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ORIGINAL RESEARCH

Loss of *KCNJ15* expression promotes malignant phenotypes and correlates with poor prognosis in renal carcinoma

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Background: *KCNJ15* belongs to the inwardly rectifying potassium channel (KIR) family. Although members of the KIR family have been proven to play important roles in a variety of developmental processes, the molecular role and clinical effects of *KCNJ15* in cancers remain unclear.

Purpose: The aim of this study was to identify the expression, biological functions and molecular mechanisms of *KCNJ15* in renal cell carcinoma (RCC).

Methods: *KCNJ15* mRNA expression was evaluated in kidney cancer tissue, paired adjacent normal tissue, and cell lines with qRT-PCR. *KCNJ15* protein expression was investigated via western blotting and immunohistochemistry. In addition, the clinical and prognostic significance of *KCNJ15* in RCC were assessed using Kaplan-Meier analysis and Cox proportional hazards analysis. In vitro, the effects of *KCNJ15* on kidney cancer cells were evaluated by means of a cell counting kit-8, transwell assay along with flow cytometry, respectively. Moreover, the potential mechanism of *KCNJ15* was demonstrated by Western blot.

Results: Here, we first found that *KCNJ15* was significantly downregulated in RCC, and this low expression was an independent prognostic factor for clear cell RCC (ccRCC). Moreover, *KCNJ15* was associated with advanced TNM stage (n=150, p=0.014) and histological grade (n=150, p=0.045). Furthermore, *KCNJ15* overexpression significantly inhibited RCC cell proliferation, migration, and colony formation, arrested the cell cycle and induced apoptosis of RCC cells in vitro. The inhibitory effect of *KCNJ15* overexpression may be regulated by its effects on the epithelial mesenchymal transition (EMT) process and matrix metalloproteinase (MMP)-7 and p21 expression.

Conclusion: These findings indicate that *KCNJ15* may be a tumor suppressor in RCC and a possible target for RCC therapy.

Keywords: *KCNJ15*, renal cell carcinoma, biomarker, tumor suppressor, prognosis

Introduction

Renal cell carcinoma (RCC), accounting for 80%–90% of the renal tumors, is a common malignant tumor of the genitourinary system.[1,2] Approximately 75%–85% of the RCC cases are clear cell RCC (ccRCC).[3,4] Around one-third of the patients diagnosed with RCC already have locally advanced or metastatic disease. The metastasis of RCC is the leading cause of the high mortality rate in patients with RCC.[2,5,6] Therefore, the study of the molecular mechanism of the occurrence, development, and metastasis of RCC and the identification of new specific early diagnostic biomarkers and therapy targets for RCC are imperative.

The inwardly rectifying potassium channel (KIR) family mainly consists of 7 subfamilies (KIR1.x–KIR7.x) and has ~20 members.[7] The inward rectification

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property of the inwardly rectifying calcium-activated potassium (KC) (Kir) channels is mediated by positively charged intracellular small molecules called polyamines.[8] KIR channels have been shown to play an important role in the resting membrane potential, K1 homeostasis maintenance, heart rate control, and hormone secretion.[9,10] In addition, KIR channel function can be regulated by many other factors in vitro and in vivo.[11]

KCNJ15, also known as IRKK, KIR1.3, and KIR4.2, is a member of the KIR4.x subfamily and encodes a potassium (K) channel. *KCNJ15* was first cloned from human embryonic kidney cells. It has eight different transcriptional mutants, but each encodes the same protein KIR4.2 (*KCNJ15* protein).[12] Previous studies have shown that *KCNJ15*, a susceptibility gene for type 2 diabetes, is upregulated by glucose and has a remarkable inhibitory effect on insulin secretion.[13–15] Moreover, *KCNJ15* is the most highly expressed among all K+ channels in the stomach and plays an essential role in the stimulation of gastric acid secretion.[16,17] In addition, *KCNJ15* is located on chromosome 21 in the Down syndrome chromosome region 1 and has been reported to be associated with Down syndrome.[18,19] However, up until now, whether *KCNJ15* plays any role in cancers has remained unclear.

In this study, we first examined the relationship between *KCNJ15* gene expression and the clinicopathological features of RCC. Furthermore, we explored the functional roles of the *KCNJ15* gene and the related molecular mechanisms in RCC. Our findings will provide a theoretical basis for the early diagnosis and specifically targeted therapy of RCC.

Materials and methods

Patient tissue specimens

In this study, 57 pairs of ccRCC tissues and paired paracancerous tissues were collected during surgery in the Second Affiliated Hospital of Lanzhou University. Tissue samples were fixed in RNAlater reagent and immediately stored in liquid nitrogen until required. All patients were pathologically diagnosed with RCC and had no history of chemotherapy or radiotherapy preoperatively.

Cell lines and culture

The human renal cancer cell lines (786-O, 769-P, Caki-1, Caki-2, and OS-RC-2) were purchased from the American Type Culture Collection (ATCC) (Manassas, VA, USA). According to the ATCC protocols, the cells were cultivated in RPMI-1640 or McCoy's 5A medium supplemented with 10% FBS (Thermo Fisher Scientific, Waltham, MA, USA) in a humidified atmosphere with 5% CO_2 at 37°C.

RNA extraction and quantitative real-time reverse transcription PCR (qRT-PCR)

Trizol reagent (Thermo Fisher Scientific) was used to extract the total RNA from renal cancer cell lines or tissues. cDNA synthesis was performed using a reverse transcription kit (TOYOBO, Co. Ltd, Osaka, Japan). After reverse transcription, *KCNJ15* mRNA expression was detected by using SYBR Premix Taq II (Takara, Shiga, Japan) with β-actin (ACTB) as an internal reference. The primers for *KCNJ15* and ACTB were as follows: *KCNJ15* primers: forward, 5′-CCACATCAGAACTCCCTTCAAACA-3′; reverse, 5′-AGTTCACTTTCAGACGAAGCACCTA-3′ and ACTB primers: forward, 5′-GAGATCAAGATCATTGCTCCTC-3′; reverse, 5′-AACTAAGTCATAGTCCGCCTAGAAG-3′.

