# TREM2 promotes the proliferation and invasion of renal cell carcinoma cells by inhibiting the P53 signaling pathway

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**Abstract.** Renal cell carcinoma (RCC) is a prevalent malignancy characterized by poor prognosis and high mortality. The role of triggering receptor expressed on myeloid cells-2 (TREM2) in RCC progression has been increasingly recognized, yet its underlying mechanisms remain to be fully elucidated. The aim of the present study was to assess the effects of TREM2 on RCC cells and its potential mechanisms. Lentiviral transfection was used to knockdown and overexpress TREM2 in RCC cells, and the expression level of TREM2 was evaluated using reverse transcription-quantitative PCR. Cell Counting Kit-8 (CCK-8) and 5-ethynyl-2'-deoxyuridine (EdU) assays were used to assess the proliferation of the RCC cells. Cell migration and invasion was evaluated using the wound healing assay and Transwell assay, respectively. Western blotting was used to assess the expression levels of TREM2, P53, p-P53, P21 and p-P21 in TREM2 knockdown or overexpression RCC cells. The results demonstrated that the expression level of TREM2 was significantly higher in cancer tissues compared with adjacent normal tissues. The results of the CCK-8 and EdU assays demonstrated that knockdown of TREM2 significantly inhibited the proliferation of RCC cells, whilst overexpression of TREM2 enhanced the proliferation of RCC cells. The results of the wound healing and Transwell assay revealed that, compared with the control group, the overexpression of TREM2 significantly increased the migration and invasion of RCC cells, whereas knockdown of TREM2 significantly decreased the migration of RCC cells. In addition, western blotting demonstrated that the phosphorylation levels of P53 and P21 proteins were significantly increased after TREM2 knockdown in RCC cells. In conclusion, TREM2 is highly expressed in RCC tissues and promotes the migration of RCC cells by inhibiting the P53 signaling pathway. The present study provides new insights into the regulatory effect of TREM2 on RCC and further reveals the potential of TREM2 as a therapeutic target for RCC.

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## Introduction

Renal cell carcinoma (RCC), a malignant tumor of the urinary system, is one of the 15 most common cancers worldwide (1). Nearly 400,000 people are affected by RCC, resulting in >100,000 deaths each year globally (2). At present, RCC is the second most common genitourinary tumor in China, second only to bladder cancer. The incidence of RCC has been on the rise annually and is notably higher in males than that in females (3,4). Early diagnosis of RCC is closely associated with improved survival rates (5). Therefore, it is the focus of RCC research to explore the mechanisms and landmark targets of RCC.

P53, a tumor suppressor protein, can regulate the cell cycle, DNA replication and cell division (6). Normally, when cells undergo uncontrolled division and proliferation, the P53 protein is activated, which in turn induces P21 expression. This process results in cell cycle arrest and inhibition of cell proliferation; however, when cell damage cannot be repaired, P53 triggers apoptosis-related genes (such as Bcl-2-associated X-protein) and programmed cell death (6-8). In addition, when P53 proteins are mutated or aggregated, their functions are lost, leading to abnormal cell proliferation and eventually inducing the occurrence of cancer (9). As a key tumor suppressor, the P53 protein is also regulated by several genes. Specifically, a large number of genes modulate the occurrence and development of RCC by regulating the P53 protein. For instance, tripartite motif 47 can promote the malignant progression of RCC by mediating the ubiquitination and degradation of P53 protein (10), and RNA-binding motif 4 inhibits the proliferation of RCC cells by enhancing the stability of P53 mRNA (11).

Triggering receptor expressed on myeloid cells-2 (TREM2), a transmembrane receptor protein of the immunoglobulin superfamily, is primarily expressed in microglia and myeloid cells (12). TREM2 has several biological functions, including cell proliferation, cell survival, inflammatory regulation and phagocytosis (13). Disease research initially focused on the association between TREM2 and Alzheimer's disease. In the central nervous system, TREM2 promotes microglial transition

to disease-associated microglia by affecting lipid metabolism (cholesterol, myelin and phospholipid metabolism), which may contribute to the occurrence of Alzheimer's disease. Therefore, TREM2 is considered a promising therapeutic target for the treatment of the disease (14). In addition, TREM2 has been reported to serve an important role in cancer. For example, Katzenelenbogen *et al* (15) reported the novel $Arg1^+$ $Trem2^+$ regulatory myeloid cells, which can mediate immunosuppression. The study reported that the elimination of these cells activated the natural killer cells and inhibited tumor growth. Furthermore, $TREM2^+$ monocyte-derived macrophages have been reported to inhibit the accumulation of natural killer cells in tumor tissues and reduce oncolytic activity, thereby promoting the malignant development of lung cancer (16). Moreover, although a study has suggested that TREM2 can promote the occurrence and development of RCC through the phosphatase and tensin homolog-phosphatidylinositol 3-kinase/protein kinase B signaling (17), whether there are additional mechanisms remains to be further explored.

The present study aimed to investigate the role of TREM2 in the progression of RCC and to explore its underlying mechanisms, particularly its interaction with the P53 signaling pathway, using methods such as reverse transcription-quantitative PCR (RT-qPCR), cell proliferation, migration and invasion assays, and western blotting, with the goal of providing novel therapeutic targets for RCC.

