Original Research

Semaphorin 6D as an independent predictor for better prognosis in clear cell renal cell carcinoma

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ABSTRACT

Introduction: Clear cell renal cell carcinoma (ccRCC) is the most common type of RCC and is associated with poor survival. However, the mechanisms underlying its development have not been thoroughly investigated. Semaphorin 6D (SEMA6D) is differentially expressed in various cancers, including lung adenocarcinoma and colorectal cancer. However, the role and mechanism of SEMA6D in ccRCC remain unexplored.

Materials and methods: We obtained 25 pairs of ccRCC tissue samples and 57 urine samples from patients with ccRCC and 52 urine samples from healthy volunteers. We performed RNA sequencing and compared the results with data from The Cancer Genome Atlas database to identify our gene of interest, SEMA6D. To verify the differential expression of SEMA6D, we used real-time quantitative polymerase chain reaction, immunohistochemistry, and enzyme-linked immunosorbent assay. Finally, we conducted in vitro proliferation, migration and invasion experiments.

Results: SEMA6D expression was significantly lower in ccRCC tissue compared to that in normal tissue. Comparative analysis of our results with data from online databases revealed that the expression level of SEMA6D in ccRCC tissue correlated with the clinical stage and pathological grade of ccRCC. Furthermore, higher SEMA6D expression was associated with improved quality of life of patients with ccRCC. In addition, the diagnostic value of SEMA6D was confirmed using data from two Gene Expression Omnibus ccRCC databases. The results showed that SEMA6D can be used as a predictor for ccRCC diagnosis, with an area under the curve of 0.9642.

Conclusion: SEMA6D may serve as a diagnostic and prognostic biomarker for ccRCC.

Introduction

Globally, there were more than 400,000 new cases of cancer were diagnosed worldwide in 2020, and nearly 1000 deaths were associated with renal cell carcinoma (RCC) [1]. The American Joint Committee on Cancer estimated that there would be over 76,000 newly diagnosed cases of RCC and over 13,000 deaths related to RCC in the United States in 2021 [2]. Clear cell renal cell carcinoma (ccRCC) is the most common type of RCC [3]. The 5-year specific survival rate of patients with ccRCC is less than 70% [4]. Therefore, it is important to develop techniques and diagnostic biomarkers for ccRCC for early detection and predicting survival time in patients.

Semaphorin 6D (SEMA6D) belongs to a large protein family, which includes both secreted and membrane proteins [5]. Semaphorins contain a semaphorin domain in the N-terminal extracellular portion [6, 7]. SEMA6D has been shown to play diverse roles in different types of cancer. For example, SEMA6D exhibits antitumor activity against lung adenocarcinoma [8]. Additionally, increased expression of SEMA6D is associated with better survival in patients with triple negative breast cancer [9]. In contrast, in case of osteosarcoma, elevated SEMA6D expression enhances cisplatin resistance [10]. SEMA6D promotes angiogenesis in colorectal and gastric cancers [11,12]. However, the

Abbreviations: RCC, renal cell carcinoma; ccRCC, clear cell renal cell carcinoma; SEMA6D, semaphorin 6D; IHC, immunohistochemistry; GEPIA, gene expression profiling interactive analysis; DFS, disease-free survival; TCGA-KIRC, cancer genome atlas kidney renal clear cell carcinoma; HPA, human protein atlas; PBS, phosphate-buffered saline; AUC, area under the curve.

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role and mechanism of SEMA6D in ccRCC have not been extensively explored.

In this study, the expression of SEMA6D in ccRCC was determined using data from online databases and the results of RNA sequencing of clinical samples. We also explored the potential role of SEMA6D as a biomarker for ccRCC diagnosis and prognosis. Collectively, our findings may contribute toward a better understanding of the mechanism of ccRCC development, progression, and prognosis.

Materials and methods

Sample collection

We collected 25 pairs of ccRCC tissue samples, the corresponding tumor-adjacent tissue, and 57 urine samples from patients with ccRCC before surgery. In addition, we collected 52 urine samples from healthy volunteers. The patients with ccRCC were not administered adjuvant anticancer therapy before surgery and diagnosed to have ccRCC after surgery by the Pathology service (PATHO) in our hospital. All patients and volunteers who participated in this study provided written informed consent. The ethics committee of the First Affiliated Hospital of Zhengzhou University approved this study (amendment no. 2019-148).

A part of the paired cancer tissue specimens was used for RNA extraction. The rest of the paired cancer tissue was stored in formalin until further use for immunohistochemistry (IHC).

