Original Article

Assessment of the prognostic value of *SPOCK1* in clear cell renal cell carcinoma: a bioinformatics analysis

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Background: Clear cell renal cell carcinoma (ccRCC) is one of the most common urological malignancies, and once metastasis occurs, it often has a poor prognosis and lacks effective treatment. Therefore, there is an urgent need to screen some new biomarkers and explore their molecular mechanisms to improve the early clinical diagnosis and targeted therapy of ccRCC. *SPOCK1* (SPARC/osteonectin, CWCV and Kazal-like domains proteoglycan 1) is a conserved multi-domain proteoglycan that plays an important role in the development of multiple cancer types; however, its prognostic value in ccRCC has not been investigated. The study of the prognostic value of *SPOCK1* in ccRCC is a good complement to the study of ccRCC biomarkers.

Methods: Databases of this study included Oncomine, Kaplan-Meier Plotter, GEPIA, GeneMANIA, cBioPortal, and TIMER. Student's *t*-test was used to analyze the differences in *SPOCK1* expression in ccRCC tissues compared with tumor-adjacent normal tissues. Kaplan-Meier curves for survival analysis were used to assess the correlation between the expression of *SPOCK1* and the prognostic outcomes. Correlation module drew the expression scatterplots between *SPOCK1* and immune cell infiltration in ccRCC, together with the Spearman's rho value and estimated statistical significance.

Results: The *SPOCK1* mRNA expression was significantly higher in ccRCC tissues (mean expression ± SD: 920.2±195.2) than in normal tissues (mean expression ± SD: 358.4±29.1, P=0.008), and high *SPOCK1* expression significantly and positively correlated with the pathological stage of ccRCC patients (F value =10.2, P<0.001). Higher expression of *SPOCK1* was also associated with significantly shorter overall survival (OS) and disease-free survival (DFS) in ccRCC patients (GEPIA: P=0.046, P<0.001, respectively; Kaplan-Meier Plotter: P=0.002, P=0.0022, respectively). The function of SPOCK1 is mainly related to tumor development and extracellular matrix remodeling, and it may participate in the epithelial-mesenchymal transition process. *SPOCK1* expression significantly and positively correlated with infiltration of several immune cells in ccRCC, including cancer-associated fibroblasts (CAFs) (Rho =0.333, P=2.16×10^{-13}), tumor-associated macrophages (TAMs) (Rho =0.18, P=1.02×10^{-4}), and tumor-associated neutrophils (TANs) (Rho =0.165, P=3.83×10^{-4}). Conversely, there was a significant and negative correlation between *SPOCK1* expression and infiltration of $CD4^+$ T cells (Rho =–0.113, P=0.015).

Conclusions: *SPOCK1* may be a potential prognostic biomarker in ccRCC.

Keywords: Bioinformatics analysis; biomarkers; clear cell renal cell carcinoma (ccRCC); *SPOCK1*

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Introduction

Clear cell renal cell carcinoma (ccRCC) accounts for >75% of RCCs and is the most common subtype of RCC (1). In recent years, the morbidity and mortality of ccRCC have been gradually increasing, and almost one-third of patients have distant metastases at the time of initial diagnosis (2). Although the current level of treatment has improved and long-term survival can be achieved with surgical treatment for patients with limited ccRCC, the prognosis is poor if distant metastases are present at the time of initial diagnosis (3). Due to its insensitivity to radiotherapy, the 2-year overall survival (OS) for patients with advanced ccRCC is approximately 20% and the 5-year OS is only 10% (4). Targeted medicines such as sunitinib, axitinib, and bevacizumab are widely used for the treatment of advanced ccRCC, but efficacy is poor and the OS of patients remains low (5). PD-1 inhibitors combined with CTLA-4 inhibitors have become one of the first-line treatment options recommended by National Comprehensive Cancer Network (NCCN) guidelines, with an objective response rate of 42%, but progression-free survival is only 11.6 months, with no significant difference compared with sunitinib (6). Therefore, there is still a lack of effective treatment for metastatic ccRCC.

