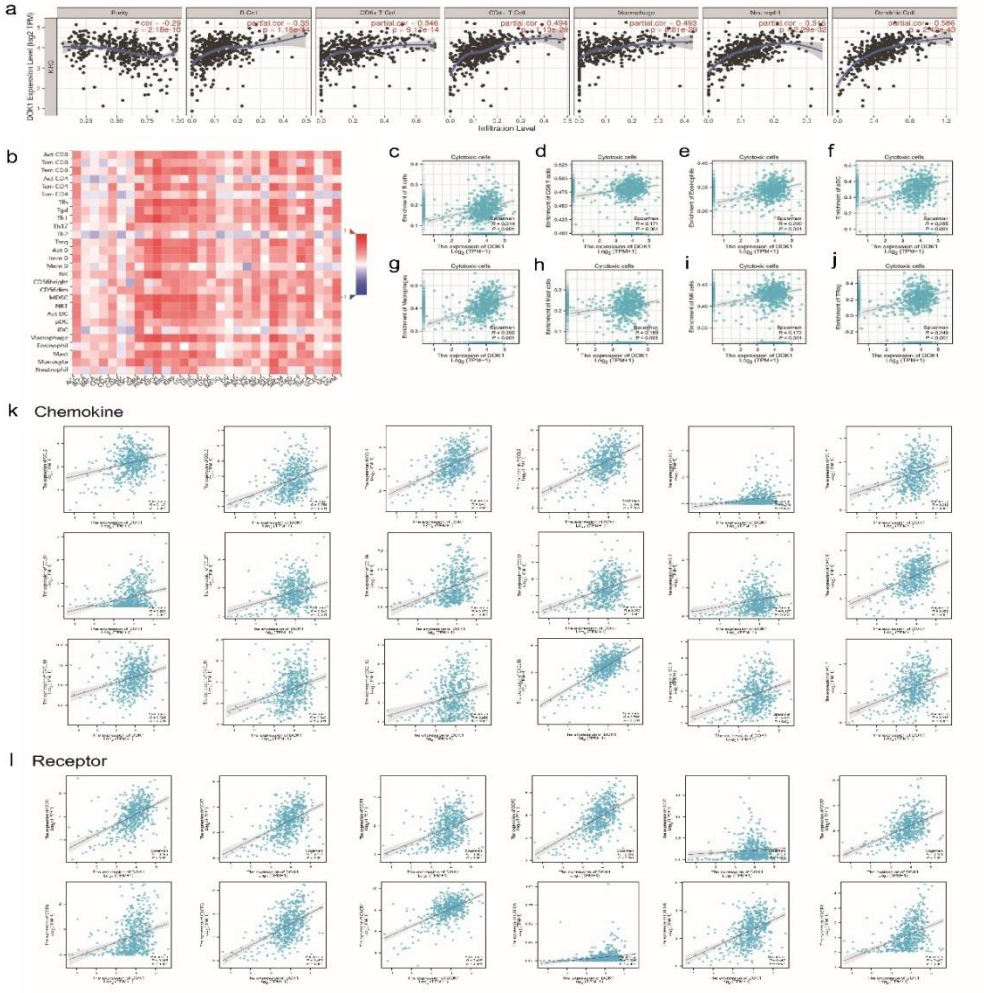


Figure 5 (a) TIMER database analysis of DOK1 correlation with tumor-infiltrating immune cells. (b) TCGA database analysis of DOK1 correlation with 28 TILs. (c–j) The scatter plot illustrated the correlation between DOK1 expression and immune cell markers in ccRCC. (k) The association between chemokines and DOK1 expression in the scatter plot. (l) The association between DOK1 expression and chemokine receptors in the scatter plot.
Note: TILs: Tumor Infiltrating Lymphocytes.