RNA sequencing

Four paired cancer and paracancerous ccRCC tissue samples were subjected to mRNA transcriptome sequencing to identify differentially expressed genes. RNA sequencing was performed by LC-Bio Technologies (Hangzhou, China). We selected the target gene at a threshold of adjusted \( p < 0.05 \) and \( \log_2 \) (fold change) (FC) > 2 or \( \log_2 \) FC < -2.

Bioinformatic analysis

Gene Expression Profiling Interactive Analysis (GEPIA) software [13] was used to analyze SEMA6D expression, clinical stage, disease-free survival (DFS), and overall survival (OS) of patients with ccRCC. The clinical data of grade and stage of ccRCC and DFS and OS of patients with ccRCC were extracted from the Cancer Genome Atlas Kidney Renal Clear Cell Carcinoma (TCGA-KIRC) database on the UCSCXena website. Data related to DFS were retrieved from the cbioPortal website (http://www.cbioportal.org). Healthy volunteers represented the control group. Additionally, the Human Protein Atlas (HPA, https://www.proteinatlas.org/) was used to search for SEMA6D-related data on protein expression and patient survival [14,15]. The genes associated with SEMA6D were downloaded from cbioPortal. Pan-cancer analysis was conducted using the TIMER website (https://cistrome.shinyapps.io/timer/). The Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway and Gene Ontology (GO) term enrichment analyses of SEMA6D-related genes were performed at threshold \( r > 2 \) and \( p < 0.05 \). The corrplot package was used to analyze the association between SEMA6D and immune genes. The ESTIMATE package was used to determine immune and stromal scores.

Cell culture

The normal kidney cell line (HK-2) and two ccRCC cell lines (786-0 and Caki-1) were obtained from Procell (Wuhan, China). The three cell lines were cultured in RPMI 1640 (HyClone, Logan, UT, USA) containing 10% heat-inactivated fetal bovine serum (HyClone) as well as 1% penicillin and streptomycin. The cells were cultured in an incubator at 37° C under a 5% CO2 atmosphere.

Overexpression of SEMA6D

The SEMA6D plasmid vector and plasmid control (Genechem, Shanghai, China) were transfected into 786-0 and Caki-1 cells using JetPrime (Polyplus-transfection, New York, NY, USA) according to the manufacturer’s instructions. All plasmids were synthesized by Genechem (Shanghai, China).

Cell proliferation, invasion, and migration assays

For the cell proliferation assay, we used Cell Counting Kit-8 (CCK-8). For CCK-8 experiments, 5,000 cells were seeded per well in 96-well plates with six repeats for each cell line. The absorbance of the sample at 450 nm was measured using the CCK-8 assay (Dojindo, Kumamoto, Japan) at 12, 24, 48, and 72 h post-seeding. Wound-healing experiments were used to monitor cell migration. The cells were grown in a six-well plate until reaching a confluence of >90%. Images were captured under 200 \( \times \) magnification at 0 and 24 h using the IX71 microscope (Olympus, Tokyo, Japan). Transwell chambers, coated with Matrigel (BD Biosciences, Franklin Lakes, NJ, USA), were used to perform the invasion assay. The cells (1 \( \times \) 10^5) were seeded into the upper chamber with RPMI 1640. The lower chamber contained 10% fetal bovine serum in RPMI1640. Five images were randomly selected, and the cells were counted under 200 \( \times \) magnification using the IX71 microscope after 24 h.

RNA extraction and quantitative real-time polymerase chain reaction (qRT-PCR)

TRIZol reagent (Thermo Fisher Scientific, Waltham, MA, USA) was used to isolate RNA from ccRCC tissue and cells, and the following procedure was used according to the manufacturer’s instructions. A Prime Script RT reagent kit with gDNA Eraser (Takara, Shiga, Japan) was used for cDNA preparation. SYBR Green (Takara) was used to perform qRT-PCR to analyze the expression of SEMA6D. GAPDH was used as an internal amplification control. The primer sequences used in this study were as follows: SEMA6D forward: 5′-GCTTTGTGCTACATACGCTGCT-3′ and reverse: 5′-ACGGGATTTGCCCCTGGAATGTG-3′; GAPDH forward: 5′-GTCTCCTCTGACTTCAACAGCG-3′ and reverse: 5′-AACCACCGTGTTGCTGTAGCCAA-3′.