Although some biomarkers, such as *CA9* (7), *Ki-67* (8), *Bcl-2* (9), and *PTEN* (10), have been proven to predict the prognosis of ccRCC, study on biomarkers still needs to be continued to improve the prognosis of ccRCC patients. *SPOCK1* (SPARC/osteonectin, CWCV and Kazal-like domains proteoglycan 1), also known as *testican-1*, is a member of the *BM-40/SPARC/osteonectin* protein family (11). *SPOCK1*, as a conserved multi-domain proteoglycan, has many biological functions, such as extracellular matrix (ECM) remodeling (12), regulation of neuronal function (13) and Aβ accumulation (14). In recent years, *SPOCK1* has begun to be widely recognized for its important function in the development of many different tumors. Our previous study showed that *SPOCK1* expression was significantly increased in several tumors, such as lung cancer, prostate cancer, and colon cancer, and was significantly associated with tumor metastasis (15). *SPOCK1* may be involved in promoting tumor progression through regulation of ECM remodeling (15). However, the functions and prognostic value of *SPOCK1* in ccRCC, especially metastatic ccRCC, still require relevant studies. The study of the prognostic value of *SPOCK1* in ccRCC is also a good complement to the study of ccRCC biomarkers. Therefore, using several large databases, we extended our study field to ccRCC to analyze the role of *SPOCK1* in ccRCC and its relevance to the prognosis of ccRCC patients. We further explored whether *SPOCK1* could be used as a potential biomarker and target for the treatment of ccRCC patients.

Methods

Study design

First, we analyzed the differences of *SPOCK1* expression in ccRCC tissues and adjacent normal tissues, as well as the differences in *SPOCK1* expression in ccRCC tissues of different stages, by obtaining clinical data of the Oncomine and GEPIA databases. Second, we used GEPIA and Kaplan-Meier Plotter databases to analyze the correlation between the *SPOCK1* expression and prognostic outcomes. Third, using the cBioPortal website, we analyzed the mutations of *SPOCK1* in ccRCC patients. Fourth, we performed a protein-protein interaction (PPI) analysis of *SPOCK1* on the STRING website to explore potential interactions between *SPOCK1* and other proteins. Finally, using the TIMER website, we explored whether there was a correlation between *SPOCK1* expression and infiltration of different immune cells in ccRCC. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

The databases we used to compare the expressions of *SPOCK1*, effect on prognostic outcomes, and underlying mechanisms between ccRCC tissues and normal tissues are detailed as follows.

Oncomine

The *SPOCK1* mRNA levels in different ccRCC subtypes were obtained from the Oncomine database (www.oncomine.org), which is an online database that provides public access to tumor-related gene expression analysis data (16). In this study, we set $P<0.01$, 2-fold, and gene rank in the top 10% as thresholds of significance. We extracted mRNA expression data from the Jones Renal dataset. We used Student's *t*-test to analyze the difference in *SPOCK1* expression in ccRCC tissues compared with tumor-adjacent normal tissues.

GEPIA

GEPIA (http://gepia.cancer-pku.cn/index.html) uses standard processing procedures to analyze the data on a

large number of tumor and normal tissue samples (17). GEPIA analyzes the OS or disease-free survival (DFS) of cancer patients based on gene expression. GEPIA uses the Log-rank test for hypothesis testing. Hazard ratio and 95% confidence interval information can also be included in survival plots. In this study, the correlation of *SPOCK1* expression in ccRCC tissues with pathological staging and prognostic outcomes was analyzed by GEPIA. We used Student's *t*-test to determine if the differences between groups were significant. In addition, we used Kaplan-Meier curves for survival analysis to further assess the correlation between the expression of *SPOCK1* and the prognostic outcomes.

Kaplan-Meier Plotter

The Kaplan-Meier Plotter (http://kmplot.com/analysis/) database was used to analyze the correlation between *SPOCK1* mRNA expression and prognostic outcomes in ccRCC patients. The database manually downloaded gene expression data and recurrence-free survival (RFS) and OS data from EGA, GEO, and TCGA. PostgreSQL server handles the database, and integrates gene expression and clinical data. To analyze the prognostic value of a particular gene, patients are divided into two groups based on the expression of biomarkers. The Kaplan-Meier survival plot was used to compare the two groups of patients, and hazard ratio with 95% confidence intervals and P value are calculated. Databases and clinical data are supervised and extended regularly. In this study, Kaplan-Meier curves for survival analysis were used to assess the correlation between the expression of *SPOCK1* and the prognostic outcomes. The database provides information on gene-prognosis correlations in a few cancer types such as liver, breast, ovarian, lung and gastric cancers (18). Information on the number of patients, median mRNA expression levels, and hazard ratios are available in this database. We considered the difference to be statistically significant when P<0.05, which is two-sided.

cBioPortal

cBioPortal (www.cbioportal.org) is a TCGA-based database that provides a large amount of cancer genomics data (19). In this study, *SPOCK1* mutation and statistical analysis data were obtained.