## Materials and methods

*Tissue samples*. RCC tumor and adjacent normal tissues were collected from patients with RCC (n=10) admitted to Wujin Hospital Affiliated with Jiangsu University (Changzhou, China) between January 2022 and June 2022. The tissue samples were stored at -80˚C prior to use. Patients were divided into the high TREM2 expression group or the low TREM2 expression group based on the median expression of TREM2. The clinicopathological data of the patients in each group are presented in Table I. The TNM staging system used in this study was the American Joint Committee on Cancer 8th edition system (18). The present study was approved by the Ethics Committee of Wujin Hospital Affiliated with Jiangsu University (approval no. 2022-SR-092), and written informed consent was obtained from all patients according to the requirements of The Declaration of Helsinki (19).

Patients diagnosed with RCC, aged between 18 and 75 years, who provided written informed consent, and had sufficient tissue available for analysis were included in the study. The exclusion criteria included: Patients with other types of cancer, those who received chemotherapy or radiotherapy prior to sample collection, and patients with severe systemic diseases or infections.

*Cell culture*. Human RCC ACHN (cat. no. SCSP-5063), human renal tubular epithelial HK-2 (cat. no. SCSP-511) and 293T (cat. no. SCSP-502) cell lines were purchased from the National Collection of Authenticated Cell Cultures (Cell Bank of Type Culture Collection of The Chinese Academy of Sciences). The 293T, ACHN and HK-2 cells were cultured in Roswell Park Memorial Institute 1640 Medium (cat. no. 22400071; Gibco; Thermo Fisher Scientific, Inc.) containing 10% fetal bovine serum (cat. no. 10099158; Gibco; Thermo Fisher Scientific, Inc.) in a thermostatic incubator (Thermo Fisher Scientific, Inc.) at 37˚C with 5% $CO_2$ for 48 h.

*Lentivirus packaging and infection*. To assess the role of RCC, lentiviral vectors were used to modulate the expression of TREM2 in RCC cells. The constructs for these experiments were generated by cloning the short hairpin (sh)RNA-targeting TREM2 (sh-TREM2, sense: 5'-GATCCGAGCCTCTTGGAAGGAGAAATCTCGAGATTTCTCCTTCCAAGAGGCTCTTTTTG-3' and anti-sense: 5'-AATTCAAAAAGAGCCTCTTGGAAGGAGAAATCTCGAGATTTCTCCTTCCAAGAGGCTCG-3') into a pSicoR vector, resulting in a lenti-shTREM2 construct for knockdown. A lentivirus vector containing scrambled shRNA (sh-NC, sense: 5'-GATCCCAACAAGATGAAGAGCACCAACTCGAGTTGGTGCTCTTCATCTTGTTGTTTTTG-3' and anti-sense: 5'-AATTCAAAAACAACAAGATGAAGAGCACCAACTCGAGTTGGTGCTCTTCATCTTGTTGG'3') was constructed as a negative control. For overexpression, the full-length TREM2 coding sequence was incorporated into a pCDH-CMV-MCS-EF1-copGFP vector, resulting in a lenti-TREM2 construct. A control vector lacking any insert (empty vector) was used as a negative control. For transfection, a total of 5 $\mu$g of each plasmid (Sangon Biotech Co., Ltd.) was used, with a 3:2:1 ratio of the lentivirus, packaging (psPAX2) and envelope (pMD2.G) plasmids. A third-generation lentiviral packaging system was used. The plasmids were transfected into 293T cells using Lipofectamine™ 2000 (cat. no. 11668019; Invitrogen™; Thermo Fisher Scientific, Inc.) at 37˚C for 48 h. After transfection, the medium was replaced with fresh growth medium to facilitate viral production. Viral particles were harvested by collecting the medium every 8 h, subsequently filtering it through a 0.45 $\mu$m filter (cat. no. HVLP02500; MilliporeSigma; Merck KGaA) to remove cellular debris. The purified viral particles were then aliquoted and stored at -80˚C.

The MOI used to infect ACHN cells was 10, with a transduction duration of 24 h. After the 24-h transduction period, the cells were washed with PBS and incubated with fresh medium for an additional 48 h. Following this incubation, ACHN cells were selected using puromycin at a concentration of 2 $\mu$g/ml for 7 days to create a stable cell line. For maintenance, the concentration of puromycin was reduced to 1 $\mu$g/ml. After the stable cell lines continued to be cultured for 3-7 days, total RNA was extracted, and the knockdown or overexpression of TREM2 was detected by RT-qPCR.

*RT-qPCR*. Total RNA was extracted from RCC tissues, ACHN cells and HK-2 cells using a TRIzol™ kit (cat. no. 15596026CN; Invitrogen; Thermo Fisher Scientific, Inc.). The extracted RNA was reverse transcribed into complementary DNA using the SuperScript™ III Reverse Transcriptase kit (cat. no. 18080085; Invitrogen; Thermo Fisher Scientific, Inc.) with the following temperature protocol: 25˚C for 10 min, 50˚C for 50 min and 85˚C for 5 min. Quantitative PCR was performed using the Hieff® qPCR SYBR® Green Master Mix (No Rox; cat. no. 11201ES08; Shanghai Yeasen Biotechnology Co., Ltd.) under the following thermocycling conditions: Initial denaturation at 95˚C for 5 min, followed by 40 cycles of 95˚C for 10 sec and 60˚C for 30 sec. The sequences of the primers

Table I. Clinicopathological data of the patients.