Immunohistochemistry

The embedded tissues from patients with ccRCC were incubated overnight at 4° C with the primary anti-SEMA6D antibody (Bioss, Beijing, China; bs-2943R) and then washed with phosphate-buffered saline (PBS). Thereafter, the samples were coated with anti-rabbit secondary antibodies for 2 h at room temperature. 3,3′-Diaminobenzidine tetrahydrochloride was used for the color reaction. The development of a dark brown color was defined as a positive result.

Enzyme-linked immunosorbent assay (ELISA)

The concentration of SEMA6D in the urine samples from patients with ccRCC and healthy volunteers was measured using the Human SEMA6D ELISA Kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). We centrifugated urine samples at 3000 rpm for 10 min and collected the upper layer, which was then stored at -80° C until further analysis. The upper layer was added to the corresponding wells that had been pre-coated with primary antibodies. To each well, recognition antigens labeled with horseradish peroxidase were added and incubated for 30 min, followed by washing with PBS. The absorbance of the sample was measured using the Varioskan Lux microplate reader (Thermo Fisher Scientific) at 450 nm. Urine samples from patients with ccRCC were collected before surgery and were all validated by PATHO.
Statistical analysis

All statistical analyses were conducted using GraphPad Prism 6.0 (GraphPad Software, Inc., USA) and SPSS Statistics 22.0 software (IBM SPSS, Chicago, IL). The data from online databases and samples collected for paired cases were analyzed using a paired sample t-test. Nonparametric cases were analyzed using Mann–Whitney U test, whereas unpaired cases were analyzed using t-test or one-way analysis of variance. Spearman’s correlation was used to analyze the association between SEMA6D and other genes. The correlation between SEMA6D and OS and DFS was determined using Kaplan–Meier analysis. The diagnostic value of SEMA6D was determined based on the area under the curve (AUC) analysis. Cox regression analysis was used to evaluate the prognostic significance of SEMA6D. Statistical significance was determined at p < 0.05.

Results

ccRCC tissue exhibited low SEMA6D expression

For differentially expressed genes, we excluded the non-meaningful genes reported in TCGA and HPA. The other genes, except for SEMA6D, are still under research. To further explore the role and mechanism of SEMA6D in ccRCC, we investigated the clinical characteristics and survival status of patients with ccRCC, and evaluated the expression of SEMA6D-associated genes and performed in vitro experiments in ccRCC cells (Fig. 1A).

The RNA-sequencing data threshold was set as follows: log2(FC) > 2 or log2(FC) < 2, and p < 0.01. We identified SEMA6D as one of the genes of interest. The expression of SEMA6D (log2(FC) = -2.09461 and p = 0.002377) was significantly downregulated in ccRCC tissue, as validated using the TCGA-KIRC database (Supplementary Fig. 1A).

The GEPIA analysis showed that the expression of SEMA6D was significantly downregulated in ccRCC tissue (Fig. 1B). In addition, there was a significant difference in the expression level of SEMA6D in patients with different stages of ccRCC (Fig. 1C). A higher expression of SEMA6D was associated with a longer survival time in patients with ccRCC (Fig. 1D, E). The HPA analysis indicated that the expression of SEMA6D was relatively higher in the kidney tissue than in other normal tissues (Fig. 1F). Moreover, patients in the high SEMA6D expression group exhibited a longer survival time than the low SEMA6D expression group (Fig. 1G). The qRT-PCR was performed to verify the results of bioinformatic analysis and RNA sequencing (Fig. 1H). Similar results were obtained in the IHC analysis (Fig. 1I). The ELISA results showed that the level of SEMA6D in urine samples from patients with ccRCC was significantly lower than that in healthy volunteers (Fig. 1J, K, Supplementary Table 1). Furthermore, the area under the ROC curve was 0.791 and the cut off value was 265.1 ng/L. In summary, the expression of SEMA6D was lower in the ccRCC tissue than in the tumor-normal tissue. Additionally, the levels of SEMA6D in urine samples from patients with ccRCC were lower than that in healthy volunteers.

Low SEMA6D levels were associated with various clinicopathological features and poor prognosis in patients with ccRCC

To further investigate the relationship between SEMA6D and the clinicopathological features of ccRCC, we analyzed the relationship between SEMA6D and the clinical features retrieved from the online database for patients with ccRCC. The SEMA6D expression level negatively correlated with survival status (Fig. 2A), grade (Fig. 2B, C), OS (Fig. 2D), stage (Fig. 2E, F), T stage (Fig. 2G, H), and DFS (Fig. 2I), based on the analysis of the KIRC database. However, no difference in the expression of SEMA6D was observed between male and female patients (Supplementary Fig. 1A). The low SEMA6D expression group tended to be in a more advanced stage of ccRCC.

