USP22 promotes proliferation in renal cell carcinoma by stabilizing survivin

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Abstract. Renal cell carcinoma (RCC) is one of the commonest urological tumors. The incidence of RCC ranks third among urological tumors, after prostate cancer and bladder tumors. However, the etiology of RCC remains unclear. Ubiquitin-specific protease 22 (USP22), a potential marker of cancer stem cells, is associated with the occurrence and progression of numerous tumors. However, the roles of USP22 in RCC have not yet been investigated. Survivin is a member of the inhibitor of apoptotic protein family involved in RCC progression. The present study first detected the expression of USP22 and survivin in RCC tissues using immunohisto-chemistry and western blotting. It was revealed that the protein levels of USP22 and survivin in RCC tissues were higher than those in adjacent normal renal tissue. Subsequently, it was demonstrated that *USP22* knockdown inhibited the growth of an RCC cell line ACHN and downregulated the protein level of survivin, accompanied by an increased level of cleaved-caspase-3. By contrast, overexpression of *USP22* promoted the growth of ACHN cells, upregulated the expression of survivin and decreased the level of cleaved-caspase-3. Notably, the changes in USP22 expression did not affect the *SURVIVIN* mRNA level. Finally, it was confirmed that USP22 interacted with survivin and stabilized it by downregulating its ubiquitination. The present results indicate that USP22 may regulate survivin via deubiquitination, thereby promoting the proliferation of RCC cells. The results of the current study suggest that USP22 may represent a novel therapeutic target for patients with RCC.

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Introduction

Renal cell carcinoma (RCC) is a common renal tumor in adults, accounting for ~90% of renal tumors and ~3% of all adult cancer cases, incidence rate of RCC was 5.78 per 100,000 women and 13.14 per 100,000 men in 2013 (1). The clinical onset of RCC is insidious; patients usually experience no obvious symptoms in the early stages. This makes RCC a problematic disease to treat, as the tumor has usually metasta-sized before surgery and often relapses following surgery (2). Nevertheless, surgery remains the primary treatment for RCC, supplemented by other treatments, including renal artery embolization, chemotherapy, immunotherapy and molecular targeted therapy (2,3). Therefore, finding targeted treatments for RCC has great clinical importance. However, at present, the molecular mechanism underlying RCC remains unclear (4,5).

The ubiquitin-proteasome system (UPS) exists in almost all eukaryotic cells. Ubiquitination is reversible, and the deubiq-uitination of substrate proteins by deubiquitinases (DUBs) can regulate the stability of proteins, thereby affecting their functions (5). Therefore, DUBs serve an important role in maintaining normal cell functions and regulating pathological processes. Ubiquitin-specific protease (USP) is one of the subfamilies of the DUB family. USP family member USP22 is highly expressed in multiple malignant tumors, including gastric, colon and prostate cancers (6). The expression level of USP22 is closely associated with metastasis potential, chemotherapeutic resistance and prognosis in patients with cancer (7). USP22 deficiency results in myeloid leukemia via an ETS-family transcription factor pu.1-dependent mechanism after the activation of carcinogenic Kras (8). However, its func-tion and mechanism in RCC have yet to be elucidated.

Survivin is a member of the inhibitor of apoptotic protein family and serves as a subunit of the chromosomal passenger complex that regulates cell division (9). Survivin expression is associated with a variety of human cancers and its high expres-sion often indicates a poor prognosis (10-12). For example, a high expression of survivin is associated with a poor overall survival and shorter cancer-specific survival in RCC (13,14). Survivin protein functions to inhibit caspase activation, thereby leading to negative regulation of apoptosis or programmed cell death (15). The stability of survivin is regulated via the ubiquitin-proteasome pathway (16). However, whether USP22 influences the ubiquitination-proteasome pathway-dependent

regulation of survivin in the development of RCC has yet to be elucidated.

The present study hypothesized that USP22 inhibits the apoptosis of renal carcinoma cells via modulating survivin level. This was first confirmed by immunohistochemical and western blotting that the protein levels of USP22 and survivin in RCC were higher compared with adjacent normal tissues. Then it was identified that USP22 upregulated survivin protein level and suppressed cell death via siRNA knockdown and overexpression experiments. Finally, the interaction between USP22 and survivin was confirmed, and that USP22 supported survivin stabilization through deubiquitination. Together, the results of the present study revealed that USP22 may inhibit the apoptosis of RCC by deubiquitinating and stabilizing survivin.

Materials and methods

Specimens. Renal carcinoma specimens (n=10) and the corresponding adjacent normal tissues were obtained by surgical excision from the Department of Urological Surgery in the Fifth Hospital of Xiamen (Xiamen, China) between March 2017 and March 2018. The inclusion criteria were as follows, histologically confirmed renal carcinoma and having adjacent normal tissues as a control. Patients with prior history of RCC that was resected within the past 5 years were excluded from the present study. Patients provided written informed consent in accordance with the legal and institutional ethical guidelines defined by the hospital by the Ethics Committee of the Fifth Hospital of Xiamen (Xiamen, China). The age of patients ranged from 36-85 years, the overall median age was 60.2 years. Adjacent normal tissues were taken from at least 2 cm apart from the tumor border.

Cell culture. Human RCC cell lines ACHN and TK-1 (American Type Culture Collection) were cultured in Dulbecco's modified Eagle's medium (Thermo Fisher Scientific, Inc.) supplemented with 10% (v/v) fetal bovine serum (Gibco; Thermo Fisher Scientific, Inc.), 100 U/ml penicillin (Thermo Fisher Scientific, Inc.) and 100 U/ml streptomycin (Thermo Fisher Scientific, Inc.) at 37°C in 5% CO_2.