STRING

The STRING (https://string-db.org/) website analyzes protein interactions through a unique set of computer prediction models (20). In this study, we performed PPI analysis through the STRING website to collect and integrate *SPOCK1* expression and potential interactions with other proteins.

GeneMANIA

GeneMANIA (http://www.genemania.org) is a resourceful website that performs functional analysis of genes with highly accurate predictive algorithms (21). In this study, we analyzed the interactions of *SPOCK1* with other proteins and predicted its functions.

TIMER

The TIMER (https://cistrome.shinyapps.io/timer/) website includes six main analysis modules that allow a systematic assessment of tumor infiltration by different immune cells (22). This study searched for *SPOCK1* and generated scatter plots to analyze the correlation between *SPOCK1* expression in ccRCC and levels of immune cell infiltration. We considered a significant correlation when P<0.05.

Statistical analysis

We used SPSS statisticical software (version 21.0, IBM Corporation, Chicago, IL, USA) and GraphPad Prism (version 6.0, GraphPad Software Inc, San Diego, CA, USA) for data analysis. Student's *t*-test was used to evaluate whether there was a significant difference between the two groups. The correlation between *SPOCK1* expression levels and DFS or OS in ccRCC patients was analyzed by Kaplan-Meier curve analysis. Statistical data were presented as the mean ± standard deviation (SD). The data differences were considered statistically significant when the P value <0.05, which is two-sided.

Results

Expression of SPOCK1 mRNA expression in ccRCC and tumor-adjacent normal tissues

We compared the *SPOCK1* mRNA expression in ccRCC

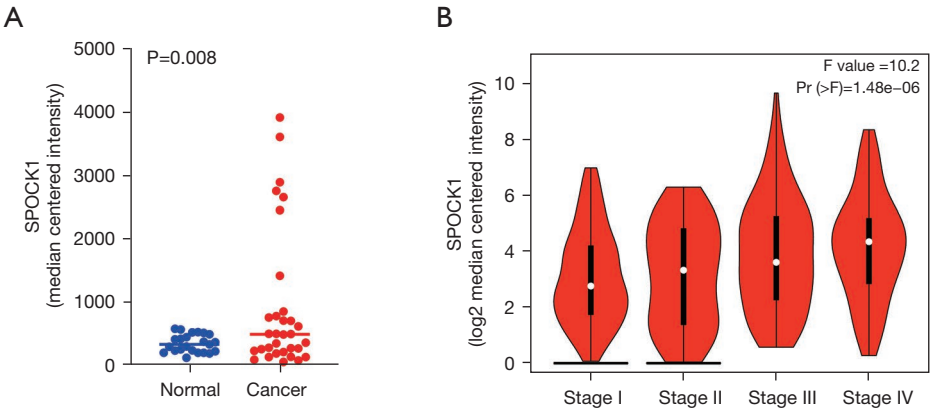

Figure 1 Correlation of *SPOCK1* expression with pathological stage in ccRCC patients. (A) Significantly higher expression of *SPOCK1* in ccRCC tissues than in normal tissues adjacent to the tumor (P=0.008); (B) significant correlation of the expression of *SPOCK1* with the pathological stage of ccRCC patients (P<0.001). ccRCC, clear cell renal cell carcinoma; *SPOCK1*, SPARC/osteonectin, CWCV and Kazal-like domains proteoglycan 1.

tissues and tumor-adjacent normal tissues through the Oncomine database. Compared with normal tissues (mean expression ± SD: 358.4±29.1), the *SPOCK1* mRNA expression was significantly higher in ccRCC tissues (mean expression ± SD: 920.2±195.2, P=0.008, *Figure 1A*).