| Variable | Total (n=10) | High expression of TREM2 (n=5) | Low expression of TREM2 (n=5) | t/$\chi^2$ | P-value |
|---|---|---|---|---|---|
| Age, years | 59.00±10.20 | 59.00±14.75 | 59.00±4.06 | 0.000 | >0.999 |
| Sex | | | | 0.400 | >0.999 |
| Male | 5 (50) | 2 (40) | 3 (60) | | |
| Female | 5 (50) | 3 (60) | 2 (40) | | |
| BMI, kg/m$^2$ | 22.78±1.67 | 22.60±1.51 | 22.90±2.00 | -0.286 | 0.782 |
| Tumor size, cm | 4.29±2.38 | 5.48±3.00 | 3.10±0.42 | 1.759 | 0.117 |
| Tumor side | | | | 0.400 | >0.999 |
| Left | 5 (50) | 2 (40) | 3 (60) | | |
| Right | 5 (50) | 3 (60) | 2 (40) | | |
| TNM stage | | | | 1.111 | >0.999 |
| T1 | 9 (90) | 4 (80) | 5 (100) | | |
| T2 | 0 (0) | 0 (0) | 0 (0) | | |
| T3 | 0 (0) | 0 (0) | 0 (0) | | |
| T4 | 1 (10) | 1 (20) | 0 (0) | | |
| Lymph node metastasis | | | | 1.111 | >0.999 |
| Negative | 9 (90) | 4 (80) | 5 (100) | | |
| Positive | 1 (10) | 1 (20) | 0 (0) | | |
| Pathology type | | | | 3.143 | >0.999 |
| Clear cell | 7 (70) | 4 (80) | 3 (60) | | |
| Papillary | 2 (20) | 0 (0) | 2 (40) | | |
| Chromophobe | 0 (0) | 0 (0) | 0 (0) | | |
| Others | 1 (10) | 1 (20) | 0 (0) | | |

Data are presented as mean ± standard deviation or n (%). TREM2, triggering receptor expressed on myeloid cells-2; BMI, body mass index; T, tumor.

used are listed in Table II. GAPDH served as a standardized control. The expression of TREM2 was quantified using the $2^{-\Delta\Delta Cq}$ method (20).

*Western blotting*. ACHN cells were lysed with radioimmunoprecipitation assay buffer (cat. no. 89900; Thermo Fisher Scientific, Inc.) at 4˚C for 30 min. Subsequently, the lysed cells were centrifuged at 12,000 x g at 4˚C for 15 min using a high-speed microcentrifuge, and the supernatant was collected. Bicinchoninic acid was used to determine the protein concentration in the supernatant. Upon denaturation at 100˚C for 5 min, protein samples (20 $\mu$g/lane) were subjected to 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The protein samples were then transferred to polyvinylidene fluoride membranes (cat. no. IPFL00005; MilliporeSigma; Merck KGaA) using the wet transfer method. The membranes were blocked with 5% skim milk at room temperature for 2 h and then primary antibodies were added for incubation overnight at 4˚C. The antibodies used included β-actin (1:5,000; cat. no. 13E5; CST Biological Reagents Co., Ltd.), TREM2 (1:1,000; cat. no. 91068; CST Biological Reagents Co., Ltd.), P53 (1:2,000; cat. no. 2527; CST Biological Reagents Co., Ltd.), p-P53 (1:1,000; cat. no. 82530; CST), P21 (1:2,000; cat. no. 2947; CST Biological Reagents Co., Ltd.) and p-P21 (1:500; cat. no. ab47300; Abcam). Subsequently, the membranes were washed three times with Tris-buffered saline with 0.1% Tween 20. The membranes were then incubated with HRP-conjugated goat anti-rabbit IgG secondary antibodies (1:5,000; cat. no. 7074; CST Biological Reagents Co., Ltd.) at room temperature for 2 h, followed by rinsing again with Tris-buffered saline with Tween 20 in triplicate. Subsequently, the Pierce™ ECL Western Blotting Substrate (cat. no. 32209; Thermo Fisher Scientific, Inc.) was evenly dripped onto the membrane. The proteins were developed using the ChemiDoc Imaging System (Bio-Rad Laboratories, Inc.) and the gray values were calculated using ImageJ software (V1.8.0; National Institutes of Health).

*Cell Counting Kit-8 (CCK-8) assay*. The proliferation ability of RCC cells was assessed by CCK-8 assay (cat. no. HY-K0301; MedChemExpress). Briefly, ACHN cells infected with the corresponding lentivirus were seeded into 96-well plates at a density of 5x10$^3$ cells/well, with six replicates per group. After 48 h, the cells were supplemented with 10 $\mu$l of the CCK-8 reagent and incubated for another 2 h. The absorbance of each well at 450 nm was detected using a microplate reader.

*5-ethynyl-2'-deoxyuridine (EdU) incorporation assay*. To further assess cell proliferation, an EdU incorporation assay was performed. EdU, a nucleoside analog of thymidine (21),

Table II. Primer sequences.

| RNA | Direction | Sequence (5'-3') |
|---|---|---|
| TREM2 | Forward | 5'-AGGGCCCATGCCAGCGTGTGGT-3' |
| | Reverse | 5'-CCAGAGATCTCCAGCATC-3' |
| GAPDH | Forward | 5'-GTCTCCTCTGACTTCAACAGCG-3' |
| | Reverse | 5'-ACCACCCTGTTGCTGTAGCCAA-3' |

TREM2, triggering receptor expressed on myeloid cells-2; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

was used to track active DNA synthesis. Cells were incubated with 10 $\mu$M EdU for 2 h at 37˚C and then fixed with 4% paraformaldehyde at room temperature for 15 min. After fixation, cells underwent a click reaction with Alexa Fluor™ 488 Azide (cat. no. A10266; Invitrogen; Thermo Fisher Scenic, Inc.) to stain the incorporated EdU according to the manufacturer's instructions at room temperature for 30 min. Subsequently, cells were counterstained with DAPI at room temperature for 10 min to label all DNA, providing a control for cell nuclei identification. Fluorescence microscopy was used to capture images of the labeled cells. The proportion of $EdU^+$ cells was quantified by comparing them to the total number of DAPI-stained nuclei.