Table 3. Correlation analysis between DOK1 and immune markers in GEPIA

Description	Gene markers	KIRC Tumor Cor	P	Normal Cor	P
CD8 T cell	CD8A	0.37	***	0.59	***
	CD8B	0.36	***	0.32	**
	TBX21	0.34	***	0.61	***
	IFNG	0.34	***	0.38	***
	CXCL9	0.32	***	0.31	**
	CXCL10	0.19	***	0.35	**
T cell (general)	CD3D	0.37	***	0.62	***
	CD3E	0.42	***	0.65	***
	CD3G	0.43	***	0.63	***
	CD2	0.39	***	0.68	***
B cell	CD19	0.65	**	0.40	***
	CD79A	0.089	*	0.40	***
Monocyte	CD86	0.52	***	0.59	***
	CD115	0.64	***	0.69	***
TAM	CCL2	-0.016	0.62	0.49	***
	CD68	0.34	***	0.60	***
	IL10	0.37	***	0.29	*
	CSF2	0.18	***	0.49	***
M1 Macrophage	INOS(NOS2)	0.065	0.16	0.14	0.23
	IRF5	0.30	***	0.35	**
	COX2(PTGS2)	0.0084	0.85	0.26	*
M2 Macrophage	CD163	0.44	***	0.59	***
	VSIG4	0.47	***	0.66	***
	MS4A4A	0.47	***	0.56	***
T cell exhaustion	PD-1(PDCD1)	0.36	***	0.48	***
	PDL-1(CD274)	0.11	*	0.42	***
	CTLA4	0.38	***	0.42	***
	LAG3	0.37	***	0.52	***
	TIM-3	0.14	**	0.39	***
	GZMB	0.25	***	0.6	***

Note. GEPIA: Gene Expression Profiling Interaction Analysis; ccRCC: renal clear cell carcinoma; Normal: correlation analysis in normal tissue of TCGA; Tumor: correlation analysis in tumor tissue of TCGA; TAM: tumor-associated macrophages. *P <0.05, **P <0.01, ***P <0.001.

Table 4. Correlation analysis between DOK1 and immune markers in TIMER2.0.

Description	Gene markers	KIRC Tumor Cor	P	Purity Cor	P
CD8 T cell	CD8A	0.406	***	0.366	***

	CD8B	0.404	***	0.372	***
	TBX21	0.36	***	0.343	***
	IFNG	0.375	***	0.332	***
	CXCL9	0.412	***	0.381	**
	CXCL10	0.332	***	0.301	***
T cell (general)	CD3D	0.445	***	0.405	***
	CD3E	0.450	***	0.408	***
	CD3G	0.499	***	0.460	***
	CD2	0.473	***	0.432	***
B cell	CD19	0.411	**	0.370	***
	CD79A	0.389	*	0.346	***
	BLK	0.402	***	0.353	***
Monocyte	CD86	0.557	***	0.532	***
	CD115 (CSF1R)	0.654	***	0.621	***
TAM	CCL2	0.053	0.224	0.033	0.473
	CD68	0.40	***	0.386	***
	IL10	0.451	0.483	0.399	***
	CSF2	0.165	***	0.147	**
M1 Macrophage	INOS (NOS2)	0.178	***	0.142	**
	IRF5	0.323	***	0.340	***
	COX2 (PTGS2)	0.220	***	0.178	***
M2 Macrophage	CD163	0.515	***	0.475	***
	VSIG4	0.566	***	0.515	***
	MS4A4A	0.505	***	0.452	***
Neutrophils	CD66b (CEACAM8)	-0.028	0.517	-0.007	0.875
	CD11b (ITGAM)	0.579	***	0.555	***
	CCR7	0.467	***	0.434	***
Natural killer cell	KIR2DL1	0.077	0.077	0.051	0.271
	KIR2DL3	0.053	0.223	0.035	0.459
	KIR2DL4	0.161	***	0.128	**
	KIR3DL1	0.055	0.210	0.055	0.234
	KIR3DL2	0.126	**	0.138	**
	KIR3DL3	0.024	0.585	0.002	0.969
Dendritic cell	HLA-DPB1	0.575	***	0.575	***
	HLA-DQB1	0.319	***	0.294	***
	HLA-DRA	0.504	***	0.494	***
	HLA-DPA1	0.483	***	0.457	***
	BDCA-1 (CD1C)	0.331	***	0.305	***
	BDCA-4 (NRP1)	0.197	***	0.132	**
	CD11c (ITGAX)	0.436	***	0.454	***
Th1	T-bet (TBX21)	0.36	***	0.343	***
	STAT4	0.385	***	0.356	***
	STAT1	0.496	***	0.454	***
	TNF-γ (IFNG)	0.375	***	0.332	***
	TNF-α (TNF)	0.384	***	0.368	***

Th2	GATA3	0.217	***	0.189	***
	STAT6	0.250	***	0.292	***
	STAT5A	0.643	***	0.611	***
	IL13	0.022	0.614	0.027	0.559
Tfh	BCL6	0.173	***	0.174	***
	IL21	0.165	***	0.142	**
Th17	STAT3	0.351	***	0.300	***
	IL17A	0.013	0.771	0.009	0.841
Treg	FOXP3	0.401	***	0.351	***
	CCR8	0.385	***	0.331	***
	STAT5B	0.203	***	0.203	***
	TGFβ (TGFB1)	0.464	***	0.403	***
T cell exhaustion	PD-1(PDCD1)	0.411	***	0.394	***
	PDL-1(CD274)	0.201	***	0.188	***
	CTLA4	0.405	***	0.386	***
	LAG3	0.422	***	0.378	***
	TIM-3 (HAVCR2)	0.214	***	0.108	***
	GZMB	0.255	***	0.219	***