Immunohistochemistry. Tissue sections were dehydrated in gradient concentration of ethanol solution and fixed with 4% paraformaldehyde at room temperature for 30 min, and then paraffin embedded. Consecutive 4-μm sections were used for analysis. Antigen retrieval was performed by microwaving sections in citrate buffer (pH 6.0). Subsequently, slides were incubated overnight with anti-USP22 (cat. no. ab71732; Abcam; 1:1,000) and anti-*SURVIVIN* (cat. no. 2808; Cell Signaling Technology, Inc.; 1:1,000) antibodies at 4°C. Subsequently, slides were block with goat serum (cat. no. AR0009; Wuhan Boster Biological Technology, Ltd; 1:20) at room temperature for 1 h and then incubated with goat anti-rabbit second antibody (cat. no. ab6721; Abcam; 1:2,000) at RT for 1 h. Streptococcal avidin-biotinylated peroxidase system (Thermo Fisher Scientific, Inc.) was used to develop the color according to the manufacturer's instructions. Tissue sections were visualized using an Olympus microscope IX50 at x20 magnification and the images were analyzed using ImageJ 1.49 version software (National Institutes of Health). The American Joint Committee on Cancer (AJCC) Staging Manual system was used to assess tumor grades (17).

DNA constructs and proliferation assay. cDNA fragments encoding USP22 were inserted into pCMV-HA vector to construct an USP22-HA overexpression vector. Short hairpin (sh)RNA targeting sequences for human USP22 (shRNA1: 5'-AAGTCCTGTATCTGCCATGTC-3', shRNA2: 5'-GTT TCACAA AGA AGCATATTC-3') or scrambled shRNA (sequence: CCTAAGGTTAAGTCGCCCTCG) were inserted into a pLKO.1-GFP-shRNA construct. ACHN and TK-10 cells were transfected with *USP22* shRNA and ACHN cells were transfected with USP22-HA vector using Turbofect (Thermo Fisher Scientific, Inc.). Cell Counting Kit-8 (CCK-8; Thermo Fisher Scientific, Inc.) was used to estimate cell numbers according to manufacturer's instructions, 48 h post-transfection. OD values (450 nm) were measured using a microplate reader (Thermo Fisher Scientific, Inc.). Cell counting assays were performed using ImageJ software version 1.49 [National Institutes of Health (NIH)] to establish growth curves. Each experiment was repeated 4 times in quintuplicate wells per sample by 2 independent experimenters.

Colony formation assay. ACHN cells were seeded in a six-well plate at 800 cells/well, *USP22*-specific shRNA and negative control shRNA were transfected into cells using Turbofect (Thermo Fisher Scientific, Inc.) the next day and the cells were cultured for another 7 days at 37°C in 5% CO_2. Cells were stained with crystal violet at room temperature for 10 min, the colonies (>1 mm) in diameter were counted using ImageJ software version 1.49 (NIH) (18).

Western blotting. Kidney tissues (about 100 mg) or ACHN cells (approximately $3x10^5$ cells) were incubated on ice with RIPA buffer (Beyotime Institute of Biotechnology) to extract the total proteins. The protein concentrations were determined with a bicinchoninic acid assay system (Thermo Fisher Scientific, Inc.). Protein samples (40 μg/lane) were separated via SDS-PAGE on a 10% gel (Beijing Solarbio Science & Technology Co., Ltd.), and then proteins were transferred onto nitrocellulose membranes. Membranes were then blocked with 5% BSA (Sigma-Aldrich; Merck KGaA) at room temperature for 1 h and incubated with primary antibodies overnight at 4°C, and then incubated with HRP-labeled goat anti-rabbit secondary antibody (cat. no. A0208; Beyotime Institute of Biotechnology; 1:3,000) or HRP-labeled goat anti-mouse secondary antibody (cat. no. A0216; Beyotime Institute of Biotechnology; 1:3,000) at room temperature for 1 h. Immunoreactive bands were visualized using ECL detection reagents (Thermo Fisher Scientific, Inc.), and protein levels were quantified using ImageJ software (version 1.49; National Institutes of Health). The antibodies were USP22 (cat. no. ab71732; Abcam; 1:2,000), *SURVIVIN* (cat. no. 2808; Cell Signaling Technology, Inc.; 1:2,000), cleaved-caspase-3 (cat. no. 9664; Cell Signaling Technology, Inc.; 1:2,000), caspase-3 (cat. no. ab197202, Abcam; 1:1,000), phosphorylated (p-)Akt (cat. no. ab38449, Abcam; 1:1,000), Akt (cat. no. ab8805, Abcam; 1:2,000), p-Erk (cat. no. ab131438, Abcam; 1:1,000), Erk (cat. no. ab32537, Abcam; 1:2,000), GAPDH (cat. no. bc002, xmbcss, www.bcssbio.com; 1:2,000), ubiquitin (cat. no. sc-8017, Santa Cruz Biotechnology, Inc.; 1:2,000), HA (cat. no. 51064-2-AP, ProteinTech Group, Inc.; HA sequences: YPYDVPDYA; 1:2,000) and β-actin (cat. no. 23660-1-AP; ProteinTech Group, Inc.; 1:2,000).

Table I. Basic clinicopathological data of renal cell carcinoma patients.