Western blot

Protein from tissues or cells was extracted using cold RIPA lysis buffer (150 mM NaCl, 50 mM Tris-base, 5 mM EDTA, 1% NP-40, and 0.25% deoxycholate, pH 7.4) with protease inhibitors (Thermo Fisher Scientific). The obtained protein was relatively quantified using a Piece BCA Protein assay kit (Thermo Fisher Scientific) and then stored in a –80°C freezer. The extracted protein samples (20 μg) were electrophoresed by SDS-PAGE and transferred to polyvinylidene fluoride membranes. The membranes were blocked with 5% non-fat skim milk in 50 mM Tris–HCl, 50 mM NaCl, and 0.1% Tween-20 (TBST) at room temperature for 2 hours and then incubated with primary antibodies including mouse polyclonal anti-*KCNJ15* antibodies (1:3,000 ratio; Santa Cruz Biotechnology Inc., Dallas, TX, USA) and rat polyclonal anti-GAPDH antibodies (1:8,000; Abcam, Cambridge, UK) at 4°C overnight. After the membranes were washed with 1× TBST buffer three times, horseradish peroxidase-labelled goat anti-rabbit antibodies (1:8,000; Abcam) and goat anti-mouse antibodies (1:8,000; Abcam) were added as secondary antibodies at room temperature with gentle shaking for 1 hour.

Immunohistochemical (IHC) analysis

IHC staining was conducted to detect *KCNJ15* expression in RCC tissues and matched paracancerous tissues. A paraffin-embedded ccRCC tissue microarray (TMA) comprising 150 cancerous tissues and 30 adjacent tissues was obtained from the Shanghai Biochip Company Ltd. (Shanghai, China). After deparaffinization and rehydration, tissue sections were cooked for antigen repair in a microwave oven with sodium citrate solution (10 mM, pH 6.0) at high temperature for 2 minutes,

medium temperature for 5 minutes, and low temperature for 10 minutes. Then, the slides were washed with PBS twice, incubated in 3% hydroxyl peroxide for 20 minutes to block endogenous peroxidase activity, and incubated with the anti-*KCNJ15* antibody (1:600, HPA016702; Sigma-Aldrich Co., St Louis, MO, USA) at 4°C overnight. Tissue sections were placed at room temperature for half an hour and incubated with a biotin-labelled goat anti-rabbit antibody for 30 minutes. The staining for antigenic detection was performed with 3,3′-diaminobenzidine (DAB) solution (Dako Denmark A/S, Glostrup, Denmark). The duration of the DAB incubation was determined by observing the degree of staining under a microscope.

Overexpression

Full-length *KCNJ15* cDNA and a negative control were cloned into a lentiviral vector (Gene Pharma, Shang Hai, China). For transfection, lentiviral constructs expressing *KCNJ15* or negative control were transfected into Caki-2 and OS-RC-2 cell lines following the manufacturer's instructions. The expression of the *KCNJ15* gene was analyzed by Western blot and qRT-PCR.

Cell proliferation assays were evaluated using the cell counting kit-8 (CCK-8) assay (Dojindo, Kumamoto, Japan). Here, cell lines were seeded at a density of 2×10^3 cells/well in a 96-well plate and allowed to grow for 24, 48, 72, and 96 hours. Then, the CCK-8 reagent was added to the plates, which were then incubated in a humidified atmosphere with 5% CO_2 at 37°C for 2 hours. Cell viability was assessed by measuring the absorbance values at 450 nm.

Cell transwell assays

Transwell chambers (8 μm pore size; BD, Franklin Lakes, NJ, USA) were used to evaluate the cell migration capacity. Each group of RCC cells were harvested 48 hours after transfection. Briefly, 4×10^4 cells were seeded in the top compartment, whereas 500 μL medium supplemented with 10% FBS was added to the lower chamber. The chambers were cultured in a humidified atmosphere with 5% CO_2 at 37°C for 24 hours. Non-migratory cells on the upper side of the chambers were gently scrubbed, whereas migratory cells attached to the bottom surface of the membrane were fixed with 4% paraformaldehyde for 20 minutes and stained with 0.05% crystal violet for 15 minutes. Migrated cells in five random fields were counted under an Olympus microscope.

Colony formation assay

For cell colony formation, 1×10^3 cells were plated in a cell culture dish and allowed to grow in a humidified atmosphere with 5% CO_2 at 37°C for 1–2 weeks until each colony consisted of >50 cells. After the plates were washed twice with PBS, the colonies were fixed with 4% paraformaldehyde for 20 minutes, stained with 0.05% crystal violet for 15 minutes, photographed, and counted.

Flow cytometric analysis of cell cycle and apoptosis

For the cell cycle assessment, the cell lines used in the study were cultured in a T75 cell culture flask until 70%–80% confluency. Cells were harvested with trypsinization and fixed in ice-cold 75% ethanol at –20°C overnight. The cells were washed twice with PBS, resuspended with the PI/RNase staining buffer (BD Pharmingen™ catalog number 550825; BD Biosciences, San Jose, CA, USA) for 30 minutes in the dark at room temperature according to the manufacturer's instructions and then detected using an FACScan flow cytometer (BD).

For the apoptosis analysis, 2×10^5 cells were stained with Annexin V and phycoerythrin using an Annexin V Apoptosis Detection kit I (BD Pharmingen™ catalog number 559763; BD Biosciences) for 15 minutes and then analyzed with flow cytometry.