Using the GEPIA database, we further analyzed the correlation between *SPOCK1* expression and the pathological stage of ccRCC patients. The results demonstrated that *SPOCK1* expression had a significant and positive correlation with the pathological stage of ccRCC patients (F value =10.2, P<0.001, *Figure 1B*).

Prognostic relevance of SPOCK1 expression in ccRCC patients

To investigate the role of *SPOCK1* expression in ccRCC development, we analyzed the correlation between *SPOCK1* expression and prognostic outcomes in ccRCC patients through the GEPIA database. The results suggested that DFS and OS were significantly shorter in ccRCC patients with high expression of *SPOCK1* (P<0.001, P=0.046, respectively, *Figure 2A*,*2B*).

Subsequently, we searched the Kaplan-Meier Plotter website to further verify whether *SPOCK1* expression correlated with the prognosis of ccRCC patients. The results showed that high *SPOCK1* expression significantly correlated with shorter DFS (P=0.0022, *Figure 2C*) and OS (P=0.002, *Figure 2D*) in ccRCC patients.

Gene mutation and protein–protein interaction analysis of SPOCK1 in ccRCC patients

Using the cBioPortal website, we analyzed the mutations of *SPOCK1* in ccRCC patients. Overall, ≥2 gene mutations were detected in ccRCC, with gene amplification being more common in ccRCC samples (*Figure 3A*). The number of *SPOCK1* mutation samples was 75 of 831 ccRCC patient samples (overall mutation rate of 9%), most of which were amplification mutations (*Figure 3B*).

In addition, we performed a PPI analysis of *SPOCK1* on the STRING website to explore potential interactions between *SPOCK1* and other proteins. There were some nodes (10) and edges (19) in the PPI network where *SPOCK1* has interactions with other proteins (*Figure 3C*). These proteins that potentially interact with *SPOCK1* are mainly associated with signaling pathways that regulate cell growth, ECM, neuroprotection, and tumor development. GeneMANIA analysis suggested that the functions of *SPOCK1* and its related molecules (such as *GTF2E2*, *SMOC1*, *SMOC2*, *SPOCK3*, *SPOCK2*, *NRP2*, *TACSTD2* and *SPARC*) are mainly related to tumor development, ECM remodeling and the epithelial-mesenchymal transition (EMT) process (*Figure 3D*).

Correlation between SPOCK1 expression and immune cell infiltration in ccRCC patients