*Wound healing assay*. ACHN cells cultured to 100% confluence in a 6-well plate were subjected to starvation treatment with a serum-free medium. After 24 h, a tip was used to scratch the cells. The scratched cells were then washed with phosphate-buffered saline and cultured in a serum-free medium for 24 h at 37˚C. Subsequently, wound healing of the cells was observed using an inverted microscope and photographed at 0 and 24 h. Two black lines were drawn on the images to facilitate calculations. The black line at 0 h represented the initial scratch or wound created in the cell monolayer, serving as the baseline for the measurements. The black line at 24 h illustrated the boundary of cell migration into the scratch area after 24 h of incubation. This line was determined based on the leading edge of the migrating cells, which is a standard practice in scratch assays to assess cell migration and wound closure rate (22). The criteria for determining this line involved identifying the foremost edge of the cells that moved closest to the original wound edge without crossing it.

*Transwell assay*. The invasion assay was performed with a Transwell chamber coated with Matrigel (BD Biosciences) at 37˚C for 1 h. The upper chamber was supplemented with ACHN cells ($5x10^4$) resuspended in serum-free medium, whilst the lower chamber was covered with Dulbecco's Modified Eagle Medium containing 10% fetal bovine serum (cat. no. 10099158; Gibco; Thermo Fisher Scientific, Inc.). The chambers were cultured in an incubator at 37˚C and 5% $CO_2$ for 24 h, during which time a certain number of cells attached to the upper chamber passed through the membrane to the lower chamber. Subsequently, the chambers were rinsed with phosphate-buffered saline, fixed with a 4% formaldehyde solution at room temperature for 15 min, and stained with crystal violet (0.01%) at room temperature for 15 min. The cells that passed through the membrane into the lower chamber were observed using a light microscope (BX53; Olympus Corporation) and images were captured.

*Statistical analysis*. These aforementioned experiments were independently repeated three times. All data were analyzed using SPSS 23.0 (IBM Corp.) and GraphPad Prism V.7.01 (Dotmatics). Continuous data from biological replicates are expressed as mean ± standard deviation. The paired or unpaired Student's t-test was used for comparisons between two groups, and one-way analysis of variance followed Tukey's post hoc test was used to compare differences among multiple groups. Categorical data are expressed as n (%) and Fisher's exact test was used for analysis. $P<0.05$ was considered to indicate a statistically significant difference.

## Results

*TREM2 is highly expressed in patients with RCC*. To assess the effect of TREM2 expression on RCC, 10 pairs of RCC and adjacent normal tissues were obtained. RNA was extracted from the tissue samples, and the expression level of TREM2 was determined by reverse transcription-quantitative PCR. The results demonstrated that the mRNA expression level of TREM2 was significantly increased in tumor tissue compared with that in normal tissues (Fig. 1A). To further confirm the higher expression level of TREM2 in cancer cells, the expression level of TREM2 in the RCC ACHN cell line and the normal renal cortex/proximal tubular HK-2 cell line were evaluated. The results revealed that the expression level of TREM2 was significantly higher in ACHN cells than that in HK-2 cells (Fig. 1B). These findings indicate that TREM2 is a potential target of RCC, and the mechanism of TREM2 in RCC deserves further exploration.

*TREM2 promotes the proliferation of RCC cells*. To assess the function of TREM2 in regulating the phenotype of ACHN cells, lentiviral transfection was used to knockdown or overexpress TREM2 in ACHN cells. After 48 h of infection, the cells were collected, and the expression of TREM2 was assessed using reverse transcription-quantitative PCR and western blotting (Fig. 2A and B). The results showed that TREM2 mRNA and protein levels were significantly lower in the sh-TREM2 group compared with the sh-NC group and significantly higher in the TREM2 overexpression group compared with the vector group. Furthermore, the results of the CCK-8 and EdU staining assays demonstrated that the knockdown of

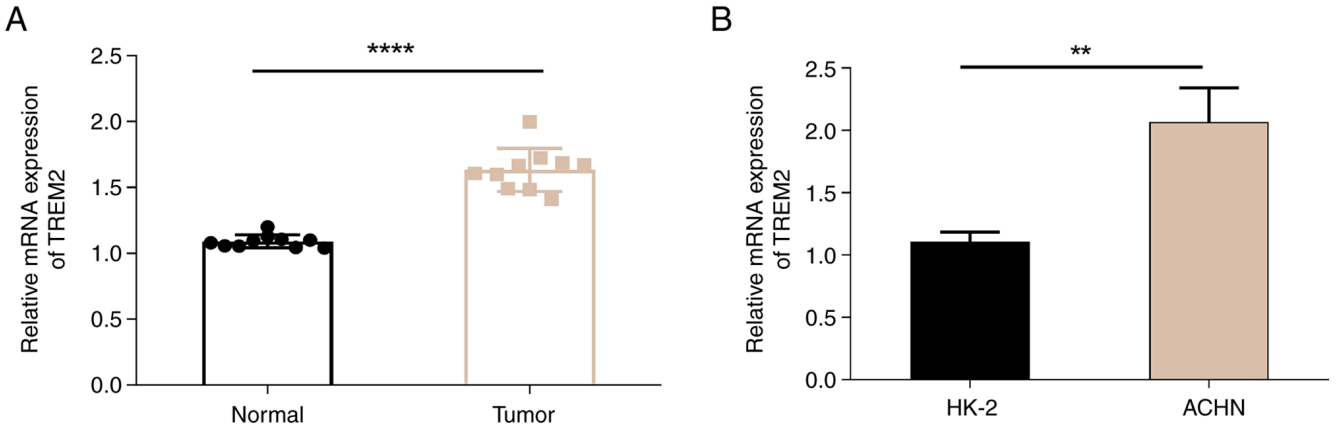


Figure 1. High expression of TREM2 in RCC tissues. Reverse transcription-quantitative PCR was used to detect the expression of TREM2 in (A) 10 cases of RCC and matched adjacent normal tissues and (B) HK-2 and ACHN cells. **P<0.01, ****P<0.0001. TREM2, triggering receptor expressed on myeloid cells-2.