Statistical analyses

All statistical analyses were performed by using SPSS version 16.0 (SPSS Inc., Chicago, IL, USA). A chi-square test was used to compare the *KCNJ15* expression levels with the clinicopathological characteristics. Survival curve analyses were performed using the Kaplan–Meier method. Differences were only considered significant when P-values <0.05.

Results

KCNJ15 expression in RCC tissues and cell lines

We first examined the relative RNA expression of *KCNJ15* in 57 fresh paired RCC samples and adjacent non-tumor tissues by qRT-PCR to investigate *KCNJ15* gene expression in RCC. As shown in Figure 1A, *KCNJ15* expression was significantly lower in RCC tissues than in adjacent non-tumor tissues (P<0.05). The protein levels were confirmed by Western blot (Figure 1B).

We next conducted IHC analysis and demonstrated that *KCNJ15* was primarily located in the cytoplasm of RCC cells and normal renal tubular epithelial cells to further validate the expression and location of *KCNJ15* in RCC tissues and adjacent non-tumor tissues. In addition, statistical analysis

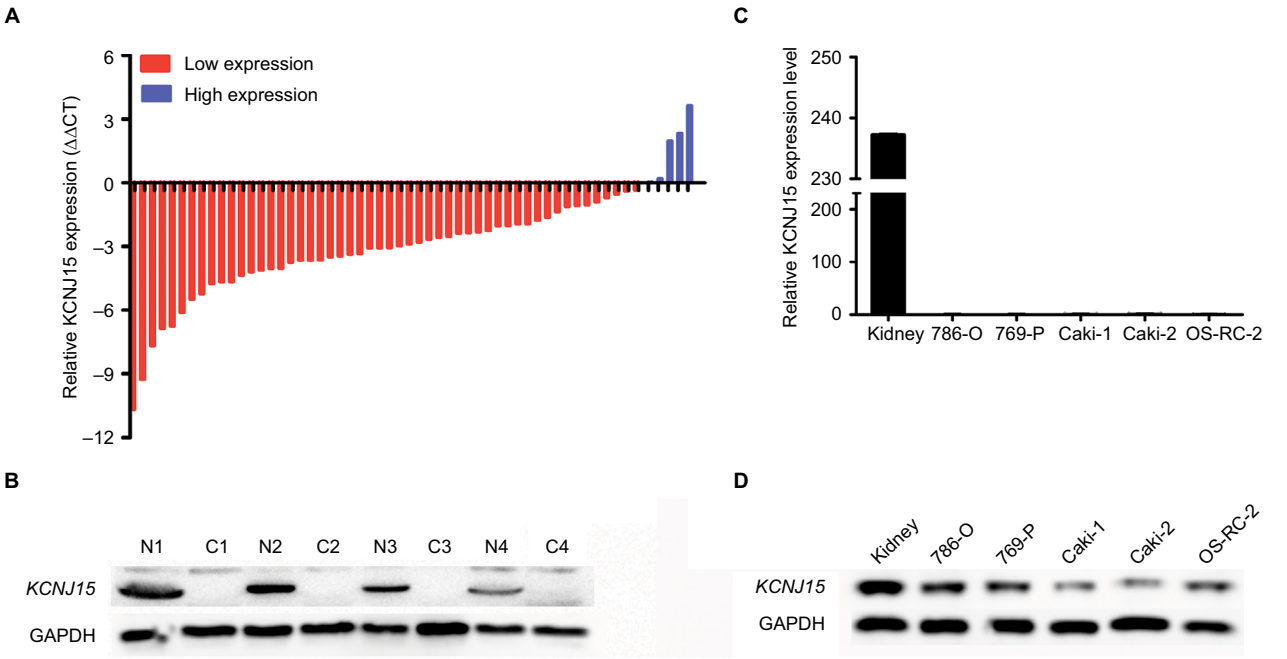

Figure 1 *KCNJ15* expression in RCC tissues, paracancerous tissues, and cell lines.
Notes: (**A**) *KCNJ15* mRNA expression levels in RCC tissues. (**B**) *KCNJ15* protein levels in RCC tissues were determined using Western blot. (**C**) *KCNJ15* mRNA expression levels were significantly lower in RCC cell lines (786-O, 769-P, Caki-1, Caki-2, and OS-RC-2) than in normal tissues. (**D**) *KCNJ15* protein expression levels in renal cancer cell lines. N (1–4) are the adjacent non-tumor tissues; C (1–4) are the the RCC tissues.
Abbreviation: RCC, renal cell carcinoma.

revealed markedly lower *KCNJ15* expression in RCC tissues than in adjacent non-tumor tissues (Figure 2A–D, $P<0.001$).

Similarly, the relative RNA and protein expression of *KCNJ15* in 5 RCC cell lines (786-0, 769-P, CAKI-1, Caki-2, and OS-RC-2) was markedly lower than that in normal paracarcinoma tissue (Figure 1C and D), indicating that low *KCNJ15* expression might be related to RCC progression.

Loss of *KCNJ15* is significantly associated with poor survival of patients with ccRCC

We conducted IHC analysis on a TMA containing 150 ccRCC cancers to determine the relationship between *KCNJ15* gene expression and prognosis of RCC patients. As shown in Table 1, among the 150 samples of paraffin-embedded ccRCC tissues, 96 samples (64%) expressed *KCNJ15* at higher levels and 54 samples (36%) expressed *KCNJ15* at low levels. Kaplan–Meier analysis demonstrated that the patients with low *KCNJ15* expression had worse overall survival than patients with high *KCNJ15* expression ($P=0.0105$) (Figure 2E), with a median survival time of 82 months for the high *KCNJ15* expression group and 71 months for the low *KCNJ15* expression group. Moreover, we found that low *KCNJ15* expression was closely correlated with the clinical stage (n=150, $P=0.014$) and histological grade ($P=0.045$) of