Patient	Sex	Age, years	Tumor grade	Treatment
1	Male	54	II	After radical resection, regular follow-ups were conducted
2	Male	52	VI	Oral administration of sorafenib (400 mg bid)
3	Female	73	III	After radical resection, regular follow-ups were conducted
4	Female	56	IV	Oral administration of sunitinib (400 mg bid)
5	Female	44	IV	Oral administration of sorafenib (400 mg bid)
6	Male	72	III	Oral administration of sorafenib (400 mg bid)
7	Male	68	II	After radical resection, regular follow-ups were conducted
8	Male	62	III	After radical resection, regular follow-ups were conducted
9	Male	36	I	After radical resection, regular follow-ups were conducted
10	Female	85	I	After radical resection, regular follow-ups were conducted

Bid: bis in die.

Co-immunoprecipitation (Co-IP). The USP22-HA and control plasmid was transfected into 293T cells and 48 h later, the cells were collected and dissolved in cold nucleolysis buffer (50 mmol/l Tris-HCl pH 8.0, 150 mmol/l NaCl, 1% NP-40 (Solarbio), 2% v/v complete protease inhibitor, and $NaVO_3$). Cells were disrupted with a homogenizer, and the supernatants were collected after 14,000 x g centrifugation for 15 min at 4°C. The supernatants were incubated with anti-USP22 antibody (cat. no. ab71732; Abcam; 1:500) or anti-IgG antibody (cat. no. ab109489; Abcam; 1:500) at 4°C and, 6 h later, protein A + G agarose beads (Santa Cruz Biotechnology, Inc; 1:200) were added to each sample and the samples were incubated overnight at 4°C. The beads were then centrifuged at 8,000 x g for 30 sec at 4°C and washed four times with 1 ml cold lysis buffer, as aforementioned. Then the beads were boiled in 30 μl sample buffer for 5 min, to release the protein from the beads. Proteins were detected via western blotting as mentioned above with USP22 (cat. no. ab71732; Abcam), SURVIVIN (cat. no. 2808; Cell Signaling Technology, Inc.).

Reverse transcription-quantitative (RT-q) PCR. Total RNA was isolated from $3x10^5$ cells using the RNAiso™ Plus kit (Tehermo Fisher Scientific, Inc.). Reverse transcriptions were conducted using a cDNA synthesis kit (Toyobo Life Science) in 20 μl reaction system. qPCR was performed using FastStart Universal SYBR Green Master (Roche, Inc.) in 25 μl reaction system with thermocycling conditions: Initial denaturation at 98°C for 30 sec; 35 cycles at 98°C for 10 sec, 60°C for 30 sec, and 72°C for 30 sec; final extension at 72°C for 2 min. The primer sequences were as follows: *β-actin* forward, 5'-AAGGAAGGCTGGAAG AGGTGC-3' and reverse, 5'-CTGGAGAGAGAGAGAGAA A-3'; *USP22* forward, 5'-GGCGGAGATCACCAGGTAT-3' and reverse, 5'-TTGTGTAGAGACTGTCCGTGGG-3'; and *SURVIVIN* forward, 5'-TGGCCTTTCAGAGCAGAGTG-3' and reverse 5'-AAGCCACAGTTAGGGGAACG-3'. RNA extraction, cDNA synthesis, and qPCR performed according to the manufacturer's protocols. Normalized relative expression levels were calculated using the $2^{-\Delta\Delta Cq}$ (cycle threshold) method (19).

Cycloheximide (CHX) analysis. ACHN cells (approximately $3x10^5$ cells) were transfected with control or USP22-HA

vector. After 48 h, CHX (500 μM) was added at different time points as indicated, and then cells were harvested. The expression of Survivin was detected via western blot analysis and quantification analysis was performed using ImageJ software (version 1.49; National Institutes of Health).

Statistical analysis. Statistical analysis was performed using GraphPad Prism 7 software (GraphPad Software, Inc.). All experiments were conducted in triplicate and repeated at ≥3 times unless otherwise specified. The data are presented as mean ± standard error of mean. The statistically significant differences between two groups were calculated with unpaired Student's t-test using GraphPad Prism 7 software (GraphPad Software, Inc.). One-way analysis of variance followed by Tukey's post hoc test were used to compare differences between multiple groups. $P < 0.05$ was considered to indicate a statistically significant difference.

Results

USP22 is highly expressed in RCC and associated with survivin expression. To determine whether the levels of USP22 and survivin are clinically correlated with RCC, 10 pairs of tissues were collected, each consisting of a patient's RCC tissue and an adjacent normal tissue as the control (Table I). The protein levels of USP22 and survivin in RCC were quantified with western blotting. Results indicated that the protein levels of USP22 and survivin in RCC tissues were significantly higher compared with those in control tissues (Fig. 1A and B). USP22 and survivin were also detected in RCC tissues and controls using immunohistochemistry. Analogous to western blotting, immunohistochemistry results also suggested upregulation of both USP22 and survivin in RCC tissues (Fig. 1C). Therefore, it was concluded that USP22 and survivin are co-expressed in RCC cells and their protein levels are upregulated in RCC.