Note. TIMER: Tumor Immune Estimation Resource; ccRCC: renal clear cell carcinoma; None, correlation without adjustment; Purity: correlation adjusted by purity; TAM, tumor-associated macrophage; Th: The helper cell; Tfh: Follicular helper T cell; Treg: regulatory T cell; Cor: R value of Spearman's correlation. *P <0.05, **P <0.01, ***P <0.001.

The relationship between DOK1 expression and chemokines and chemokine receptors in ccRCC was seen in Figs. 5k and 5l, respectively. We analyzed the relationship between DOK1 expression and cells associated with immune infiltration, such as B, T, and NK cells. Understanding these correlations allows us to discern the activity and distribution of immune cells in the tumor microenvironment through the analysis of DOK1 expression. The analysis revealed a positive correlation between DOK1 expression and infiltration of T cells, macrophages, and NK cells, which may suggest a potential role for DOK1 in immune escape and macrophage M2 polarization within the cancer microenvironment. This can further be utilized to predict the prognosis and therapeutic outcomes for cancer patients. Dendritic cells are key antigen-presenting cells in the immune system, playing a crucial role in activating T cells and initiating immune responses. A positive correlation between DOK1 and activated dendritic cells may imply that DOK1 could play a significant role in the activation and function of dendritic cells. The recruitment, maturation, and activation of mast cells in the tumor microenvironment significantly impact the occurrence and progression of tumors. A positive correlation between DOK1 and activated mast cells may indicate the regulatory effect of DOK1 on mast cells within the tumor microenvironment.

3.6 Relationship between DOK1 expression and immune checkpoint in ccRCC

We assessed the association between DOK1 and PD1/PD-L1 (programmed death ligand 1) and CTLA4 (cytotoxic T lymphocyte-associated antigen-4) in ccRCC. Figure 6a illustrates the substantial positive correlation between the expression of DOK1 in ccRCC and PD-1/PD-L1 and CTLA4. Individuals exhibiting elevated DOK1 expression were also found to have elevated PD-1/PD-L1 and CTLA4 expression (Fig. 6b-g). PD-1/PD-L1 and CTLA-4 are involved in immune escape, immunosuppression, and regulation of T-cell function in tumors, as well as serving as important targets for immunotherapy. The association of DOK1 with PD-1/PD-L1 and CTLA-4 may reveal the potential role of DOK1 in immune escape and immunotherapy in tumors, and provide important

clues for the development of new immunotherapy strategies.