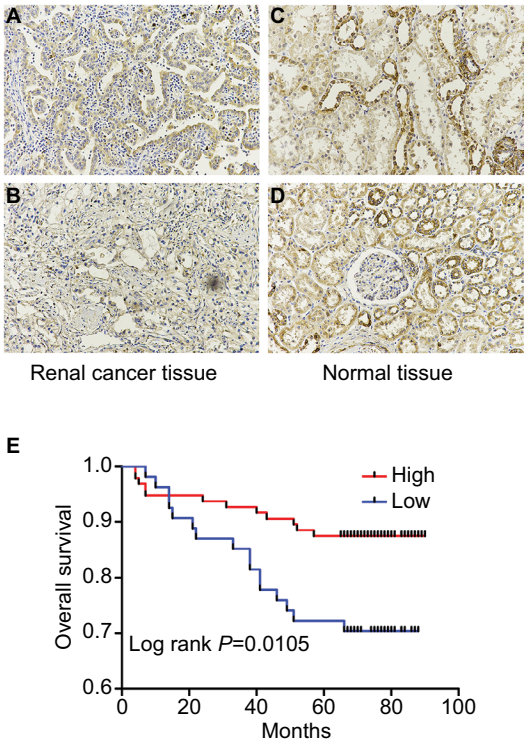

Figure 2 *KCNJ15* is downregulated in ccRCC.
Notes: (**A**, **B**) Immumohistochemical staining of *KCNJ15* protein expression in tumor renal tissues. (**C**, **D**) Immumohistochemical staining of *KCNJ15* protein expression in adjacent tissues. (**E**) Kaplan–Meier survival analysis indicated that high expression of *KCNJ15* increased the overall survival rate of patients with RCC. Data are presented as mean ± SD (log-rank $P<0.05$).
Abbreviations: ccRCC, clear cell RCC; RCC, renal cell carcinoma.

ccRCC patients. However, *KCNJ15* expression in ccRCC patients was not correlated with age (n=150, *P*=0.337), gender (n=150, *P*=0.237), or tumor size (*P*=0.195) (Table 1).

We performed a Cox regression analysis to determine whether *KCNJ15* expression is an independent prognostic factor for ccRCC. As shown in Table 2, multivariate analysis indicated that clinical stage (*P*=0.000, HR =0.167, 95% CI =0.077–0.36), histological grade (*P*=0.009, HR =0.350, 95% CI =0.160–0.767), and *KCNJ15* expression (*P*=0.012, HR =0.375, 95% CI =0.175–0.803) were independent prognostic factors for overall survival in ccRCC (Table 2). These results showed that downregulation of *KCNJ15* might serve as an independent predictor for patients with ccRCC.

Generation of overexpression cell lines

KCNJ15 was transfected into Caki-2 and OS-RC-2 cells to restore *KCNJ15* expression to analyze the functions of *KCNJ15* in RCC. As shown in Figure 3, the protein and mRNA levels of *KCNJ15* were clearly higher in Caki-2 and OS-RC-2 cells transfected with *KCNJ15* than in the untransfected control (NC) group, which was transfected with the empty vector.

Table 1 Correlation between *KCNJ15* expression and clinicopathological characteristics of bladder cancer patients

Characteristics	Case number (n=150)	*KCNJ15*		*P*-value
		High (n=96)	Low (n=54)	
Gender				0.237
Male	108	66	42	
Female	42	30	12	
Age, years				0.337
≥60	59	35	24	
<60	91	61	30	
TNM stage				0.014*
T_{is-1}	119	82	37	
T_{2-3}	31	14	17	
Histological grade				0.045*
G_{1-2}	104	72	32	
G_{3-4}	46	24	22	
Tumor size, cm				0.195
≥5	70	41	29	
<5	80	55	25	

Note: *P<0.05.

KCNJ15 decreases cell proliferation and colony formation in Caki-2 and OS-RC-2 cell lines

We performed CCK-8 cell proliferation assays to investigate the effect of *KCNJ15* expression on the proliferation and growth of RCC cell lines. Here, the proliferation rate of *KCNJ15*-transfected cells was significantly lower than that in the control group (Figure 4A and D). Moreover, the colony formation assays demonstrated that *KCNJ15* overexpression prevented the formation of colonies in both Caki-2 and OS-RC-2 cell lines (*P*<0.05) (Figure 4B, C, E, and F). These results revealed that the overexpression of the *KCNJ15* gene inhibited RCC cell growth in vitro.

KCNJ15 overexpression prevents migration in Caki-2 and OS-RC-2 cell lines

We performed the transwell assays in two cell lines (Caki-2 and OS-RC-2) to determine the migration capacity of RCC cells overexpressing *KCNJ15*. In the migration assays, the number of migrated cells in the *KCNJ15* overexpression groups was significantly lower than that in the control cells (*P*<0.01, Figure 5), suggesting that *KCNJ15* overexpression inhibited cell migration in RCC.

KCNJ15 overexpression induces cell cycle arrest

We performed flow cytometric analysis to further investigate whether the effect of *KCNJ15* overexpression on cell proliferation reflected a cell cycle arrest. As expected, more *KCNJ15* overexpression cells were in the G0/G1 phase and fewer were in the S phase when compared with the NC cells (Figure 6), suggesting that *KCNJ15* overexpression suppressed cell proliferation by inducing cell cycle arrest at the G1 phase.

KCNJ15 overexpression induces apoptosis in Caki-2 and OS-RC-2 cell lines

We conduced flow cytometric analysis to verify whether the overexpression of the *KCNJ15* gene affected cell apoptosis.