USP22 knockdown inhibits the expansion and monoclonal colony formation of ACHN cells. To knockdown *USP22* in RCC cells, two pairs of *USP22*-targeting shRNA were constructed and their knockdown efficiency was validated via western blotting (Fig. 2A). Next, the role of USP22 in the proliferation of human renal carcinoma cell line ACHN and
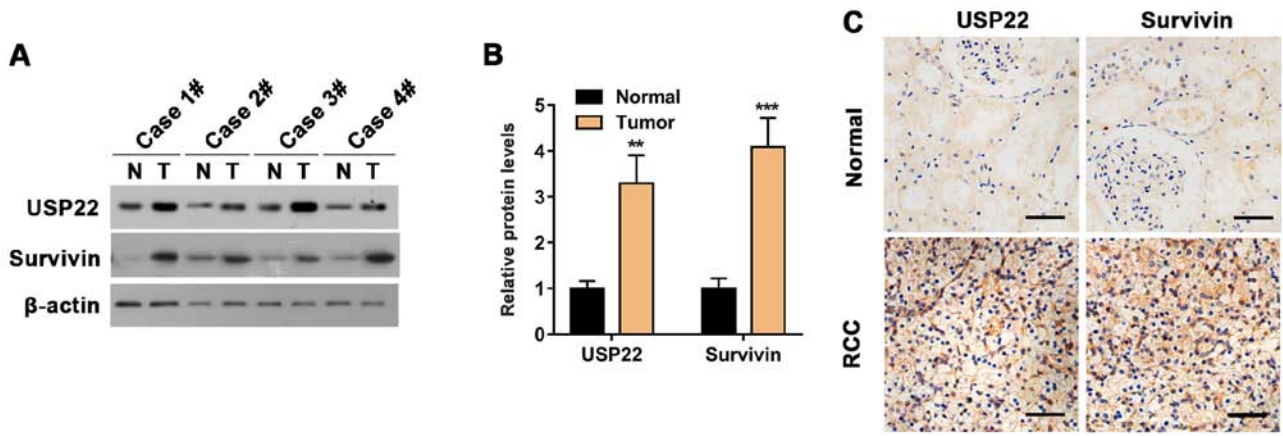
Figure 1. USP22 is highly expressed in renal cell carcinoma tissues. (A) Expression levels of USP22 and survivin in RCC tissues and normal adjacent renal tissues examined via western blotting. (B) Quantitative analysis of the western blot analysis. Data are presented as mean ± standard error of mean. n=10. **P<0.01 and ***P<0.001 as determined by Student's t-test. (C) Representative immunohistochemistry image of the USP22 and survivin expression in RCC tissues and adjacent normal renal tissues (scale bar=50 μm). USP22, ubiquitin-specific protease 22; RCC, renal cell carcinoma; T, tumor; N, normal.
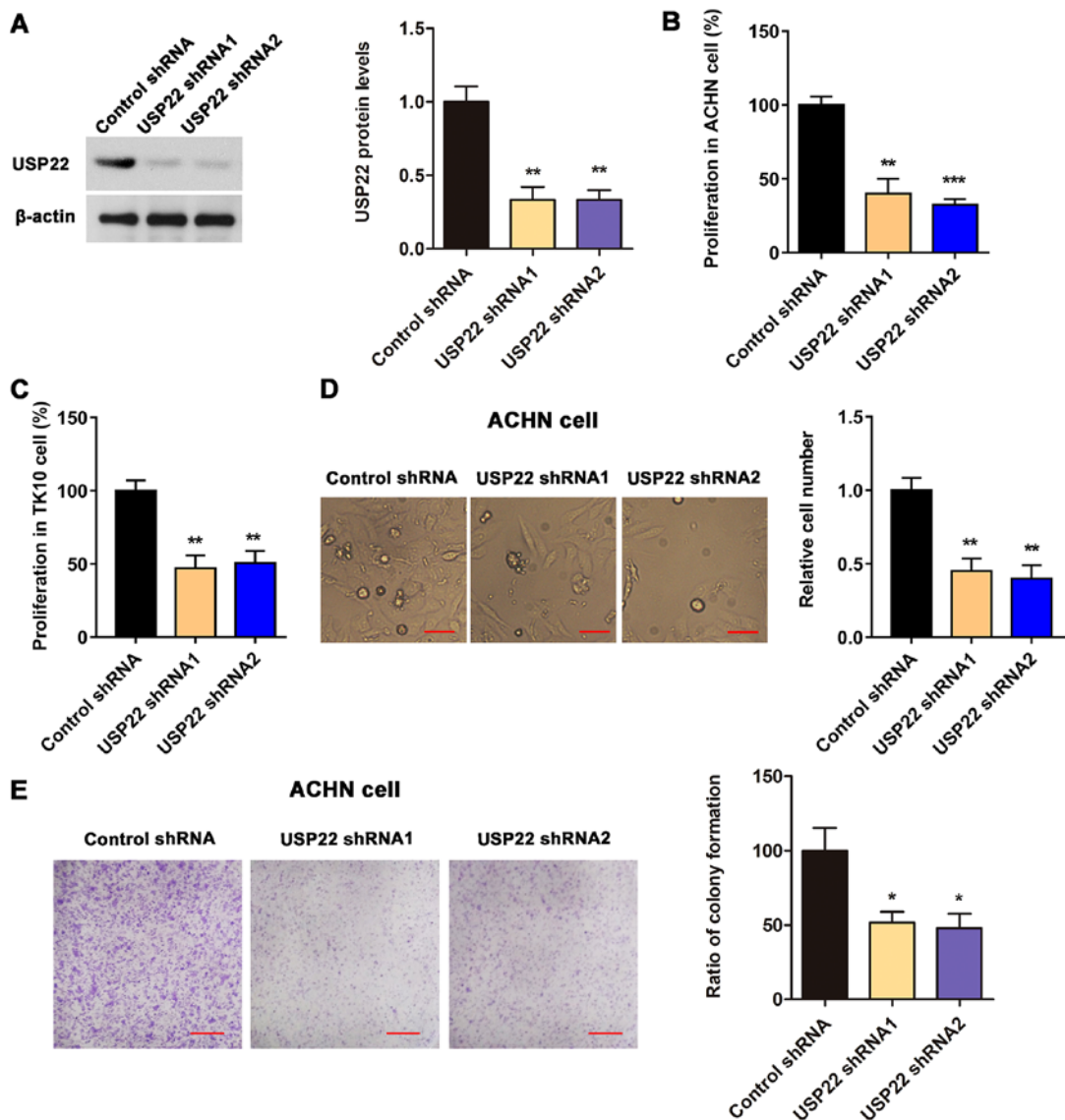