The level of immune cell infiltration in tumors correlates

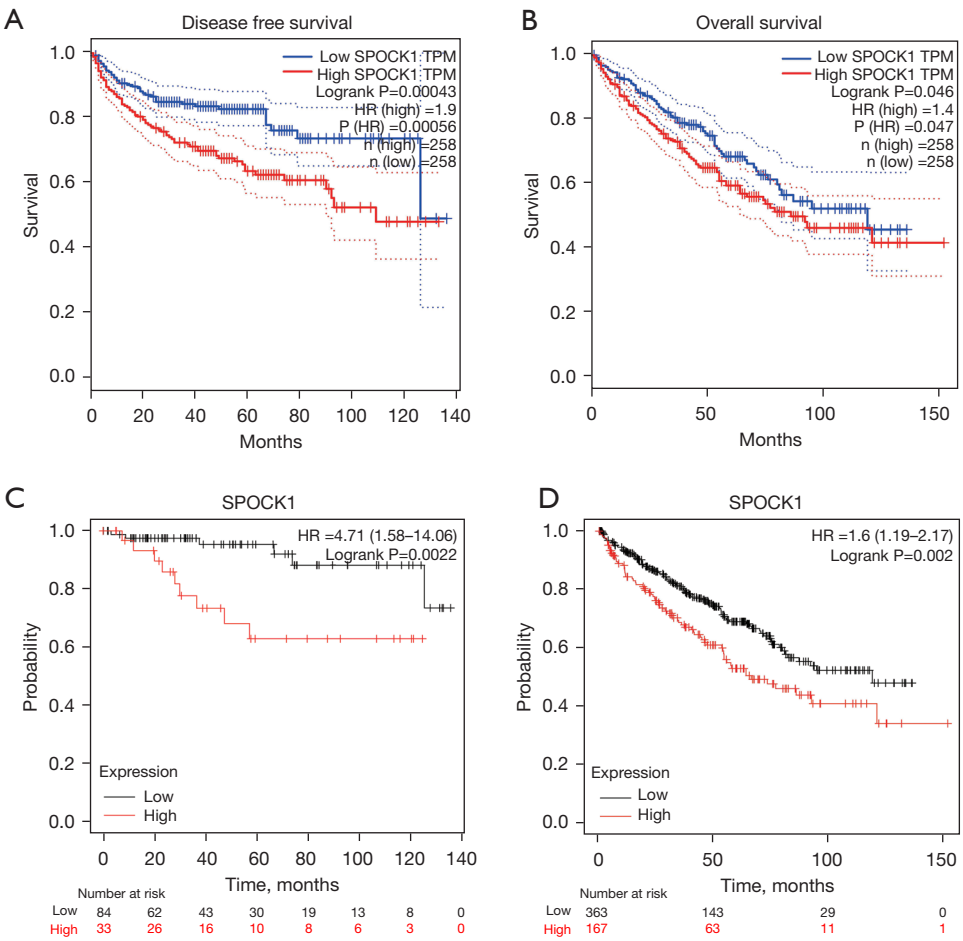

Figure 2 Prognostic value of mRNA expression of *SPOCK1* in ccRCC patients. (A) High transcriptional levels of SPOCK1 were significantly associated with shorter DFS (P<0.001, GEPIA); (B) high transcriptional levels of *SPOCK1* were significantly associated with shorter OS (P=0.046, GEPIA); (C) high mRNA expression of *SPOCK1* was significantly associated with shorter DFS (P=0.0022, Kaplan-Meier Plotter); (D) high mRNA expression of *SPOCK1* was significantly associated with shorter OS (P=0.002, Kaplan-Meier Plotter). ccRCC, clear cell renal cell carcinoma; DFS, disease-free survival; OS, overall survival; HR, hazard ratio; TPM, transcripts per million; *SPOCK1*, SPARC/osteonectin, CWCV and Kazal-like domains proteoglycan 1.

with tumor proliferation and metastasis, a process that may be mediated through regulation of the tumor microenvironment (TME). Using the TIMER website, we explored whether there was a correlation between *SPOCK1* expression and infiltration of different immune cells in ccRCC (*Figure 4*). Our results suggested that high *SPOCK1* expression was associated with significantly higher levels of infiltration of some immune cells in ccRCC, such as cancer-associated fibroblasts (CAFs) (Rho =0.333, $P=2.16\times10^{-13}$), tumor-associated macrophages (TAMs) (Rho =0.18, $P=1.02\times10^{-4}$), and tumor-associated neutrophils (TANs) (Rho =0.165, $P=3.83\times10^{-4}$). In contrast, high *SPOCK1* expression negatively correlated with the levels of infiltration of $CD4^+$ T cells, B cells, and $CD8^+$ T cells in ccRCC. Among them, a significant negative correlation was found with $CD4^+$ T cell infiltration (Rho =–0.113, P=0.015).

Discussion

Despite the advances in the treatment of ccRCC, the prognosis of patients with advanced ccRCC remains very poor, with 5-year OS <10% (23). There has been much exploration of the pathogenesis of ccRCC, but effective treatments for metastatic ccRCC are still lacking.