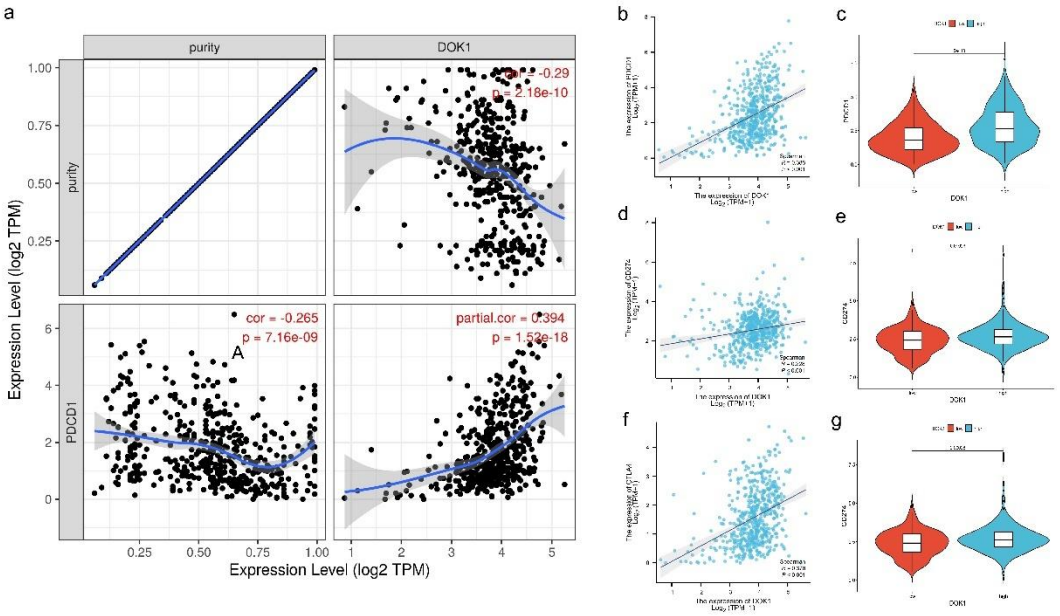


Figure 6 (a) The correlation between DOK1 and PD-1 expression in ccRCC adjusted for purity in TIMER. (b) The correlation between DOK1 and PD-1 expression in ccRCC. (c) PD-1 expression in the DOK1 differential expression groups. (d) The correlation between DOK1 and PD-L1 expression in ccRCC. (e) PD-L1 expression in the DOK1 differential expression groups. (f) The correlation between DOK1 and CTLA4 expression in ccRCC. (g) CTLA4 expression in the DOK1 differential expression groups.

Note: PD-1: programmed death 1; TIMER: The Tumor Immune Estimation Resource; PD-L1: programmed death ligand 1; CTLA4: cytotoxic T lymphocyte-associated antigen-4.

Discussion

According to the GLOBOCAN 2022 global cancer statistics, renal cell carcinoma ranked as the 14th most prevalent malignant neoplasm worldwide in 2020, representing 2.2% of all newly diagnosed cancers[1]. ccRCC is the predominant subtype of renal cell carcinoma, constituting approximately 60% to 85% of cases[3, 20]. While surgical resection remains the primary treatment modality for renal cell carcinoma, it is noteworthy that up to 40% of patients may experience local recurrence and distant metastasis. Therefore, early diagnosis and the identification of prognostic biomarkers are critical for enhancing patient survival outcomes[21, 22].

In the current study, we employed bioinformatics analyses utilizing TIMER, Oncomine, UALCAN, and TCGA public databases to investigate the association between DOK1 expression and the prognosis of clear cell renal cell carcinoma (ccRCC). The results showed that compared with normal kidney tissue, the mRNA and protein levels of DOK1 were significantly increased in ccRCC tissue. Additionally, the upregulation of DOK1 mRNA expression exhibited a positive correlation with advanced T stage, lymph node metastasis, and poorer overall survival (OS) as well as disease-specific survival (DSS). These results implied that DOK1 might serve as a possible ccRCC predictive biomarker.

The DOK1 gene was discovered to have a tumor suppressor role that inhibited cell proliferation in breast cancer[23], ovarian cancer[24], Burkitt's lymphoma[25], head and neck cancer (HNC)[26], chronic lymphocytic leukemia (CLL)[27], and lung cancer[28]. This was in contrast to the results in ccRCC we obtained from the database. Figure 1a visualized that DOK1 has different expression in the different cancers. We further performed in vitro experiments for validation. The constructed shRNA targeting DOK1 (shR-DOK1) and shControl were transfected into the 786-O and 769-P cell lines. High DOK1 expression promoted ccRCC cell invasion, migration, and proliferation,

as shown by a number of in vitro functional tests. GSEA enrichment analysis showed that DOK1 plays a role in the G1 and G1/S phases of the cell cycle, which affected cell proliferation capacity. The Flow cytometry experiments further validated it. Knocking down DOK1 resulted in a prolonged G1 phase and shortened S phase of cells, which inhibited cell proliferation. These results indicated that high expression of DOK1 promotes the proliferation of renal cell carcinoma cells, leading to poor prognosis of ccRCC.

Utilizing the DOK1 protein-protein interaction (PPI) network and enrichment analysis, we conducted an examination of the co-expressed genes associated with DOK1. Our findings indicate that DOK1 expression is significantly correlated with various proteins, particularly the CT10 kinase-like regulator (CRKL). CRKL has been identified as an adapter protein with two SH3 domains after a Src homology 2 (SH2) domain[29]. This protein plays a crucial role in mediating several cellular processes, including phagocytosis, adhesion, migration, differentiation, cytoskeletal remodeling, proliferation, and pathogen uptake[30]. Notably, elevated levels of CRKL expression have been observed in numerous tumor cell types[29, 31-33]. Furthermore, CRKL participates in the fibroblast growth factor (FGF)/fibroblast growth factor receptor (FGFR) signaling pathway[30]. The binding of FGF to FGFR initiates the regulation of downstream signaling pathways that are essential for both pro-mutagenic processes (such as embryogenesis, growth, and development) and non-pro-mutagenic processes (including neuromodulation and metabolic regulation)[34, 35]. Aberrant expression and mutations in FGFR can lead to inappropriate activation of this signaling pathway, resulting in uncontrolled pro-mutagenesis and subsequent tumorigenesis. Cancers associated with these alterations include lung, gastric, breast, colorectal, chronic granulocytic leukemia, cholangiocarcinoma, glioblastoma, chondrosarcoma, liposarcoma, and bladder cancer[36, 37]. In recent years, FGFR has emerged as a prominent target for pharmacological research, with inhibitor drugs designed to target FGFR showing promise in mitigating the aberrant activation of the FGF/FGFR signaling pathway, thereby offering potential therapeutic benefits for the aforementioned malignancies[38, 39]. By elucidating the relationship between DOK1/CRKL and the FGF/FGFR signaling pathway, we hypothesize that targeted inhibition of the DOK1/CRKL pathway may provide therapeutic advantages in the treatment of renal cell carcinoma.