Figure 2. USP22 knockdown inhibits the proliferation and colony formation of RCC cell lines. (A) *USP22* shRNA efficiency validated via western blot analysis in ACHN cells. (B) Effect of *USP22* knockdown on ACHN cell proliferation by CCK8 assay. (C) Effect of USP22 depletion on TK-10 cell proliferation by CCK8 assay. (D) Effect of USP22 depletion on numbers of ACHN cells. Scale bar, 20 μm. (E) Effect of USP22 depletion on cell colony formation by colony forming assays. USP22, ubiquitin-specific protease 22; sh-, short hairpin. Scale bar, 100 μm. Data are presented as mean ± standard error of mean. n=4. *P<0.05, **P<0.01 and ***P<0.001 as determined by one-way ANOVA.
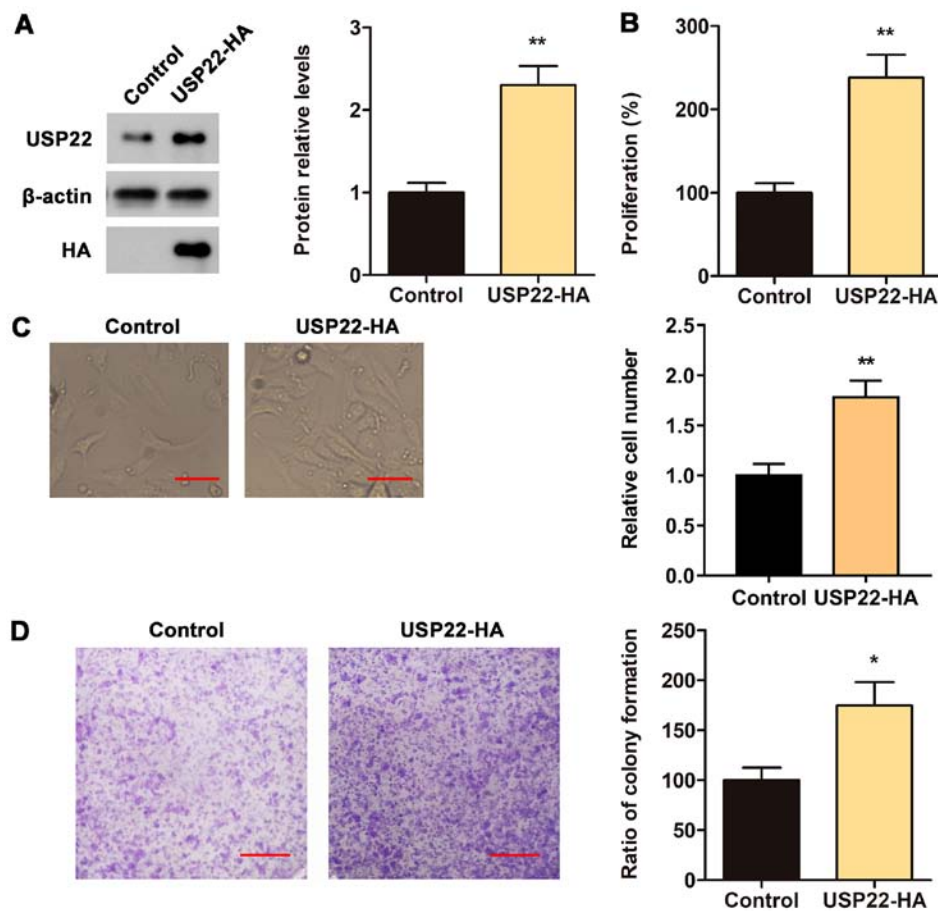
Figure 3. Overexpression of *USP22* promotes the proliferation and colony formation of ACHN cells. (A) Verification of overexpression efficiency of *USP22* in ACHN cells. (B) Effect of overexpression of *USP22* on cell proliferation by CCK8 assay. (C) Effect of overexpression of *USP22* on ACHN cell numbers. Scale bar, 20 μm. (D) Effects of overexpression of USP22 on colony formation by colony forming assays. Data are presented as mean ± standard error of mean n=4. *P<0.05 and **P<0.01 as determined by Student's t-test. Scale bar, 100 μm. USP22, ubiquitin-specific protease 22.

TK-10 cells was evaluated with CCK8 assays. The results indicated that USP22 knockdown significantly inhibited the proliferation of ACHN and TK-10 cells (Fig. 2B-C). Although USP22 silencing demonstrated similar inhibitory effects on both ACHN and TK-1 human RCC cells, only ACHN cells were subjected to the subsequent experiments as the most commonly utilized cell line in RCC research.