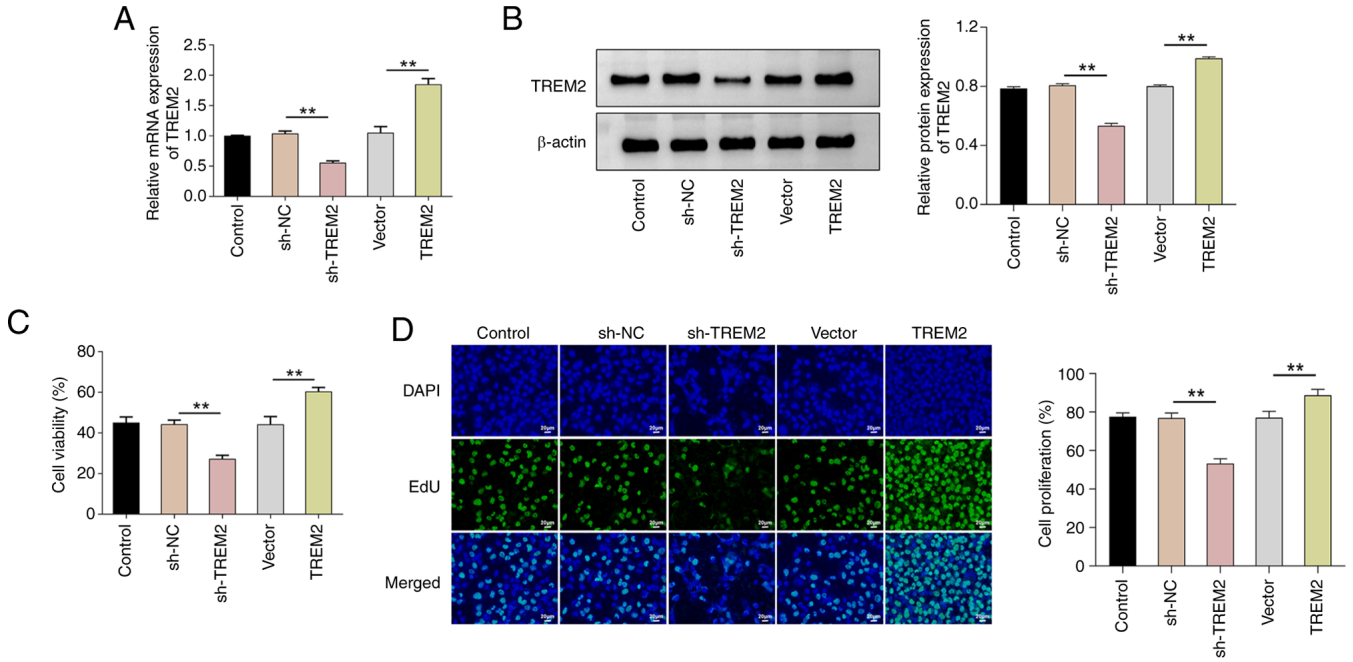


Figure 2. TREM2 promotes the proliferation of RCC cells. ACHN cells were infected with the corresponding lentiviruses (sh-NC, sh-TREM2, vector and TREM2). After 48 h, cells were collected and the results of the knock-down and overexpression of TREM2 was assessed using (A) reverse transcription-quantitative PCR and (B) western blotting. (C) Cell Counting Kit-8 and (D) EdU assays were used to detect the effect of the knockdown or overexpression of TREM2 on the viability and proliferation of RCC cells. **P<0.01. TREM2, triggering receptor expressed on myeloid cells-2; RCC, renal cell carcinoma; sh, short hairpin; NC, negative control; EdU, 5-ethynyl-2'-deoxyuridine.

TREM2 significantly decreased cell viability and proliferation in comparison with the sh-NC group; whilst overexpression of TREM2 significantly increased cell viability and proliferation in comparison with the vector group (Fig. 2C and D). Therefore, the findings indicate that TREM2 could promote the proliferation of RCC cells.

*TREM2 promotes the migration and invasion of RCC cells.* Migration and invasion are an important indices to assess the malignant phenotype of cancer cells (23). To evaluate the effect of TREM2 on the migration of RCC cells, a wound healing assay was performed to observe the wound healing of cells at 0 and 24 h. Briefly, in comparison with the sh-NC group, the knockdown of TREM2 significantly inhibited the wound healing rate of ACHN in RCC cells. Conversely, overexpression of TREM2 promoted the wound healing rate of ACHN in RCC cells compared with the vector group (Fig. 3A). These results suggest that TREM2 could enhance the migration of RCC cells. A Transwell assay further revealed that knockdown of TREM2 significantly inhibited the cell invasion compared with that in the sh-NC group, while overexpression of TREM2 resulted in a significantly higher cell invasion compared with the vector group (Fig. 3B). Overall, the findings indicate that TREM2 promotes the migration and invasion of RCC cells.