Our analysis demonstrated a significant correlation between DOK1 expression in ccRCC and the infiltration of various immune cell populations, particularly M2 macrophages, $CD8^+$ T lymphocytes, and regulatory T cells (Tregs).

Macrophages are a class of multifunctional immune cells that are essential for maintaining tissue homeostasis, defending against pathogenic organisms, and facilitating wound healing processes[40]. As the pivotal component of the tumor microenvironment, macrophages significantly influence tumor growth, angiogenesis, immune regulation, metastasis, and resistance to chemotherapy[41, 42]. The primary functions of M2 macrophages include the modulation of immune responses, facilitation of tissue repair processes, and the promotion of tumor progression[43]. Mostly located on the surface of monocytes and macrophages, CD163 is an important member of the scavenger receptor superfamily. It is known to be a very specific marker for macrophages linked to M2 tumors. When it comes to inflammation, CD163 is an immunomodulator that is essential to the tumor-associated macrophage population and has a significant impact on tumor growth and metastasis[44, 45]. Malignant tumors include breast, bladder, lung, and colorectal cancer are intimately linked to the expression of CD163 molecules, which has drawn more attention recently[46-48]. A negative prognosis was suggested by our study's finding that DOK1 expression and CD163 had a positive correlation. This indicates that high DOK1 expression was frequently associated with an increase in M2-type TAMs.

As the primary cytotoxic T lymphocytes (CTL), CD8+ T cells are an essential part of the adaptive immune system[49]. During the development and growth of tumors, CD8+ T cells typically exhibit immunosuppressive effects. Using cell transformation to damage tumor-infiltrating lymphocytes (TIL) and reduce the body's ability to clear tumors. According to numerous studies, CD8+ T cell activity in the tumor

microenvironment rises noticeably as the disease stage advances[50, 51].

Treg plays a key role in developing and preserving self-tolerance as well as controlling immunological homeostasis. They have a suppressive effect on the immune response, protect the body from autoimmune diseases, and inhibit the anti-tumor immune response[52]. Immune response against tumors can be strengthened by removing Treg or inhibiting its effect[53, 54].

Our research revealed that enhanced CD8+ T cells and Treg were frequently present in kidney cancer cases with elevated expression of DOK1. It implied that DOK1 may inhibit immune infiltration, which could impair immunological response and cause renal cancer and poor prognosis in patients.

The TISIB, TIMER, and GEPIA databases showed that there was a substantial correlation between the cellular response to chemokines and an increase in DOK1 expression. Chemokines are important factors in the evolution of disease and have a major influence on patient prognosis and treatment as they are involved in a number of cancer growth processes, including angiogenesis, metastasis, cancer cell proliferation, stem cells, and invasiveness[55, 56]. The chemokine network has various functions in tumor immune response and cancer biology, and it has become a viable target for immunotherapy[57, 58]. A positive correlation was found between DOK1 expression and the chemokine CC subfamily (CCL2, CCL3, CCL4, CCL5, CCL7, CCL11, CCL17, CCL19, CCL22), the chemokine CXC subfamily (CXCL3, CXCL11, CXCL13, CXCL16, XCL1, XCL2), and the chemokine C subfamily (XCL1, XCL2). Also, DOK1 expression was positively correlated with chemokine receptor CCR subfamily (CCR1, CCR2, CCR4, CCR5, CCR6, CCR8), and CXCR subfamily (CXCR3, CXCR4, CXCR5, CXCR6).

Among them, CCL2, as an important chemokine in the tumor microenvironment, promoted tumor cell growth and survival through autocrine or paracrine secretion. The positive correlation of DOK1 with the CCL2-CCR2 axis predicted the potential role of DOK1 in tumor invasion and metastasis.