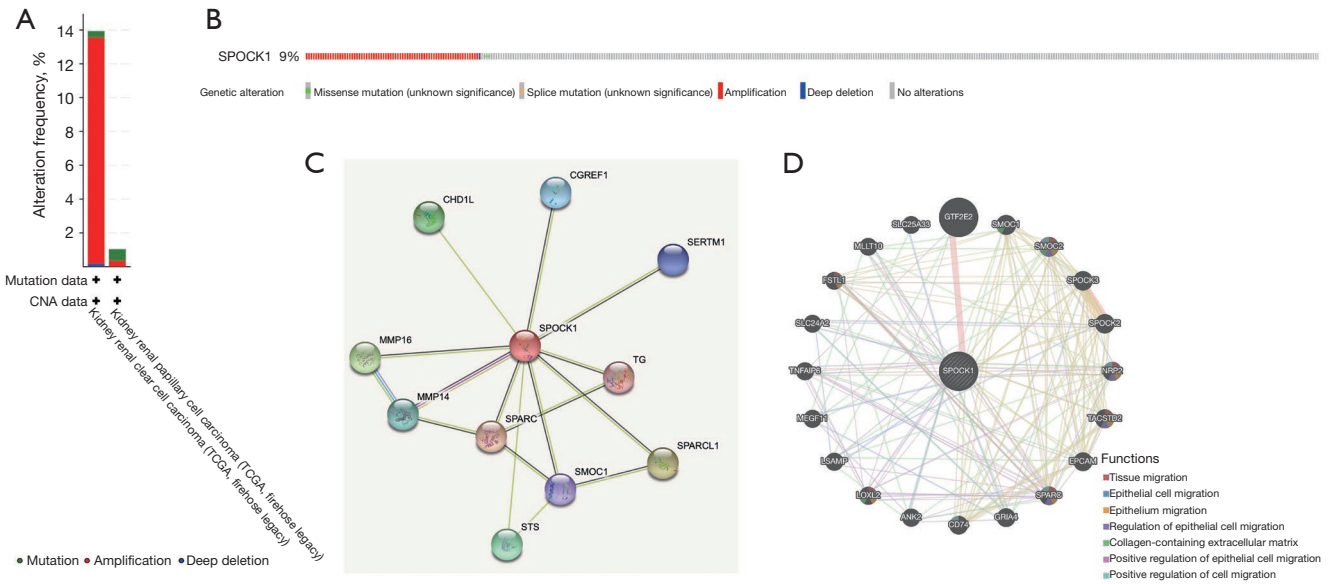

Figure 3 Gene mutation and expression analysis of *SPOCK1* in ccRCC patients (cBioPortal and STRING databases). (A,B) Among 831 samples from ccRCC patients, 75 samples were mutated (mutation rate of 9%), and most of them were amplification mutations; (C,D) protein-protein interaction network analysis of *SPOCK1* with other related proteins. +, select molecular profiles: both mutations and copy number alterations; ccRCC, clear cell renal cell carcinoma; CNA, copy-number alterations; *SPOCK1*, SPARC/osteonectin, CWCV and Kazal-like domains proteoglycan 1.

Therefore, there is a strong need to continue exploring pharmacological targets and prognostic biomarkers for ccRCC. Although a review has confirmed that *SPOCK1* plays an important role in a variety of tumors such as prostate, lung, and colorectal cancers, its role in ccRCC remains to be elucidated (15). Therefore, we performed a comprehensive assessment of *SPOCK1*'s prognostic value in ccRCC in terms of different gene expressions in ccRCC tissues and tumor-adjacent normal tissues, gene mutations in ccRCC, and the correlation between high *SPOCK1* expression and prognostic outcomes and infiltration of immune cells in ccRCC.

Compared with tumor-adjacent normal tissues, *SPOCK1* expression was significantly higher in ccRCC tissues, and its expression had a significant positive correlation with ccRCC pathological stage. In addition, ccRCC patients with high *SPOCK1* transcript levels had significantly shorter DFS and OS. These findings suggested that *SPOCK1* has a key role in the progression of ccRCC. We previously reported that *SPOCK1* expression is significantly upregulated in various tumors, such as non-small cell lung cancer, prostate cancer, colorectal cancer, liver cancer, and breast cancer, and that high *SPOCK1* expression often indicates poor prognosis of patients with cancer, because of its role in tumor cell proliferation, colony formation and metastasis (15). Therefore, *SPOCK1* may be involved in the progression of ccRCC and may be a biomarker to predict the prognosis of ccRCC patients.

Further genetic analysis in the present study showed that the gene mutation rate of *SPOCK1* in ccRCC was 9% and mainly comprised gene amplification. In addition, we performed a PPI analysis of *SPOCK1* on the STRING website to explore potential interactions between *SPOCK1* and other proteins. The results revealed that the proteins potentially interacting with *SPOCK1* were mainly associated with signaling pathways regulating cell growth, ECM, neuroprotection, and tumor development. The results of our GeneMANIA analysis, on the other hand, showed that the functions of *SPOCK1* and its related molecules (such as *GTF2E2*, *SMOC1*, *SMOC2*, *SPOCK3*, *SPOCK2*, *NRP2*, *TACSTD2* and *SPARC*) are mainly related to tumor development, ECM remodeling and the EMT process. These results are consistent with the role of *SPOCK1* in the development of other tumors and its possible molecular mechanisms (15). Therefore, we speculate that *SPOCK1* may also be involved in ccRCC by participating in ECM remodeling, regulating downstream signaling pathways, and ultimately leading to ccRCC progression. Of course, more