*TREM2 promotes the proliferation of RCC cells by inhibiting the P53 signaling pathway.* The P53 signaling pathway serves an important role in regulating the cell cycle and the

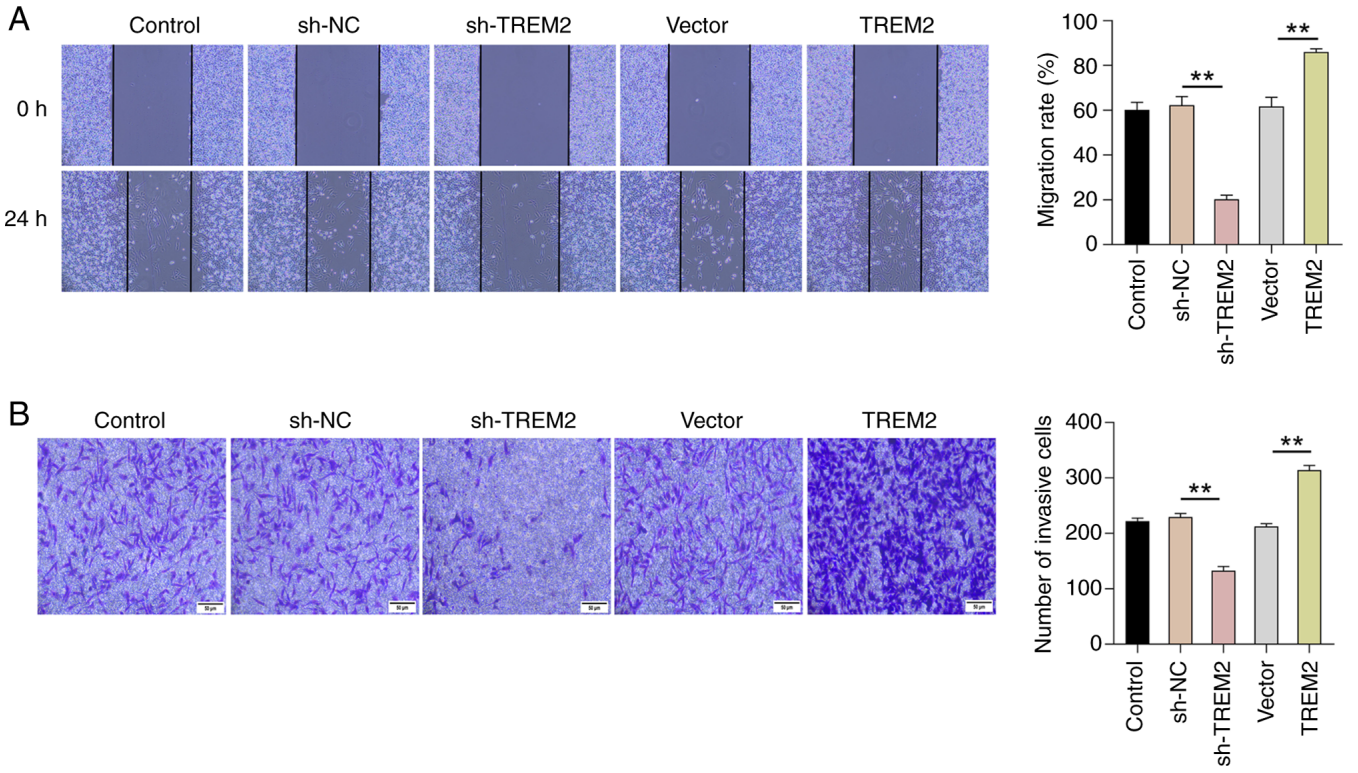


Figure 3. TREM2 promotes migration of RCC cells. (A) Wound healing assay and (B) Transwell assays were used to assess the effect of the knockdown or overexpression of TREM2 on the migration and invasion of RCC cells. (A) Magnification, x100. (B) Scale bar, 50 $\mu$m. $^{**}$P<0.01. TREM2, triggering receptor expressed on myeloid cells-2; RCC, renal cell carcinoma; sh, short hairpin; NC, negative control.

proliferation, invasion and migration of cells (9). The effect of TREM2 on the activation of the P53 signaling pathway was evaluated to assess whether TREM2 regulates tumor occurrence and development through the P53 signaling pathway. Western blotting revealed that knockdown or overexpression of TREM2 had no significant effect on the total protein levels of P53 and P21; however, in comparison with the sh-NC group, knockdown of TREM2 significantly increased the phosphorylation levels of P53 and P21, whilst overexpression of TREM2 significantly decreased the phosphorylation levels of P53 and P21 (P<0.01, Fig. 4). In summary, the results indicated that TREM2 may promote the proliferation, invasion and migration of RCC cells by inhibiting the activation of the P53 signaling pathway.

## Discussion

In the present study, TREM2 was significantly upregulated in RCC tissues, which is consistent with the findings of a previous study (17). Such a result strengthens the hypothesis that TREM2 serves a crucial role in the pathophysiology of RCC. Furthermore, the results of the present study align with emerging research indicating that TREM2 is associated with several cancers, including lung cancer, colorectal cancer, thyroid cancer and RCC (16,17,24,25), highlighting its potential as a common mediator in oncogenic processes. Notably, the findings of the present study extend the current understanding by demonstrating that TREM2 was not only elevated in RCC but also served a significant role in regulating key molecular pathways. A novel layer of complexity to its role in cancer was added in the observed TREM2 upregulation in RCC tissues, particularly in how it influenced malignant cell proliferation, a pivotal characteristic of cancer cells (26). The present study made a unique contribution to the oncological field by providing evidence of the regulatory effects of TREM2 on the phosphorylation levels of P53 and P21. P53 and P21 are critical regulators of cell cycle and apoptosis (27), suggesting a mechanism by which TREM2 could promote oncogenesis and tumor progression. These insights into the molecular interactions of TREM2 not only offer a deeper understanding of its function in RCC but also underscore its potential as a therapeutic target based on its significant impact on tumor biology.