It was also determined that the cell number and cloning rate of ACHN cells transfected with *USP22* shRNA was significantly lower compared with the cells transfected with control shRNA (Fig. 2D-E, P<0.01 in cell number and P<0.05 in cloning rate), indicating that USP22 may contribute to the proliferation of RCC.

Overexpression of USP22 promotes the proliferation and monoclonal colony formation of ACHN cells. To confirm the aforementioned findings described, a USP22-HA vector was constructed to test whether the overexpression of USP22 promotes the proliferation of ACHN cells. First, the expression of the USP22-HA vector was validated via western blotting. The results indicated that USP22-HA plasmids significantly increased the protein level of USP22 in ACHN cells (Fig. 3A). CCK8, cell number counting and monoclonal colony formation assays were performed to determine the proliferation rate of the cells. The results demonstrated that

the overexpression of USP22 significantly increased the proliferation rate and the cell cloning rate of ACHN cells (Fig. 3B-D). These data further confirmed that USP22 may promote the proliferation of RCC.

USP22 regulates the protein levels of survivin and cleaved-caspase-3. A previous report has revealed that USP22 regulates the proliferation of hepatocellular carcinoma via the survivin signal pathway (20). In order to study the mechanism of USP22 in regulating the proliferation of RCC, the protein level of survivin was detected in ACHN cells following *USP22* knockdown or overexpression. It was revealed that *USP22* knockdown significantly decreased the protein level of survivin, and that the overexpression of *USP22* had a reverse effect (Fig. 4A). Furthermore, it was found that cleaved caspase-3, a downstream protein of survivin, was decreased following *USP22* knockdown and increased by *USP22* overexpression (Fig. 4A-D); however, no significant changes were observed in the signal pathway of cell proliferation, including p-AKT, AKT, p-Erk, Erk and ratio of p-AKT/AKT and p-Erk/Erk (Fig. 4A). Notably, it was demonstrated that the mRNA level of *SURVIVIN* in ACHN cells was not changed by either knockdown or overexpression of *USP22* (Fig. 4E). Furthermore, *SURVIVIN* overexpression rescued the effect of *USP22* shRNA on cell proliferation and monoclonal colony
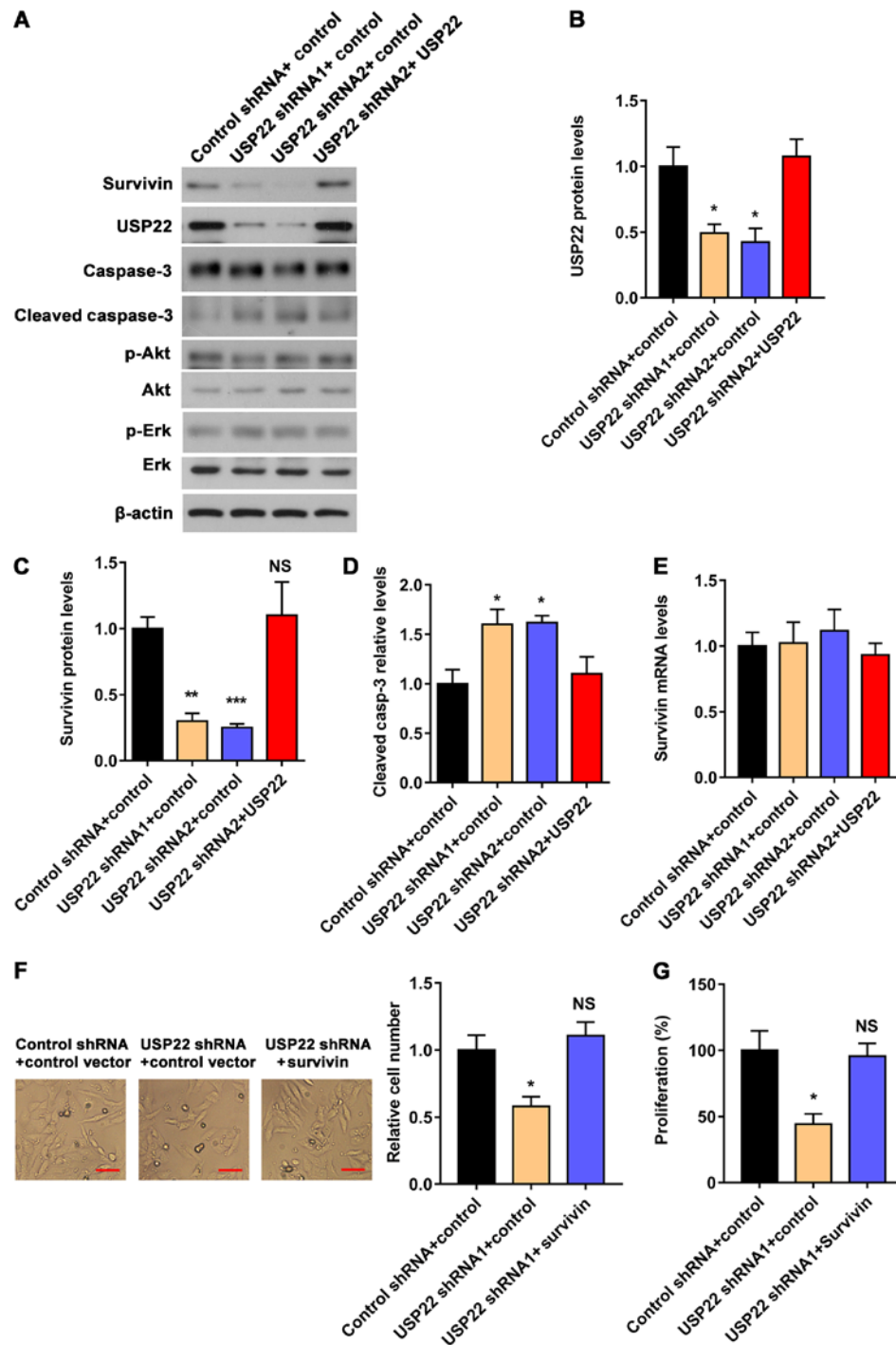
Figure 4. USP22 regulates the expression of survivin and the cleavage of caspase-3 but does not affect *SURVIVIN* at the mRNA level. (A) Western blot analysis of the protein levels of survivin, USP22, caspase-3, cleaved caspase-3, Akt, p-Akt, Erk and p-Erk in ACHN cells transfected with control shRNA, *USP22* shRNA1, *USP22* shRNA2 or USP22-HA. (B) Quantitative analysis of the USP22 protein levels. (C) Quantitative analysis of the survivin protein levels. (D) Quantitative analysis of the cleaved caspase-3 levels. (E) Reverse transcription-quantitative PCR analysis of the mRNA levels of survivin in ACHN cells transfected with control shRNA, *USP22* shRNA1, *USP22* shRNA2 or USP22-HA. (F) Effect of *SURVIVIN* overexpression on cell numbers in *USP22*-knockdown ACHN cells. Scale bar, 20 μm. (G) Effect of *SURVIVIN* overexpression on the proliferation of *USP22*-knockdown ACHN cells by CCK8 assay. Data are presented as mean ± standard error of mean. n=4. *P<0.05, **P<0.01 and ***P<0.001 as determined by one-way ANOVA. USP22, ubiquitin-specific protease 22; sh-, short hairpin.