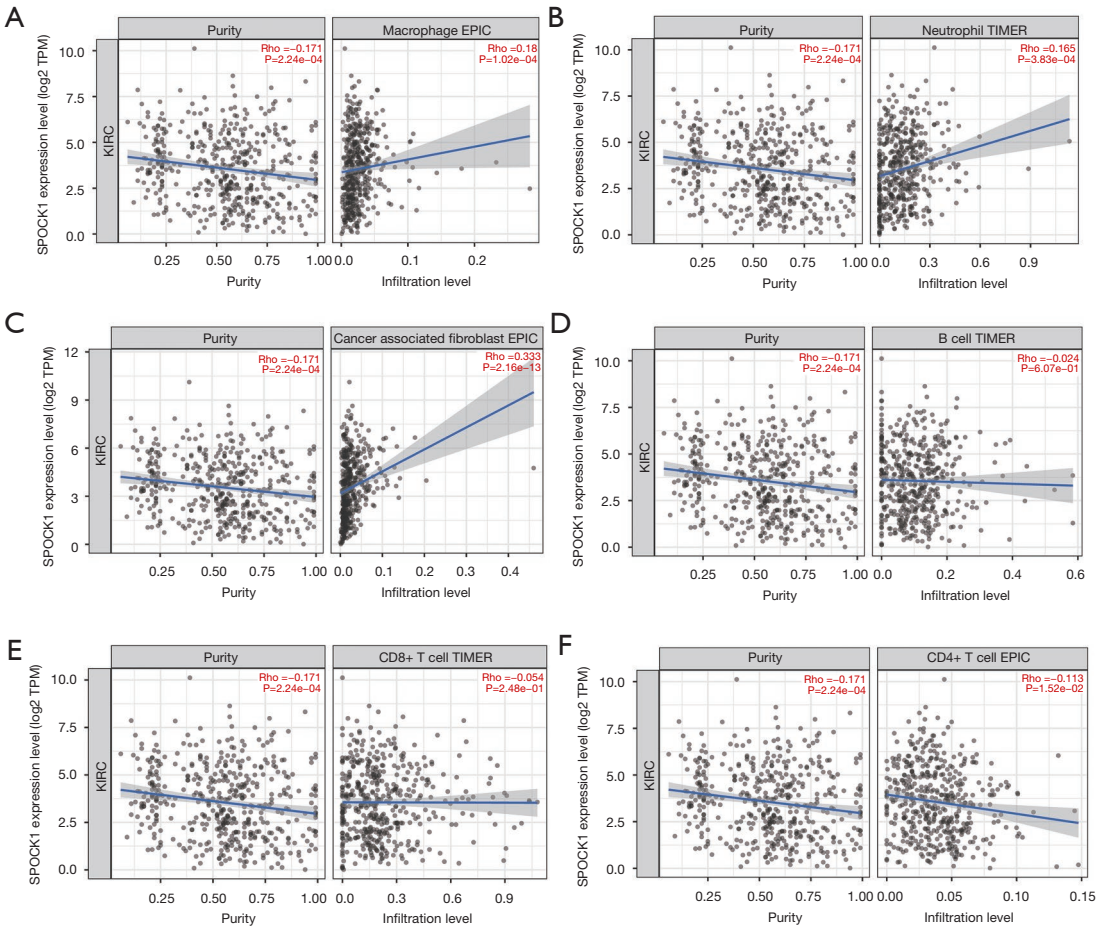

Figure 4 Correlation between *SPOCK1* expression and immune cell infiltration (TIMER and EPIC algorithms). (A) Tumor-associated macrophages; (B) tumor-associated neutrophils; (C) cancer-associated fibroblasts; (D) B cells; (E) $CD8^+$ T cells; (F) $CD4^+$ T cells. TIMER, tumor immune estimation resource; EPIC, estimating the proportions of immune and cancer cells; TPM, transcripts per million; *SPOCK1*, SPARC/osteonectin, CWCV and Kazal-like domains proteoglycan 1.

studies on the role of *SPOCK1* in ccRCC development are needed to confirm this conclusion.