Table 2 Univariate and multivariate analyses for overall survival

Clinicopathological variables	Univariate			Multivariate		
	RR	95.0% CI	*P*-value	RR	95.0% CI	*P*-value
Age (</≥50), years	0.514	0.243–1.086	0.081	–	–	–
Gender (male/female)	0.899	0.396–2.04	0.798	–	–	–
Tumor size	0.689	0.279–1.699	0.419	–	–	–
Histological grade	0.260	0.122–0.555	0.000	0.350	0.160–0.767	0.009
TNM stage	7.842	3.695–16.642	0.000	0.167	0.077–0.360	0.000
KCNJ15 (low/high)	0.390	0.184–0.825	0.014	0.375	0.175–0.803	0.012

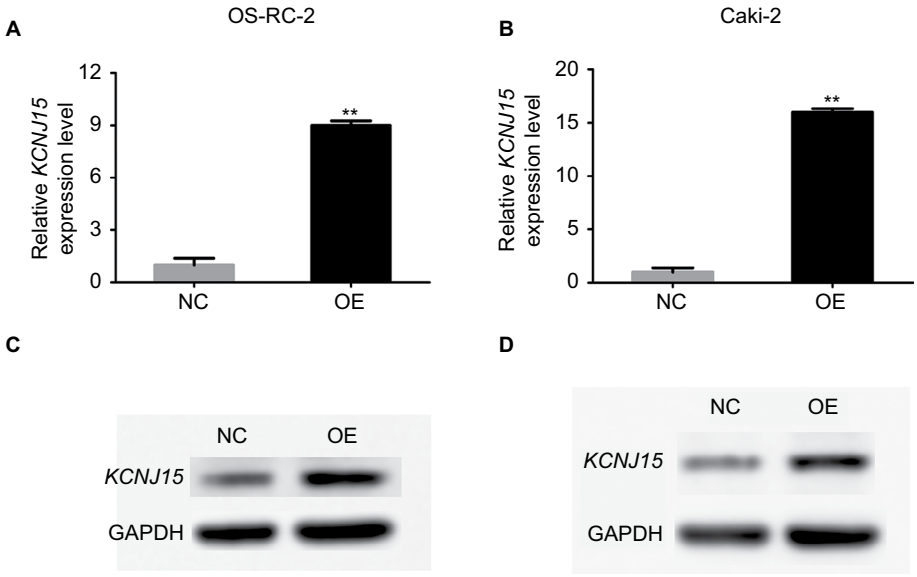

Figure 3 *KCNJ15* is overexpressed in Caki-2 and OS-RC-2 renal cancer cell lines.
Notes: qRT-PCR and Western blot analysis of *KCNJ15* in empty vector-transfected control (NC) and target gene-transfected cells (OE) are depicted for OS-RC-2 (**A**, **C**) and Caki-2 (**B**, **D**) cells. Data are presented as mean ± SD. ***P*<0.01
Abbreviation: qRT-PCR, quantitative real-time reverse transcription PCR.

Quantitative analysis of apoptosis revealed that *KCNJ15* overexpression increased the cell apoptosis rates by ~11.8% in the Caki-2 cell line ($P<0.01$; Figure 7A and B) and 10% in the OS-RC-2 cell line ($P<0.01$; Figure 7C and D) relative to the apoptosis rates in the NC groups.

Effect of *KCNJ15* on signaling pathways

We finally analyzed the expression of proteins in related signaling pathways by Western blot to explore the molecular mechanisms of *KCNJ15*. As shown in Figure 8, *KCNJ15* overexpression upregulated p21 expression and downregulated N-cadherin, vimentin, and matrix metalloproteinase (MMP)-7 expression but had no impact on the expression of mechanistic target of rapamycin (mTOR), phosphorylated (P-) mTOR, glycogen synthase kinase (GSK)-3β, P-GSK-3β, or phosphoinositide 3-kinase (PI3K) in both cell lines (Caki-2 and OS-RC-2).

Discussion

This is the first report showing that *KCNJ15* expression is obviously lower in RCC than in adjacent tissues and serves as an independent prognostic indicator for reduced overall survival in patients with ccRCC. In addition, downregulated *KCNJ15* expression is associated with clinical characteristics including advanced TNM stage (n=150, $P=0.014$) and histological grade (n=150, $P=0.045$). These results indicate that *KCNJ15* plays an important role in RCC occurrence and development. Furthermore, we examined cell proliferation, migration, cell cycle, and apoptosis in the RCC cell lines after increasing *KCNJ15* expression levels and revealed that *KCNJ15* overexpression inhibited cell proliferation and migration, suppressed G0/G1-phase entry, and induced cell apoptosis in vitro.

Previous evidence has demonstrated that *KCNJ15* is expressed in many organs such as the kidney, lung, and pancreas during human development[18] and might play a crucial role in the negative regulation of insulin secretion by maintaining the resting membrane potential of pancreatic β cells.[14] Additionally, *KCNJ15*/Kir4.2 and its intracellular polyamines are essential for electric field sensing in galvanotaxis. Furthermore, *KCNJ15*/Kir4.2 might affect the phosphoinositide 3-kinase/protein kinase B (PI3K/AKT) pathway.[11,20]

We assessed the expression of related signaling proteins to examine the mechanism of function of *KCNJ15*. The p21 protein is a member of the CIP family and an inhibitor of cyclin-dependent kinases and has been shown to be able to control cell cycle progression and negatively regulate cell proliferation. In this study, *KCNJ15* overexpression upregulated p21 expression, suggesting that *KCNJ15* inhibited cell proliferation and induced cell cycle arrest via upregulation of p21 protein expression.