formation of ACHN cells (Fig. 4F and G). These results indicate that USP22 regulates ACHN cell expansion via modulating survivin at the post-transcriptional level.

USP22 interacts with survivin and regulates the ubiquitination of survivin. To further investigate the association between USP22 and survivin, the interaction between USP22 and survivin was evaluated via co-immunoprecipitation. It was revealed that the USP22 antibody, but not control IgG, could co-IP with survivin, suggesting that USP22 interacts with survivin (Fig. 5A). Next, the effect of USP22 on the ubiquitination of surviving was examined. ACHN cells
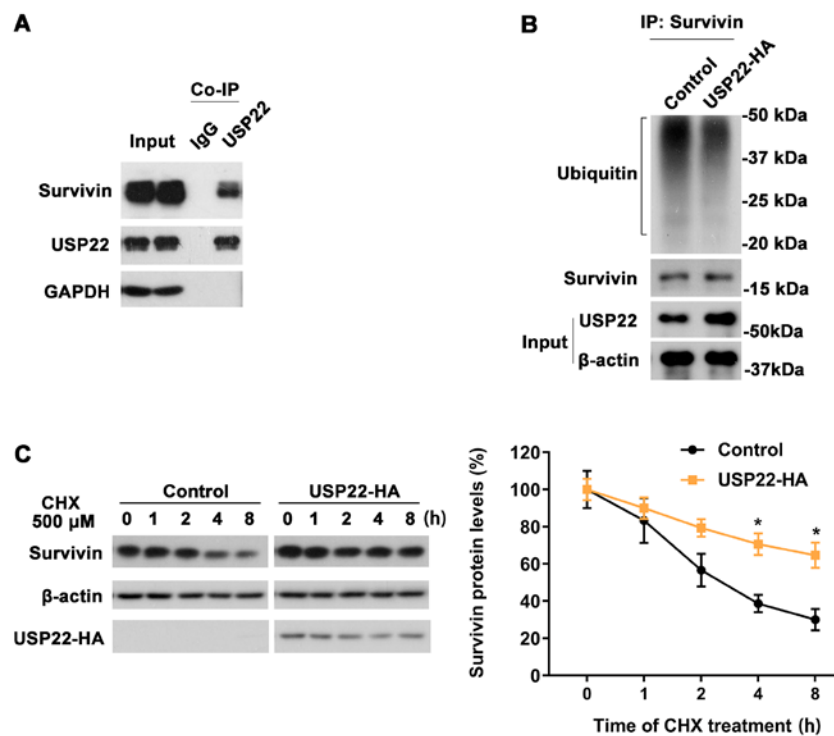
Figure 5. USP22 interacts with survivin and regulates survivin stability. (A) Interaction between the survivin and USP22 proteins tested by Co-IP in ACHN cells. (B) Survivin ubiquitination in ACHN cells co-transfected with USP22-HA or control. (C) Degradation of survivin examined in ACHN cells co-transfected with USP22-HA or control. Data are presented as mean ± standard error of mean. n=4. *P<0.05 as determined by one-way ANOVA. USP22, ubiquitin-specific protease 22; Co-IP, Co-immunoprecipitation; CHX, cycloheximide.
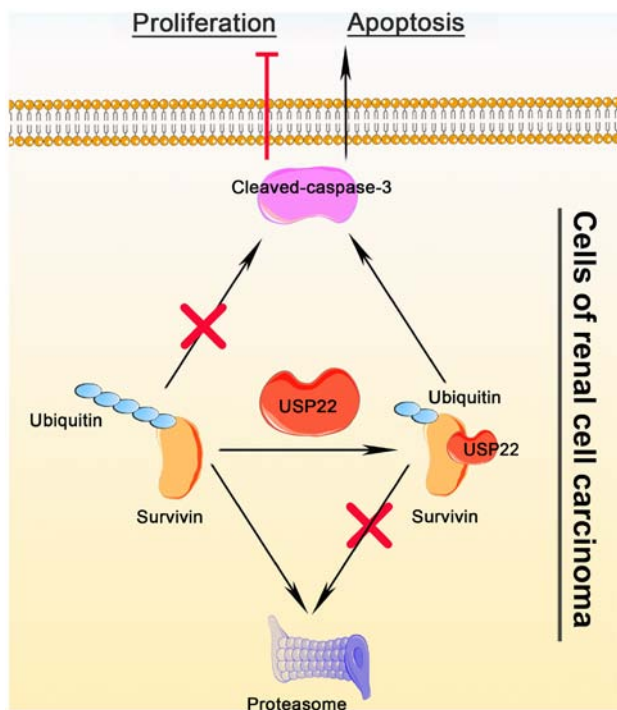