To verify the prognostic value of SEMA6D, analyses were conducted for clinical outcomes from the TCGA database. The results showed that DFS was shorter in the low SEMA6D group (p = 0.033, Fig. 2J). A similar result was observed in the T1+T2 group (p = 0.014, Fig. 2K). However, there were no differences in DFS in the groups of patients with stage I–II and stage III–IV ccRCC (Supplementary Fig. 1B, 1C).

We also performed OS analysis to identify the association between SEMA6D expression in different ccRCC subgroups. Based on the expression level of SEMA6D, we divided patients with ccRCC into high SEMA6D expression group and low SEMA6D expression group. The results showed that SEMA6D was positively correlated with OS in patients with ccRCC (p < 0.001, Fig. 2L), female patients with ccRCC (p = 0.011, Fig. 2M), and patients with stage III–IV ccRCC (p = 0.014, Fig. 2N). However, there was no difference in DFS in the group of male patients (Supplementary Fig. 1D) and patients with stage I–II ccRCC (Supplementary Fig. 1E, F).

Diagnostic value of SEMA6D in patients with ccRCC and validation of the results using Gene Expression Omnibus (GEO) analysis

To verify whether SEMA6D may serve as a diagnostic biomarker in ccRCC, ROC curves for the clinicopathological variables were developed. The expression of SEMA6D may help distinguish patients with ccRCC, with an AUC of 0.964 (95% confidence interval, CI: 0.944–0.984; p < 0.0001, Fig. 3A). The results also showed that the sensitivity of SEMA6D in distinguishing OS-good from OS-poor, G1–2 from G3–4, stage I–II from stage III–IV, T1–2 from T3–4, and survival status in patients with ccRCC with AUCs of 1, 0.632, 0.622, 0.585, and 0.565, respectively (95% CI: 1–1, 0.585–0.680, 0.574–0.671, 0.535–0.635, and 0.5155–0.6146; p < 0.0001, p < 0.0001, p < 0.0001, p = 0.0012, and p = 0.0093; Fig. 3B, 3C, 3D, 3E, and 3F respectively).

To further verify the ability of SEMA6D to be a diagnostic biomarker for ccRCC, we used two groups from the GEO ccRCC database, GSE92482 and GSE126964. The expression of SEMA6D in the two databases was higher in the normal tissue than in the tumor tissue (Fig. 3G, H). The AUC for GSE92482 was 0.8819 (95% CI: 0.724–1.000; p = 0.0015, Fig. 3I), whereas that for GSE126964 was 0.9769 (95% CI: 0.940–1.000; p < 0.0001, Fig. 3J). SEMA6D expression as a ccRCC risk factor was calculated using univariate analysis. The results for DFS showed that SEMA6D could be used as a meaningful prognostic factor for patients with ccRCC (Fig. 3K).

Relationship between SEMA6D and immune-related genes and the Notch pathway

cBioPortal (https://www.cbioportal.org/) was used to select SEMA6D-related genes at a threshold of p < 0.05 and r > 0.4 or r < -0.4. The GO term enrichment analysis was also performed (Supplementary Fig. 2). The GO term enrichment results showed that SEMA6D-related genes perform functions mainly associated with antigen processing. The corplot package was used to analyze the relationship between SEMA6D and other immune-related genes, yielding a positive relationship with CD274 and a negative relationship with IFNG (Fig. 4A). MCP-counter and ssGSEA were used to estimate the proportions of immune cell types associated with different levels of SEMA6D in ccRCC, showing positive associations with endothelial cells and others, but negative associations with CD8T cells and others (Fig. 4B, Supplementary Fig. 3). The immune score negatively correlated with the expression of SEMA6D (r = -0.269) (Fig. 4C), whereas the stromal score positively correlated with the expression of SEMA6D (Fig. 4D).