The epithelial–mesenchymal transition (EMT) pathway is considered to participate in cancer progression and metastasis. Our data revealed that overexpression of *KCNJ15* caused

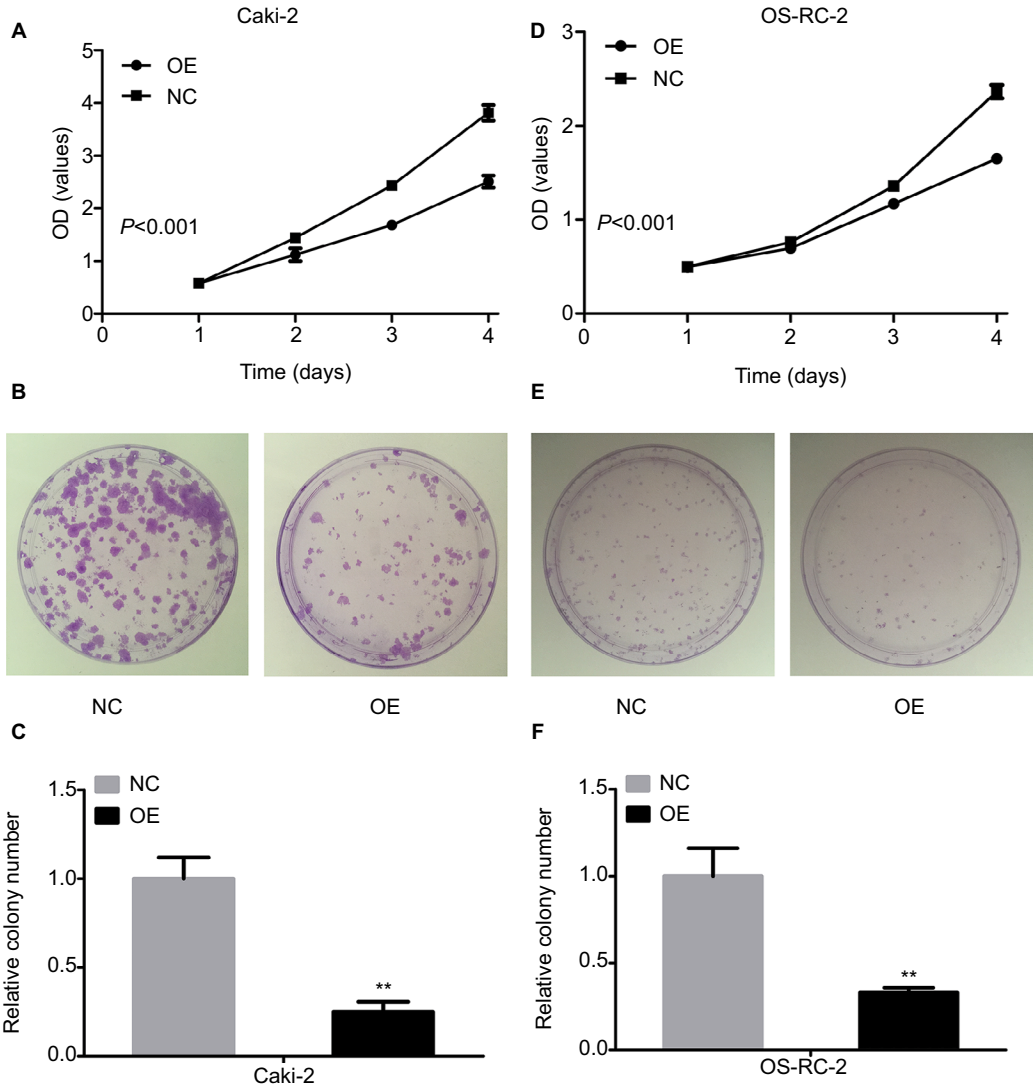

Figure 4 *KCNJ15* inhibits the growth of Caki-2 and OS-RC-2 cell lines.
Notes: (**A** and **D**) The cell proliferation rate of Caki-2 and OS-RC-2 *KCNJ15* overexpression cells as assessed by the OD value was detected by CCK-8 assay. (**B** and **E**) The number of colonies of Caki-2 and OS-RC-2 cell lines with *KCNJ15* overexpression. (**C** and **F**) Histogram of the number of colonies of Caki-2 and OS-RC-2 cell lines with *KCNJ15* overexpression. **$P<0.01$ compared with the NC group. Each assay was performed in triplicate. NC, untransfected control; OE, target gene-transfected cells.
Abbreviation: CCK-8, cell counting kit-8.

the downregulation of N-cadherin and vimentin protein expression, suggesting that *KCNJ15* was involved in the regulation of RCC migration and invasion through suppressing the transition of epithelial cells into mesenchymal cells.

MMPs are protein hydrolases that can promote the metastasis and invasion of tumor cells by degrading the extracellular matrix. Our Western blot results showed that MMP-7 protein expression was decreased by the overexpression of *KCNJ15*, indicating that *KCNJ15* inhibited the invasion of RCC via upregulating MMP-7 expression.

Taken together, these findings suggested that *KCNJ15* might serve as a tumor inhibitor and play an inhibitory role in RCC carcinogenesis and progression. However, the occurrence of kidney cancer is a complex, multifactorial, and multi-step biological process. Therefore, further research is needed to unravel the exact mechanism by which this occurs.

Conclusion

This experiment demonstrates that *KCNJ15* gene expression was downregulated in RCC tissues and could serve as an independent prognostic predictor of ccRCC. *KCNJ15* inhibited metastasis by suppressing the EMT process and MMP-7 expression. Furthermore, *KCNJ15* may have induced cell cycle G1 arrest through the upregulation of the p21 signaling pathway. These results indicate that *KCNJ15* might be a new candidate prognostic biomarker and target for RCC therapy.