In the present study, the results of the CCK-8 assay revealed that TREM2 could promote the proliferation of ACHN cells, which aligns with the findings of Zhang *et al* (17). The invasion and migration ability of cancer cells is an important index to evaluate the malignancy of tumors (26,28) and it is of importance to explore the molecular regulatory mechanism of cancer cell invasion and migration for identifying therapeutic targets for cancer. In the present study, the wound healing and Transwell assays were used to determine the regulatory effect of TREM2 on the migration and invasion of RCC cells. The results revealed that knockdown of TREM2 expression significantly inhibited the migration and invasion of ACHN cells, whereas overexpression of TREM2 promoted migration and invasion. These results indicate that TREM2 may promote the development of RCC by facilitating the migration and invasion of RCC cells. Moreover, as reported in several previous studies, TREM2 can regulate the invasion and migration of many cancer cells, but its function varies in different

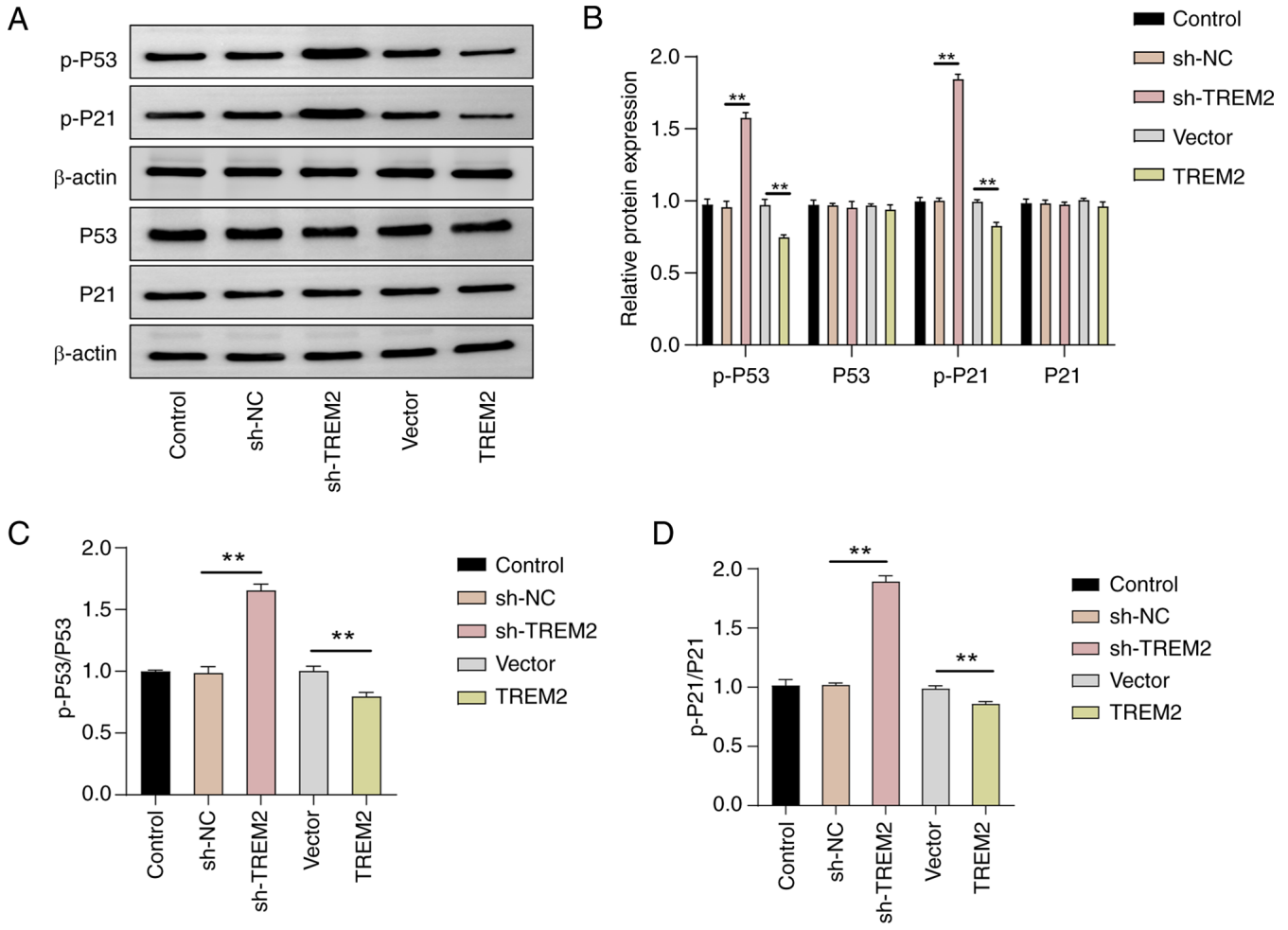


Figure 4. TREM2 promotes the proliferation of renal cell carcinoma cells by inhibiting the P53 signaling pathway. (A) Western blotting was used to assess the effects of the knockdown or overexpression of TREM2 on the protein and phosphorylation levels of P53 and P21, as well as the phosphorylation levels. (B) Semi-quantitative results of P53, p-P53, P21 and p-P21 protein bands. Relative ratio of (C) p-P53/P53 and (D) p-P21/P21. **P<0.01. TREM2, triggering receptor that is expressed on myeloid cells-2; sh, short hairpin; NC, negative control.

tumors (24,29,30). For example, TREM2 promotes the invasion and migration of gastric cancer cells (24), but inhibits the viability of colorectal and liver cancer cells (24,29,30). Therefore, the role of TREM2 in different cancers must be carefully evaluated when selecting it as a target for cancer treatment.