CCL3, also known as macrophage inflammatory protein 1-alpha, promoted dendritic cell (DC) recruitment for presentation of tumor antigens and facilitates T cell activation in the tumor microenvironment. The positive correlation of DOK1 with CCL3 also suggested a multifaceted role for DOK1 in the tumor microenvironment such as promotion of T cell responses and modulation of immune cell subset recruitment and adhesion.

Immune cells including T and B cells typically express PD-1 (programmed cell death 1) on their surface. Tumor surfaces typically express PD-L1 (Programmed Cell Death 1 Ligand 1), which can attach to PD-1. It can adversely affect lymphocyte activation, suppress T cell proliferation and cytokine release, and cause immunological escape[59, 60]. Analysis of the TIMER database and GSEA enrichment revealed a substantial positive correlation between DOK1 and PD-1 expression.

CTLA-4 (Cytotoxic T Lymphocyte-Associated Antigen-4), also called CD152, is mostly found in activated CD4+ and CD8+ T lymphocytes. It has a crucial role in activating Treg and clumping together in tumor tissues to inhibit anti-tumor immunity[61, 62]. Blocking CTLA-4 can blunt the activity of tumor-infiltrating Tregs and thereby boosts the intratumor immune response[63]. Patients with high DOK1 expression also showed high levels of CTLA-4 expression. This revealed the potential role of DOK1 in tumor immune escape, immunomodulation, and immunotherapy. DOK1 might affect the efficacy of immune checkpoint inhibitors by regulating the PD-1 pathway and the CTLA-4 pathway, which provided potential targets for the development of new immunotherapeutic strategies.

We investigated the prognostic predictive function of DOK1 in ccRCC. However, there were still some limitations. Although we utilized a database to study the pathway of action of DOK1 and its correlation with immune infiltration in ccRCC patients, its specific molecular mechanisms and roles need to be verified by further experiments.

Conclusion

Up-regulation of DOK1 was a biomarker of poor prognosis in ccRCC, suggesting its potential role in the development and progression of the disease. In vitro cytological experiments confirmed the important role of DOK1 expression in the malignant biological behavior of ccRCC. The

vital role of DOK1 in tumor immune infiltration also provided new ideas for personalized treatment of ccRCC.

Declarations

Data availability statement: The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Ethics approval and consent to participate: This study was approved by the ethics committee of the affiliated Yantai Yuhuangding hospital of Qingdao university.

Consent for publication: The authors warranted that their paper is original and had full authority to give this consent. All data generated or analysed during this study are included in this published article.

Availability of data and materials: All data and materials were obtained in the article.

Competing Interests: The authors declared that they had no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Reference

1. Bray, F., et al., Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin, 2024. 74(3): p. 229-263.
2. Siegel, R.L., et al., Cancer statistics, 2022. CA Cancer J Clin, 2022. 72(1): p. 7-33.
3. Bahadoram, S., et al., Renal cell carcinoma: an overview of the epidemiology, diagnosis, and treatment. G Ital Nefrol, 2022. 39(3).
4. De Meerleer, G., et al., Radiotherapy for renal-cell carcinoma. Lancet Oncol, 2014. 15 (4): p. e170-7.
5. Xu, D.D., et al., Synergistic effects of low-dose chemotherapy and T cells in renal cell carcinoma. Oncol Rep, 2020. 44(3): p. 897-908.
6. Casuscelli, J., et al., Molecular Classification of Renal Cell Carcinoma and Its Implication in Future Clinical Practice. Kidney Cancer, 2017. 1(1): p. 3-13.
7. Keefe, S.M., K.L. Nathanson, and W.K. Rathmell, The molecular biology of renal cell carcinoma. Semin Oncol, 2013. 40(4): p. 421-8.
8. Mashima, R., et al., The roles of Dok family adapters in immunoreceptor signaling. Immunol Rev, 2009. 232(1): p. 273-85.
9. Brummer, T., C. Schmitz-Peiffer, and R.J. Daly, Docking proteins. Febs j, 2010. 277(21): p. 4356-69.
10. Sunny, S. and P.B. Jayaraj, Protein-Protein Docking: Past, Present, and Future. Protein J, 2022. 41(1): p. 1-26.
11. Li, W., et al., Downstream of tyrosine kinase/docking protein 6, as a novel substrate of tropomyosin-related kinase C receptor, is involved in neurotrophin 3-mediated neurite outgrowth in mouse cortex neurons. BMC Biol, 2010. 8: p. 86.
12. Vakser, I.A., Challenges in protein docking. Curr Opin Struct Biol, 2020. 64: p. 160-165.
13. Porter, K.A., et al., What method to use for protein-protein docking? Curr Opin Struct Biol, 2019. 55: p. 1-7.
14. Chandrashekar, D.S., et al., UALCAN: A Portal for Facilitating Tumor Subgroup Gene Expression and Survival Analyses. Neoplasia, 2017. 19(8): p. 649-658.
15. Pontén, F., K. Jirström, and M. Uhlen, The Human Protein Atlas--a tool for pathology. J Pathol, 2008. 216(4): p. 387-93.
16. Nagy, Á., G. Munkácsy, and B. Győrffy, Pancancer survival analysis of cancer hallmark genes. Sci Rep, 2021. 11(1): p. 6047.
17. Li, T., et al., TIMER2.0 for analysis of tumor-infiltrating immune cells. Nucleic Acids Res, 2020. 48(W1): p. W509-w514.
18. Tang, Z., et al., GEPIA: a web server for cancer and normal gene expression profiling and interactive analyses. Nucleic Acids Res, 2017. 45(W1): p. W98-w102.
19. Ru, B., et al., TISIDB: an integrated repository portal for tumor-immune system interactions. Bioinformatics, 2019. 35(20): p. 4200-4202.
20. Linehan, W.M. and C.J. Ricketts, The Cancer Genome Atlas of renal cell carcinoma: findings and clinical implications. Nat Rev Urol, 2019. 16(9): p. 539-552.
21. Abu-Ghanem, Y., et al., The Impact of Histological Subtype on the Incidence, Timing, and Patterns of Recurrence in Patients with Renal Cell Carcinoma After Surgery-Results from RECUR Consortium. Eur Urol Oncol, 2021. 4(3): p. 473-482.
22. Čechová, M., et al., Histologic tumor necrosis