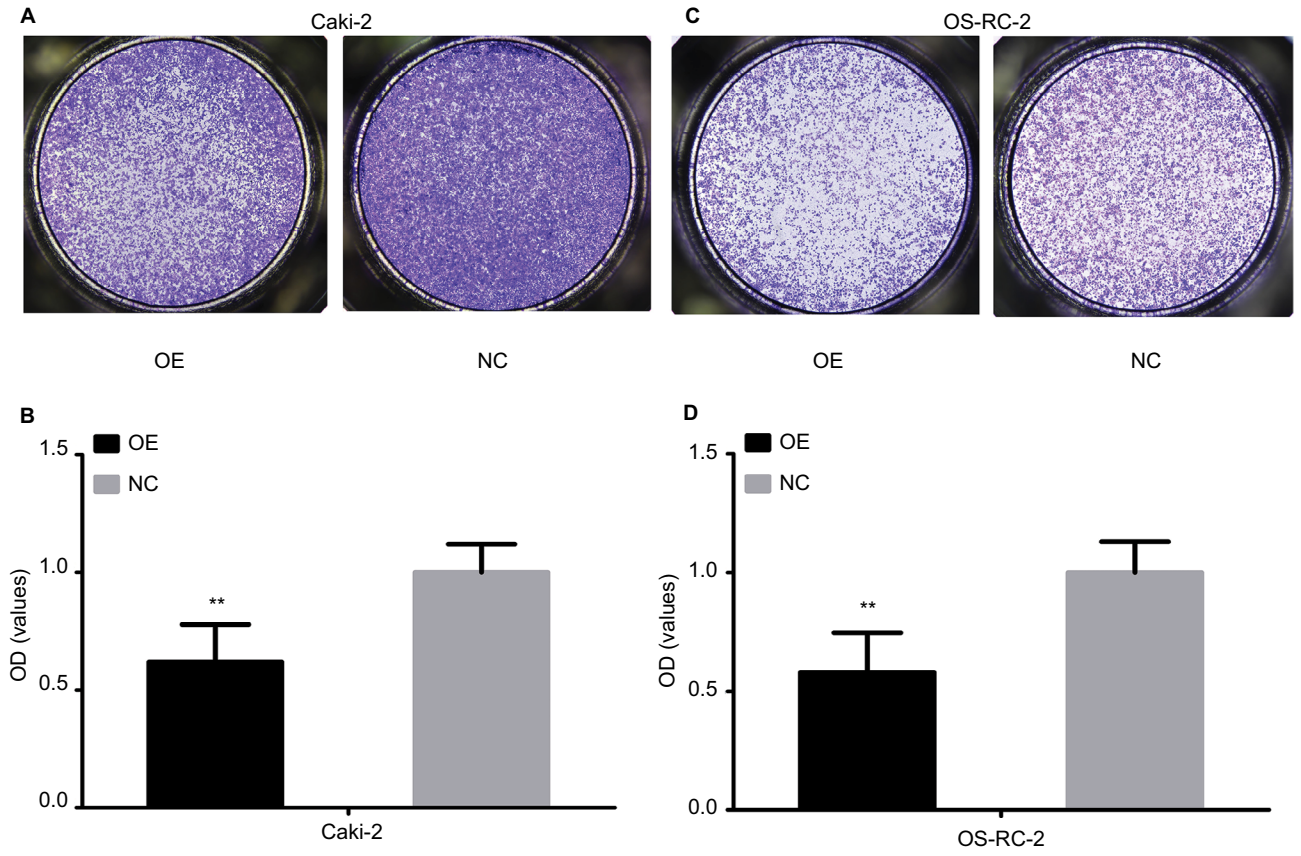

Figure 5 Overexpression of *KCNJ15* inhibits the migration ability of RCC cell lines in vitro.
Notes: (**A** and **B**) The effect of *KCNJ15* overexpression on Caki-2 cell migration was examined by transwell assay. (**C** and **D**) The effect of *KCNJ15* overexpression on OS-RC-2 cell migration was examined by transwell assay. Data are presented as mean ± SD. ***P*<0.01. Each experiment was repeated three times independently. NC, un-transfected control; OE, target gene-transfected cells.
Abbreviation: RCC, renal cell carcinoma.

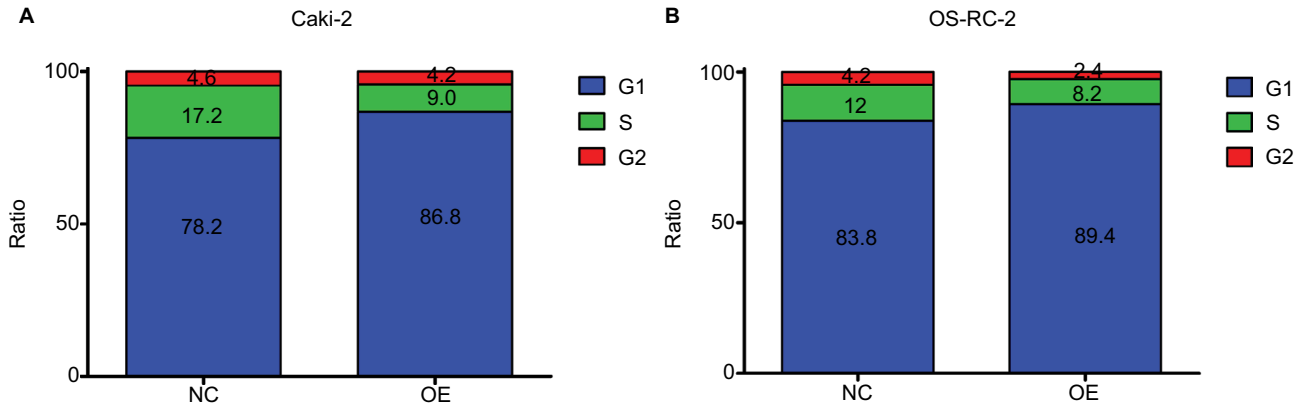

Figure 6 The effect of *KCNJ15* expression on the cell cycle.
Notes: (**A** and **B**) Both Caki-2 and OS-RC-2 cell lines exhibited cell cycle arrest at the G1 phase. All assays were performed three times independently. NC, un-transfected control; OE, target gene-transfected cells.