In the present study, the relationship between TREM2 and RCC progression was evaluated, focusing on the P53/P21 signaling axis. This signaling axis serves a crucial role in cell cycle regulation, DNA replication and cell division (31). The cell cycle of cancer cells is frequently abnormal, accelerating cell division and leading to uncontrollable cell proliferation and tumor formation (32). P53, a key regulator of the cell cycle, is often altered in several forms of cancer, marking a significant step in carcinogenesis (6,33,34); however, the present study revealed that the total expression levels of the P53 protein were not affected by TREM2, suggesting that the regulatory role of TREM2 in cancer proliferation is not directly involved in altering P53 expression. Protein phosphorylation, a vital post-translational modification of the P53 protein, serves a crucial role in its nuclear localization and activation of downstream signaling pathways (35). In the present study, it was observed that TREM2 knockdown could increase the phosphorylation levels of P53, enhancing its tumor-suppressive functions; however, TREM2 overexpression decreased P53 phosphorylation, potentially facilitating oncogenic activities. Additionally, P21, as a downstream effector of the P53 pathway, serves a key role in halting the cell cycle by inhibiting cyclin D1 and cyclin-dependent kinase (36). The results of the present study indicate that TREM2 could influence the progression of RCCs by modulating the phosphorylation states of P53 and P21, thereby affecting their functional states without altering their protein expression levels. This modulation suggests that TREM2 could exert its effects on tumor biology through a specific, post-translational mechanism. According to Lei *et al* (37), such mechanisms of TREM2-mediated post-translational mechanism serve a significant role in regulating cancer progression, supporting the pivotal roles of TREM2 in modulating key signaling pathways in cancer. For example, TREM2-mediated phosphorylation affected Akt in prostate adenocarcinoma and RCC, and NF-κB in papillary thyroid carcinoma and gastric cancer (38). To the best of our knowledge, the present study is the first to demonstrate that TREM2 regulates RCC progression by specifically targeting the P53/P21 signaling axis, particularly through the modulation of protein phosphorylation rather than through changes

in protein quantity. Further in-depth investigation is needed to elucidate exactly how TREM2 interacts with the molecular mechanisms regulating the phosphorylation of P53 and P21, thereby providing a deeper understanding of its role in cancer pathology and its potential as a therapeutic target.

Although the present study elucidated the role of TREM2 in promoting the proliferation, and migration of RCC cells through controlled *in vitro* experiments, it is crucial to recognize the limitations posed by the lack of *in vivo* studies and comprehensive clinical data. The complexity of the tumor microenvironment in living organisms, including multiple cell types and dynamic signaling interactions, serves a crucial role in influencing cancer cell behaviors. These intricacies are often not fully replicated in *in vitro* models. Additionally, the present study did not include longitudinal clinical data or follow-up data of patients with RCC, which are essential for assessing outcomes, such as survival rates and therapy responses. Furthermore, TREM2 serves a crucial role in modulating immune responses within the tumor microenvironment, influencing not only inflammation and tissue remodeling but also the broader immune landscape in cancers like RCC. Specifically, TREM2 has been associated with immunosuppressive functions, including the regulation of myeloid cell activity and the suppression of $CD8^+$ T-cell infiltration (16,38,39). Future studies should use flow cytometry, immune assays, RNA sequencing, animal models and clinical trials to thoroughly explore the immunosuppressive impact of TREM2 on RCC, highlighting its potential as a therapeutic target. Finally, the interactions between TREM2 and other genes and signaling pathways involved in RCC remain underexplored. Understanding these interactions could reveal complex regulatory networks that contribute to the pathogenesis of RCC and may identify novel therapeutic targets. To thoroughly explore these interactions, a comprehensive experimental approach is necessary, which should include the following: RNA sequencing to analyze gene expression patterns; protein-protein interaction assays to detect direct interactions; genetic manipulation to observe effects of TREM2 modulation; phosphorylation studies to assess signaling changes; reporter assays for evaluating transcription factor activity; pathway inhibition tests to determine pathway dependencies; and animal model analyses to study the *in vivo* relevance. These methods will collectively enhance the understanding of the role of TREM2 in RCC and its potential as a therapeutic target.

In conclusion, the present study demonstrated that TREM2 may promote the malignant biological behavior of RCC cells through the P53 signaling pathway. Furthermore, the results revealed the relationship between TREM2 and RCC, providing new insights into the regulation of RCC by TREM2 and further demonstrating the potential of TREM2 as a therapeutic target for RCC.

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**Availability of data and materials**

The data generated in the present study may be requested from the corresponding author.

**Authors' contributions**

LZ, QX and BW designed the study. ZL collated the data, performed data analyses. QX and BW drafted the manuscript. QX and BW confirm the authenticity of all the raw data. All authors have read and approved the final manuscript.

**Ethics approval and consent to participate**

The present study was approved by the Ethics Committee of Wujin Hospital Affiliated with Jiangsu University (Changzhou, China; approval no. 2022-SR-092), and written informed consent was obtained from all patients according to the requirements of the approved guidelines.

**Patient consent for publication**

Not applicable.

**Competing interests**

The authors declare that they have no competing interests.

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