Figure 6. Schematic diagram of survivin-mediated apoptotic inhibition by USP22 in RCC. In RCC cells, USP22 interacts with and deubiquitinates survivin, preventing its ubiquitin proteasome-mediated degradation and inhibiting cell apoptosis. USP22, ubiquitin-specific protease 22; RCC, renal cell carcinoma.

were transfected with either USP22-HA or control plasmid. After 48 h of expression, the two groups were immunoprecipitated with anti-survivin antibodies and immunoblotted with an anti-ubiquitin antibody. The results indicated that the ubiquitination level of survivin was decreased after the overexpression of USP22 (Fig. 5B). Cycloheximide chase assays were performed in 293T cells and it was revealed that the degradation of survivin was markedly decreased following overexpression of USP22 (Fig. 5C). Together, these results indicated that USP22 was associated with survivin and regulated the stability of survivin via modulating its ubiquitination level (Fig. 6).

Discussion

The present study reported, for the first time to the best of the authors' knowledge, that USP22 is highly expressed in RCC tissues. Upregulation of USP22 has been observed in various cancers (21-23). Together with the present study, these findings suggest that a high level of USP22 may be a common characteristic in cancers. To investigate the roles of USP22 in RCC, *USP22* shRNA and USP22-HA vector were used to manipulate the expression of *USP22* in ACHN cells, and it was revealed that the proliferation and monoclonal colony formation abilities of tumor cells were inhibited via *USP22* knockdown and promoted via *USP22* overexpression. These *in vitro* data support the hypothesis that USP22 may be used as a therapeutic target for RCC.

Previous studies demonstrate survivin is highly expressed in numerous tumors, including RCC (14,21-23). This was confirmed using RCC tissues and it was revealed that survivin and USP22 have similar expression patterns. Survivin regulates cell division and is associated with the development of tumors (9,24,25). The present study hypothesized that during

the development of RCC, USP22 upregulates survivin, and thus decreases the apoptosis of RCC cells. To explore the association between USP22 and survivin, the present study examined the survivin protein level after manipulating the expression of *USP22* using shRNAs. It was demonstrated that *USP22* knockdown significantly decreased the protein level of survivin, and *USP22* overexpression had the opposite effect, suggesting USP22 as a novel modulator of survivin. Notably, the mRNA level of survivin was unaltered, indicating that USP22 mediates survivin protein at the post-transcriptional level.

The interactions between USP22 and survivin were investigated via a Co-IP assay and it was found that USP22 directly or indirectly binds survivin. This is the first time that USP22 has been reported to be associated with survivin. USP22 is a deubiquitinating enzyme involved in regulating the ubiquitination of many disease-associated proteins (21). It was revealed that USP22 stabilized survivin via deubiquitination. In addition, the upregulation of the apoptosis inhibitor survivin was accompanied by a decrease in the cleaved caspase-3 level, which may explain why the proliferation and colony formation of RCC cells were enhanced following the overexpression of survivin. The finding that survivin expression rescued the effect of USP22 knockdown in cell proliferation also supported the conclusion that the effect on proliferation of USP22 depends on survivin. Future studies will focus on the research and development of small-molecule inhibitors for USP22 as a novel molecular therapeutic approach for RCC.

In conclusion, the present study demonstrated that USP22 decreased apoptosis in RCC via modulating survivin stability. The findings indicated that USP22 may be used as a novel therapeutic target for patients with renal cancer.

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Not applicable.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

YL, MZ and JH were guarantors of integrity for the entire study. YL, HZ, BS were responsible for the conception of the study, and YL, HZ, BS, YP and FL were responsible for the study design. Definition of intellectual content was conducted by YL, HZ and BS. Literature research was performed by YL, HZ, BS and YP, clinical studies by MC, MZ and JH, and experimental studies by YL, HZ, BS, YP and FL. Data

acquisition was conducted by YL, HZ, BS and YP, and data analysis by YL, HZ, BS and YP. YL, HZ, BS and YP conducted statistical analyses. YL, MZ and JH prepared the manuscript and the manuscript was edited by YL, MZ and JH. The manuscript was reviewed by YL, MZ and JH. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The human studies were approved by the ethical review board at the Fifth Hospital of Xiamen and patients provided written informed consent in accordance with the legal and institutional ethical guidelines defined by the hospital by the Ethics Committee of the Fifth Hospital of Xiamen.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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