Original Article

Circular RNA SMARCA5 is overexpressed and promotes cell proliferation, migration as well as invasion while inhibits cell apoptosis in bladder cancer

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Background: This study aimed to investigate the function of circular RNA SMARCA5 (circ-SMARCA5) on cell proliferation, apoptosis, migration and invasion in bladder cancer.

Methods: Ten pairs of human bladder cancer tissue and adjacent tissue, five human bladder cancer cell lines (including TCCSUP, 5637, J82, UM-UC-3 and T-24) and normal human urothelial cell line SV-HUC-1, were obtained for the detection of circ-SMARCA5. Control overexpression and ShRNA, circ-SMARCA5 overexpression and ShRNA were constructed and transfected into UM-UC-3 cells as Control(+), Control(–), Circ-SMARCA5(+) and Circ-SMARCA5(–) groups. The role of circ-SMARCA5 was investigated in terms of cellular proliferation, apoptosis, migration, and invasion.

Results: Circ-SMARCA5 was overexpressed in tumor tissue compared to paired adjacent tissue and it was also overexpressed in TCCSUP, 5637, J82 and UM-UC-3 cells compared to normal human urothelial cell line SV-HUC-1. In UM-UC-3 cells, cell proliferation ability, migration rate and invasion cell count were increased in Circ-SMARCA5(+) group compared to Control(+) group, while reduced in Circ-SMARCA5(–) group compared to Control(–) group. Regarding the cell apoptosis, apoptosis rate and apoptotic protein C-Caspase 3 expression were decreased in Circ-SMARCA5(+) group than those in Control(+) group but raised in Circ-SMARCA5(–) group compared to Control(–) group, meanwhile, the anti-apoptotic protein Bcl-2 expression was elevated in Circ-SMARCA5(+) group than that in Control(+) group but reduced in Circ-SMARCA5(–) group compared to Control(–) group.

Conclusions: Circ-SMARCA5 is overexpressed, and promotes cell proliferation, migration and invasion, but represses apoptosis in bladder cancer.

Keywords: Circular RNA; circSMARCA5; bladder cancer; cell proliferation; cell apoptosis; migration; invasion

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Introduction

Bladder cancer is one of the most common malignant cancer globally, with approximately 400,000 new cases each year worldwide, and estimated 272,400 new cases during 2015 in China (1-3). According to tumor stage, bladder cancer could be divided in two major classes: non-muscle-invasive disease (around 70% of all cases) and muscle-invasive bladder cancer (around 30% of all cases) (4,5). Although the majority of patients are diagnosed as non-muscle-invasive bladder cancer, and transurethral resection, as a kind of primarily treatment, has good efficacy on removing tumors, while there are still 50–70% of these tumors are prone to relapse after resection and develop invasive bladder cancer (4,5). Recently, despite various treatments including surgery, chemotherapy and radiotherapy have been applied on bladder cancer, the treatment efficacy is limited, and bladder cancer is still a big challenging problem not only worldwide but also in China (4). Therefore, it is necessary to explore detailed mechanisms involved in the initiation and development of bladder cancer, which might provide new hope for the treatment.

Circular RNA (CircRNA), a class of noncoding RNA family, is characterized by covalently closed loop formed by back-spicing event, and it is commonly named according to the parental genes or particular functions (6-8). Increasing studies have shown that circRNAs are aberrantly expressed in various cancers and they play crucial roles in regulating cancer biological processes (6,7,9). Circular SMARCA5 (circ-SMARCA5), locates on chr4:144464662-144465125, is encoded by SWI/SNF-related matrix-associated actin-dependent regulator of chromatin A5 (SMARCA5) gene (10). Some attentions have been paid on the role of circ-SMARCA5 in cancers, and several previous studies indicate that circ-SMARCA5 serves as an important regulator in these carcinomas such as prostate cancer and hepatocellular carcinoma, whereas no evidence about the function of circ-SMARCA5 on bladder cancer are found (10,11). Thus, we conducted this study to investigate the expression of circ-SMARCA5 in bladder cancer tissues and cells, and further explore the function of circ-SMARCA5 on regulating cell proliferation, apoptosis, migration and invasion in bladder cancer cells, which might provide valuable information for proposing circ-SMARCA5 as therapeutic target in bladder cancer.

Methods

Samples and cells

Ten pairs of human bladder cancer tissue and adjacent tissue were obtained during the surgery. Five human bladder cancer cell lines including TCCSUP, 5637, J82, UM-UC-3 and T-24 were purchased from Cell Bank of Type Culture Collection of Chinese Academy of Sciences (Shanghai, China), normal human urothelial cell line SV-HUC-1 was purchased from American type culture collection (Manassas, USA). TCCSUP cell line was cultured in 90% Eagle's Minimum Essential Medium (EMEM) (Invitrogen, USA) and 10% fetal bovine serum (FBS) (Gibco, USA), 5637 and T-24 cell lines were cultured in 90% Roswell Park Memorial Institute (RPMI) 1640 Medium (Sigma, USA) and 10% FBS (Gibco, USA), J82 and UM-UC-3 cell lines were cultured in 90% Minimum Essential Medium (MEM) (Gibco, USA) and 10% FBS (Gibco, USA), and SV-HUC-1 cell line was cultured in 90% Ham's F-12K (Kaighn's) Medium (Gibco, USA) and 10% FBS (Gibco, USA). All cells were incubated under 95% air and 5% CO_2 at 37 ℃. The Ethics Committee of our hospital approved this study before its initiation, and the number of the approval was 20170082. The study was carried out in accordance with the principles expressed in the Declaration of Helsinki. Besides, all subjects provided written informed consents before the initiation of our study.