Ethical approval and consent

This study was implemented according to the Declaration of Helsinki and approved by the ethics committee of Shenzhen Second People's Hospital (approval number 20170512001). Written informed consents were obtained from the patients.

Acknowledgments

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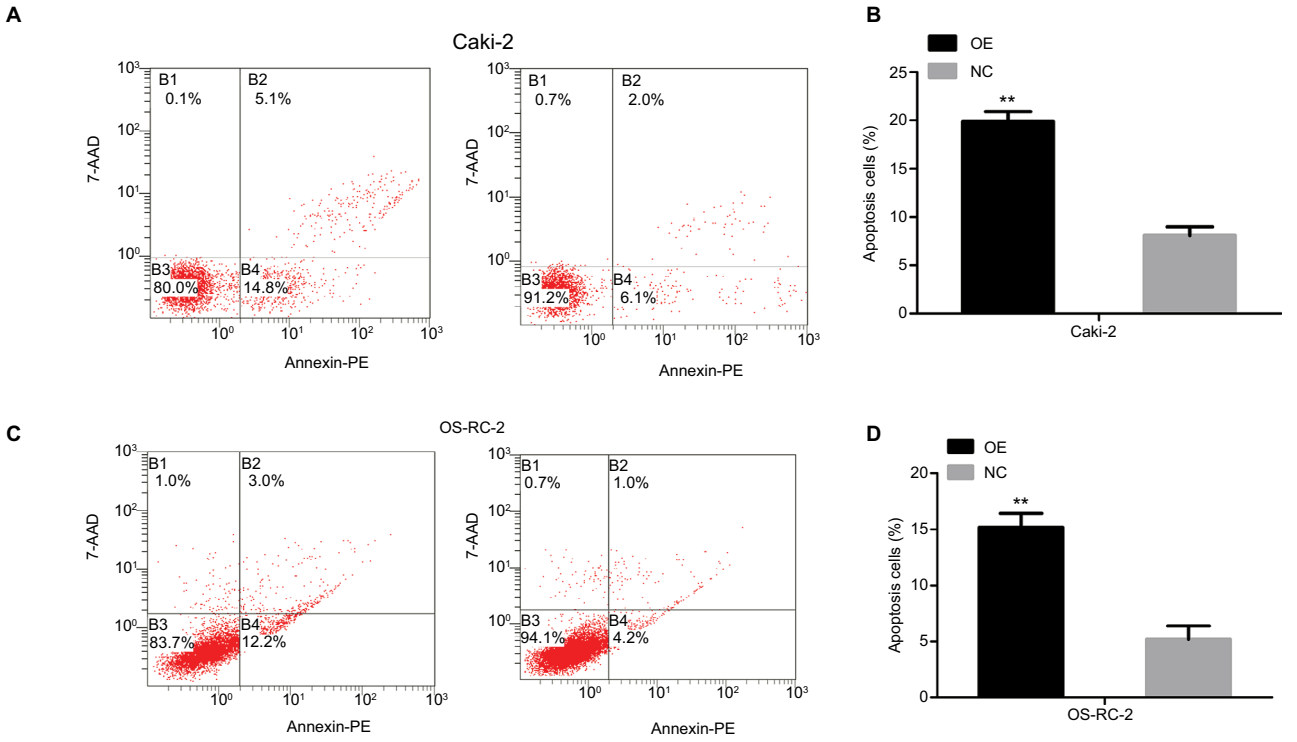

Figure 7 *KCNJ15* overexpression increases the apoptosis rate of renal cancer cell lines.
Notes: (**A** and **C**) The apoptosis rate of Caki-2 and OS-RC-2 cells was determined by flow cytometric analysis. (**B** and **D**) Histogram of the apoptosis rate of Caki-2 and OS-RC-2 overexpression cell lines. Data are presented as mean ± SD. ***P*<0.01 compared with the NC group. NC, un-transfected control; OE, target gene-transfected cells.
Abbreviation: 7-AAD, 7-amino-actinomycin

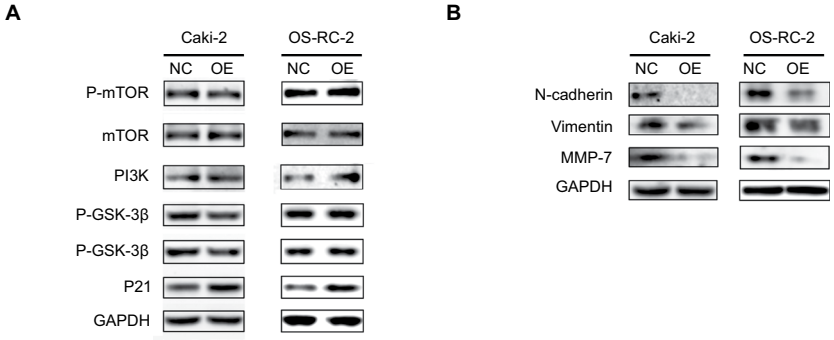

Figure 8 The effect of *KCNJ15* overexpression on the PI3K/AKT, EMT, and MMP-7 pathways.
Notes: (**A**) Western blot analysis was performed to examine the expression of P-mTOR, mTOR, PI3K, P-GSK-3β, GSK-3β, and p21 in Caki-2 and OS-RC-2 cell lines. (**B**) The expression of EMT-associated proteins (N-cadherin and vimentin) and MMP-7 was determined by Western blot.
Abbreviations: EMT, epithelial–mesenchymal transition; GSK, glycogen synthase kinase; MMP, matrix metalloproteinase; mTOR, mechanistic target of rapamycin; PI3K, phosphoinositide 3-kinase.

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Disclosure

The authors report no conflicts of interest in this work.

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