As an immunogenic tumor, ccRCC responds to interleukin-2 and interferon-based therapy, efficacy is low. The effect of immune checkpoint inhibitor therapy is also limited, which may be related to the TME (24). Numerous studies have shown that the TME may be involved in tumorigenesis, progression and metastasis (25,26). In the TME, different immune cells have roles in promoting tumor progression or inhibiting tumor development, thus participating in the process of tumor development (27,28). As important immune cells in the TME, TAMs play a key role in tumor development (29). TAMs have two main phenotypes, M1 and M2, of which M1-TAMs are thought to be involved in antigenic presentation and inflammation, and M2-TAMs are thought to be involved in the process of tumor development and have relevance in resistance to antitumor drugs (30,31). The results of a meta-analysis by Shen *et al.* suggested that TAMs might be prognostic biomarker and new targets for immunotherapy in ccRCC (32). TANs are important inflammatory cells in the TME, and it has been reported that they promote angiogenesis, metastasis, and immune regulation in ccRCC by secreting cytokines and chemokines (33). CAFs are one of the most important components of the TME, and they secrete a number of cytokines, growth factors and ECM-related proteins that promote proliferation, drug resistance and invasion of ccRCC cells (34). $CD4^+$ T cells, B cells, and $CD8^+$ T cells are very important immune cells, and their functional inactivation may be associated

with tumor immune escape (35). The results of this study indicated that high *SPOCK1* expression significantly correlated with infiltration of some immune cells in ccRCC, including TAMs, CAFs and TANs (P<0.001). In contrast, high *SPOCK1* expression negatively correlated with the level of infiltration of $CD4^+$ T cells, B cells, and $CD8^+$ T cells in ccRCC. Among them, a significant negative correlation was found with $CD4^+$ T cells infiltration (P=0.015). This suggests that *SPOCK1* may contribute to the development of ccRCC by enhancing the immune infiltration of TAMs, CAFs and TANs and suppressing both B cell and T lymphocyte infiltration. It has been shown that T lymphocyte infiltration in tumor tissue has a positive correlation with the efficacy of immune checkpoint inhibitors, and that proteoglycans may prevent T cell infiltration through ECM remodeling, resulting in poor efficacy of immune checkpoint inhibitors (36). Therefore, inhibition of *SPOCK1* in combination with PD-1 inhibitors may be available for ccRCC treatment.

Our study still has some limitations. All the data we analyzed were obtained from databases, so more clinical studies are needed to validate these results. Although the databases provide a correlation of *SPOCK1* expression with prognostic outcomes, further cohort studies are needed to analyze whether this correlation is independent of other clinical variables. In the future, we will further explore the specific mechanisms of *SPOCK1* in ccRCC development through cellular and animal experiments, which will provide stronger evidence on whether *SPOCK1* can be used as a potential prognostic biomarker and therapeutic target for ccRCC.

Conclusions

Compared with tumor-adjacent normal tissues, *SPOCK1* expression was significantly higher in ccRCC tissues, and its expression had a significant positive correlation with ccRCC pathological stage. In addition, ccRCC patients with high *SPOCK1* transcript levels had significantly shorter DFS and OS. Further genetic analysis showed that the mutation rate of *SPOCK1* in ccRCC was 7% and mainly gene amplification. In addition, the results of PPI analysis suggested that the function of *SPOCK1* is mainly related to tumor development and ECM remodeling, and may be involved in the EMT process. High *SPOCK1* expression significantly correlated with infiltration of some immune cells in ccRCC, including TAMs, CAFs and TANs. In contrast, high *SPOCK1* expression negatively correlated with the level of infiltration of $CD4^+$ T cells, B cells, and $CD8^+$ T cells in ccRCC. Of these, a significant negative correlation was found with $CD4^+$ T cell infiltration. Our study suggests new ideas to explore regarding the potential for *SPOCK1* to a prognostic biomarker for ccRCC.

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Footnote

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://tau.amegroups.com/article/view/10.21037/tau-22-161/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

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