rate as a predictor of recurrence after surgical treatment for localized clear cell renal cell carcinoma. Rozhl Chir, 2022. 101(3): p. 114-118.
23. Tuna, E., et al., Analysis of the DOK1 gene in breast cancer. Mol Biol Rep, 2020. 47(3): p. 1605-1612.
24. Mercier, P.L., et al., Characterization of DOK1, a candidate tumor suppressor gene, in epithelial ovarian cancer. Mol Oncol, 2011. 5(5): p. 438-53.
25. Lee, S., et al., Dok1 expression and mutation in Burkitt's lymphoma cell lines. Cancer Lett, 2007. 245(1-2): p. 44-50.
26. Saulnier, A., et al., Inactivation of the putative suppressor gene DOK1 by promoter hypermethylation in primary human cancers. Int J Cancer, 2012. 130(11): p. 2484-94.
27. Lee, S., et al., Frameshift mutation in the Dok1 gene in chronic lymphocytic leukemia. Oncogene, 2004. 23(13): p. 2287-97.
28. Berger, A.H., et al., Identification of DOK genes as lung tumor suppressors. Nat Genet, 2010. 42(3): p. 216-23.
29. Xie, P., et al., CRKL dictates anti-PD-1 resistance by mediating tumor-associated neutrophil infiltration in hepatocellular carcinoma. J Hepatol, 2024. 81(1): p. 93-107.
30. Park, T., Crk and CrkL as Therapeutic Targets for Cancer Treatment. Cells, 2021. 10(4).
31. Fu, L., et al., CRKL protein overexpression enhances cell proliferation and invasion in pancreatic cancer. Tumour Biol, 2015. 36(2): p. 1015-22.
32. Lin, F., et al., CRKL promotes lung cancer cell invasion through ERK-MMP9 pathway. Mol Carcinog, 2015. 54 Suppl 1: p. E35-44.
33. Yu, M., et al., CRKL promotes proliferation, migration, invasion of laryngeal squamous cell carcinoma. Eur Rev Med Pharmacol Sci, 2020. 24(10): p. 5473-5480.
34. Ghedini, G.C., et al., Future applications of FGF/FGFR inhibitors in cancer. Expert Rev Anticancer Ther, 2018. 18(9): p. 861-872.
35. Turner, N. and R. Grose, Fibroblast growth factor signalling: from development to cancer. Nat Rev Cancer, 2010. 10(2): p. 116-29.
36. Babina, I.S. and N.C. Turner, Advances and challenges in targeting FGFR signalling in cancer. Nat Rev Cancer, 2017. 17(5): p. 318-332.
37. Liu, G., et al., Inhibition of FGF-FGFR and VEGF-VEGFR signalling in cancer treatment. Cell Prolif, 2021. 54(4): p. e13009.
38. De Luca, A., et al., FGFR Fusions in Cancer: From Diagnostic Approaches to Therapeutic Intervention. Int J Mol Sci, 2020. 21(18).
39. Zhang, P., et al., Targeting FGFR for cancer therapy. J Hematol Oncol, 2024. 17(1): p. 39.
40. Pan, Y., et al., Tumor-Associated Macrophages in Tumor Immunity. Front Immunol, 2020. 11: p. 583084.
41. Gao, J., Y. Liang, and L. Wang, Shaping Polarization Of Tumor-Associated Macrophages In Cancer Immunotherapy. Front Immunol, 2022. 13: p. 888713.
42. Xiang, X., et al., Targeting tumor-associated macrophages to synergize tumor immunotherapy. Signal Transduct Target Ther, 2021. 6(1): p. 75.
43. Chen, T., et al., M2 macrophages in kidney disease: biology, therapies, and perspectives. Kidney Int, 2019. 95(4): p. 760-773.
44. Etzerodt, A. and S.K. Moestrup, CD163 and inflammation: biological, diagnostic, and therapeutic aspects. Antioxid Redox Signal, 2013. 18(17): p. 2352-63.
45. Skytthe, M.K., J.H. Graversen, and S.K. Moestrup, Targeting of CD163(+) Macrophages in Inflammatory and Malignant Diseases. Int J Mol Sci, 2020. 21(15).
46. Troiano, G., et al., Prognostic significance of CD68(+) and CD163(+) tumor associated macrophages in head and neck squamous cell carcinoma: A systematic review and meta-analysis. Oral Oncol, 2019. 93: p. 66-75.
47. Wei, C., et al., Crosstalk between cancer cells and tumor associated macrophages is required for mesenchymal circulating tumor cell-mediated colorectal cancer metastasis. Mol Cancer, 2019. 18(1): p. 64.
48. Zhou, W., et al., Abundance of CD163-Positive Tumor-Associated Macrophages in the Early Gastric Cancer Predicts the Recurrence after Curative Resection. Dig Dis, 2020. 38(6): p. 458-465.
49. Laidlaw, B.J., J.E. Craft, and S.M. Kaech, The multifaceted role of CD4(+) T cells in CD8(+) T cell memory. Nat Rev Immunol, 2016. 16(2): p. 102-11.
50. Dolina, J.S., et al., CD8(+) T Cell Exhaustion in Cancer. Front Immunol, 2021. 12: p. 715 234.
51. Jansen, C.S., et al., An intra-tumoral niche