The KEGG pathway enrichment analysis was also performed (Fig. 4E). The Notch signaling pathway is involved in various inflammatory diseases and cancers [16,17]. The KEGG pathway enrichment analysis showed that it was one of the most altered pathways. To further explore the relationship between SEMA6D and the Notch pathway, we extracted all important genes involved in the Notch pathway from the KEGG database, at threshold p < 0.05 and r > 0.2 or r < -0.2
Fig. 1. SEMA6D was downregulated in clear cell renal cell carcinoma (ccRCC) tissues and survival was reduced. (A) A schematic of the work flow of this study. (B) SEMA6D expression was lower in the normal tissues than in the ccRCC tissues, based on GEPIA. (C) SEMA6D was differentially expressed in patients with different stages of ccRCC, based on GEPIA. (D) The high SEMA6D expression group exhibited longer disease-free survival than the low expression group, based on GEPIA. (E) The high SEMA6D expression group had a longer overall survival than the low-expression group, based on GEPIA. (F) The expression of SEMA6D was relatively high in the kidney tissues, based on the HPA analysis. (G) The high SEMA6D expression group of patients with ccRCC presented a longer survival time, based on HPA. (H) The mRNA expression of SEMA6D was relatively high in the normal tissue compared with that in the cancer tissue based on clinical ccRCC samples. (I) Results of the immunohistochemical analysis showed that the expression of SEMA6D was lower in the normal tissue than in the cancer tissue obtained from clinical samples. (J, K) The expression of SEMA6D in urine samples was higher in healthy volunteers than in patients with ccRCC. *p < 0.05; **p < 0.001.
The Notch pathway positively correlated with the stromal score at $r = 0.564$ (Fig. 4G). The correlation between the Notch pathway and immune gene levels was also determined. The Notch pathway showed a positive relationship with $\text{GZMB}$ and others, and a negative relationship with $\text{CD274}$ (Fig. 4H).

SEMA6D inhibited the proliferation, migration, and invasion of ccRCC cells.

The results of qRT-PCR showed that 786-0 and CAKI-1 cells weakly expressed SEMA6D compared with HK-2, a normal kidney cell line (Supplementary Fig. 5). We explored the role of SEMA6D overexpression in the ccRCC cell lines. Efficiency was measured using qRT-PCR (Fig. 5A). The results of CCK-8 suggest that cell proliferation is inhibited by SEMA6D overexpression (Fig. 5B). SEMA6D overexpression prevented the migration and invasion abilities of ccRCC cells (Fig. 5C, D). These data indicate that the overexpression of SEMA6D downregulates the metastatic potential of ccRCC cells.

Discussion

Recently, the clinical features of ccRCC, such as the low rate of early-
stage diagnosis and resistance to chemotherapy, have gained attention [18]. To date, the standard treatment for ccRCC is surgical resection along with targeted immunotherapy [19]. To increase early-stage detection, it is essential to identify reliable biomarkers and prognostic indicators for ccRCC [20]. In this study, we verified our RNA-sequencing results using data from the TCGA-KIRC database to identify specific genes related to ccRCC. We identified the co-differentially expressed SEMA6D, based on data available in online databases, and validated using qRT-PCR and IHC.

The target gene SEMA6D has been reported to have several important roles in tumor immune responses [21,22]. Recent studies have shown that SEMA6D could promote tumor progression; its high expression is related to malignant clinicopathological indexes of gastric cancer [23]. Furthermore, SEMA6D levels may be closely correlated with esophageal cancer and lung adenocarcinoma [24,25] and can regulate angiogenesis in colorectal cancer [26]. It has also been reported that SEMA6D could regulate chemoresponse in breast cancer [27]. Here, the results of the univariate analysis showed that OS was more affected by SEMA6D than DFS.

The OS means the time from randomization to death from any cause, while the DFS means the time from randomization to first recurrence or death. We found that DFS is more difficult to record than OS. The record of DFS requires that the discovery of tumor recurrence is made in time and that the death of the patient is certain. As is well understood, many

Fig. 3. Diagnostic value of SEMA6D expression in patients with ccRCC. (A) ROC curve showed that SEMA6D could distinguish ccRCC from para-cancerous tissue. (B–F) ROC curve analysis of the expression of SEMA6D in subgroups of patients with ccRCC in relation to OS status, grades G1+G2 vs. G3+G4, stage I+II vs. stage III+IV, T1+2 vs. T3+4, and survival status. (G, H) The GSE92482 and GSE126964 databases showed that SEMA6D expression was lower in ccRCC tumor tissue than in normal tissue. (I, J) ROC curve indicating that SEMA6D effectively distinguished ccRCC from normal tissues in GSE92482 and GSE126964. (K) Univariate analysis was conducted to assess risk factors for disease-free survival (DFS) and its association with SEMA6D in clear cell renal cell carcinoma (ccRCC). *p < 0.05; **p < 0.01; ***p < 0.001.
Fig. 4. Relationship between SEMA6D and immune-related genes and the Notch pathway. (A) The relationship between SEMA6D and other immune-related genes. (B) MCP-counter was used to estimate the level of immune cell types prevalent in clear cell renal cell carcinoma (ccRCC). (C, D) The relationship between the expression of SEMA6D and immune score, and stromal score. (E) The KEGG pathway analysis was performed. (F) The relationship between the Notch pathway and immune-related genes. (G) The relationship between the Notch pathway and immune-related genes.
cancer patients experience complications in other organ systems. Many cancer patients also die outside of hospital, making it difficult to determine the cause of their death. These factors have a negative effect on the judgement of DFS. This may explain the difference in values for OS and DFS. In future studies, we will increase our sample size to enrich our data, as well as gather the survival data of the patients included in this study.