maintains and differentiates stem-like CD8 T cells. Nature, 2019. 576(7787): p. 465-470.
52. Göschl, L., C. Scheinecker, and M. Bonelli, Treg cells in autoimmunity: from identification to Treg-based therapies. Semin Immunopathol, 2019. 41(3): p. 301-314.
53. Qianmei, Y., et al., Recent advances in the role of Th17/Treg cells in tumor immunity and tumor therapy. Immunol Res, 2021. 69(5): p. 398-414.
54. Tanaka, A. and S. Sakaguchi, Regulatory T cells in cancer immunotherapy. Cell Res, 2017. 27(1): p. 109-118.
55. Cai, X., et al., Chemokine-like factor 1: A promising therapeutic target in human diseases. Exp Biol Med (Maywood), 2020. 245(16): p. 1518-1528.
56. Zlotnik, A. and O. Yoshie, The chemokine superfamily revisited. Immunity, 2012. 36(5): p. 705-16.
57. Bule, P., et al., Chemokine-Directed Tumor Microenvironment Modulation in Cancer Immunotherapy. Int J Mol Sci, 2021. 22(18).
58. Salazar, N. and B.A. Zabel, Support of Tumor Endothelial Cells by Chemokine Receptors. Front Immunol, 2019. 10: p. 147.
59. Keir, M.E., et al., PD-1 and its ligands in tolerance and immunity. Annu Rev Immunol, 2008. 26: p. 677-704.
60. Liu, J., et al., PD-1/PD-L1 Checkpoint Inhibitors in Tumor Immunotherapy. Front Pharmacol, 2021. 12: p. 731798.
61. Rowshanravan, B., N. Halliday, and D.M. Sansom, CTLA-4: a moving target in immunotherapy. Blood, 2018. 131(1): p. 58-67.
62. Van Coillie, S., B. Wiernicki, and J. Xu, Molecular and Cellular Functions of CTLA-4. Adv Exp Med Biol, 2020. 1248: p. 7-32.
63. Zhang, H., et al., Regulatory mechanisms of immune checkpoints PD-L1 and CTLA-4 in cancer. J Exp Clin Cancer Res, 2021. 40(1): p. 184.