The role and mechanism of SEMA6D in ccRCC have not been extensively investigated. We determined that the functions of SEMA6D are closely related to antigen processing and the Notch signaling pathway, according to the results of the GO term and KEGG pathway enrichment analyses. To investigate the role of SEMA6D as a biomarker for ccRCC, we collected urine samples from both healthy volunteers and patients with ccRCC to compare the SEMA6D levels between them [28].

Fig. 5. Overexpression of SEMA6D inhibited the proliferation, migration, and invasion of ccRCC cells in vitro. (A) SEMA6D mRNA expression was tested using qRT-PCR. (B) CCK-8 assay showing cell growth curves. (C) Wound healing assay was conducted to measure cell immigration. (D) Invasion assay performed using 786-0 and CAKI-1 cells. *p < 0.05; ***p < 0.001.
Here, the ELISA results showed that there was a significant difference in the SEMA6D levels between the control and experimental groups. The process of obtaining urine samples is less painful and more acceptable than obtaining blood samples for patients. In future studies, we will include more urine samples from both healthy volunteers and patients with ccRCC to further validate our findings.

To the best of our knowledge, this is the first study to show that the expression of SEMA6D was lower in ccRCC tissue than in normal tissue, but also variable in ccRCC tissue. The patients in the high SEMA6D expression group exhibited a longer survival time than those in the low expression group. The expression of SEMA6D varied in different stages of ccRCC. The normal kidney tissue displayed a relatively high SEMA6D expression than other tissues. However, patients with stage IV ccRCC presented the lowest SEMA6D expression. Thus, SEMA6D may serve as a reliable biomarker and may also be used as a target gene for research on the development of ccRCC. Next, we conducted cell experiments to explore the role of SEMA6D in ccRCC. The results of proliferation, migration, and invasion experiments showed that SEMA6D plays an anti-tumor role in ccRCC. It is also essential to further investigate the function and mechanism of SEMA6D in ccRCC using in-depth and in vivo studies. In conclusion, determining the function and mechanism of SEMA6D may help develop novel approaches to treat ccRCC.

Conclusions

In the present study, we observed that the expression of SEMA6D was low in ccRCC tissues compared with that in healthy tissues. High SEMA6D expression was also associated with a better prognosis in patients with ccRCC. Thus, SEMA6D may serve as a diagnostic and prognostic biomarker for ccRCC. Overexpression of SEMA6D inhibited the proliferation, migration, and invasion of ccRCC cells in vitro.

Disclosure

The authors report no conflicts of interest in this work. Written informed consent was obtained from the patients for publication of the results. A copy of the written consent is available for review by the Editor-in-Chief of this journal on request.

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CRediT authorship contribution statement

Jiachen Duan: Visualization, Data curation, Formal analysis, Writing – review & editing. Mengyuan Jin: Visualization, Data curation, Formal analysis, Writing – review & editing. Baoping Qiao: Visualization, Data curation, Formal analysis, Writing – review & editing.

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Supplementary materials

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[26] Y. Lee, S.J. Kim, J. Choo, et al., miR-23a-3p is a key regulator of IL-17C-induced tumor angiogenesis in colorectal cancer, Cells 9 (6) (2020) 1363, https://doi.org/10.3390/cells9061363.

[27] D.E. Baxter, L.M. Allinson, W.S. Al Amri, et al., MiR-195 and Its Target SEMA6D regulate chemoresponse in breast cancer, Cancers 13 (2021) 5979, https://doi.org/10.3390/cancers13235979, Basel.

[28] A.L. Pastore, G. Palleschi, L. Silvestri, et al., Serum and urine biomarkers for human renal cell carcinoma, Dis. Markers 2015 (2015), 251403, https://doi.org/10.1155/2015/251403.