Measurement of circ-SMARCA5 expression in bladder cancer

Circ-SMARCA5 expression in human bladder cancer tissue and adjacent tissue, as well as in human bladder cancer cell lines and normal human urothelial cell line was measured by quantitative polymerase chain reaction (qPCR).

Transfection

Control overexpression, circ-SMARCA5 overexpression, control shRNA, and circ-SMARCA5 shRNA plasmids were constructed by Shanghai Gene Bio-Tech Company (Shanghai, China) with pCD25-ciR and pGPU6 plasmids. And 0.8 μg of each plasmid was transfected into UM-UC-3 cells as Control(+), Circ-SMARCA5(+), Control(–), and

Circ-SMARCA5(–) groups with HilyMax (Dojindo, Japan). Circ-SMARCA5 expression in each group was measured at 24 h after transfection to determine the transfection success or not using qPCR. Additionally, the sequences of circ-SMARCA5 overexpression and circ-SMARCA5 shRNA were as follows: sense sequence, 5'-CACCGAAAAACAAAAGGAGGCCGAAGCCTCCTTTTGTTTTTC-3'; anti-sense sequence, 5'-AAAAGAAAAACAAAAGGAGGCTTCGGCCTCCTTTTGTTTTTC-3'.

Measurement of proliferation, apoptosis, migration and invasion

Cell proliferation ability was measured at 0, 24, 48 and 72 h after transfection using Counting Kit-8 (CCK-8) (Sangon Biotech, China) according to the instruction of manufacturer. Cell apoptosis rate was measured at 48 h by FITC Annexin V Apoptosis Detection Kit II with propidium iodide (AV/PI) (BD, USA) according to the instruction of manufacturer. Apoptotic protein Cleaved Caspase 3 (C-Caspase 3) and anti-apoptotic protein B-cell lymphoma-2 (Bcl-2) were measured at 48 h using Western Blot. Cell migration rate was calculated by dividing migration distance across the gap at 24 h by that at 0 h under microscope (Olympus, Japan) using wound healing assay referring to a previous report (12). In addition, invasive cell count was calculated using Matrigel invasion assay with Matrigel basement membrane matrix (BD, USA) and 8-um Polyvinylidene Fluoride contained transwell filter chamber (Coring, USA) referring to a previous report (12).

qPCR

Circ-SMARCA5 expression was determined by qPCR assay, which was performed as the following steps: (I) extraction of total RNA was performed with TRIzol™ Reagent (Invitrogen, USA); (II) RNase R (Epicentre, USA) was used to digest the linear RNA in each sample of total RNA (1 μg), followed by the agarose gel electrophoresis to check the residual linear RNA; (III) if no band of linear RNA SMARCA5 was observed, the reverse transcription to cDNA was performed by PrimeScript™ RT reagent Kit (Takara, Japan); (IV) qPCR was conducted by PrimeScript™ RT reagent Kit(TAKARA, Japan), and qPCR amplification was performed at 95 ℃ for 3 mins, followed by 40 cycles of 95 ℃ for 5 s, 61 ℃ for 30 s, and then 72 ℃ for 30 s. The results of qPCR were calculated by 2-ΔΔCt formula. Notably, we chose the forward and reverse primers according to the unique structure of circular RNA, and the sequences amplified by our primers would contain the sequence that expands the back-splicing junction in circular RNA structure, which was not included in linear RNA. Thus, only the circular RNA was detected in our study. Meanwhile, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as the internal reference and its qPCR was performed with total RNA. Additionally, the SMARCA5 expression was also detected by qPCR assay. Information of qPCR primers were as follows: Circ-SMARCA5 (hsa_circ_0001445), forward (5'-3'): AGATGGGCGAAAGTTCACTTAGA, reverse (5'-3'): GATTCTGATCCACAAGCCTCCT. GAPDH, forward (5'-3'): GACCACAGTCCATGCCATCAC, reverse (5'-3'): ACGCCTGCTTCACCACCTT. SMARCA5, forward (5'-3'): CCGAGGATTAAACTGGCTCA, reverse (5'-3'): GAGGCCCAGGAATGTTTCTA.

Western Blot

Western Blot assay was performed as the following steps: (I) RIPA Lysis and Extraction Buffer (Thermo, USA) was added to cell samples on ice, and concentration of total protein was assessed by bicinchoninic acid (BCA) kit (Pierce Biotechnology, USA); (II) thermal denaturation was conducted at 98 ℃ for 5 min; (III) 20 μg proteins were added to the sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) (Thermo, USA); (IV) after electrophoresis, proteins were transferred to polyvinylidene fluoride membranes (PVDF) membrane (Millipore, USA); (V) membranes were blocked with 5% bovine serum albumin (BSA) (Sigma, USA) (37 ℃, 1 h), and then incubated with primary antibody (4 ℃, overnight) and appropriate horseradish peroxidase (HRP)-conjugated secondary antibody (room temperature, 2 h). Finally, the bands were visualized by Pierce™ECL Plus Western Blotting Substrate (Thermo, USA). Information of antibodies used were as follows: Primary antibody: Caspase-3 Rabbit mAb (CST, USA) (dilution: 1:1,000); Cleaved Caspase-3 Rabbit mAb (CST, USA) (dilution: 1:1,000); Bcl-2 Rabbit mAb (CST, USA) (dilution: 1:1,000); GAPDH Rabbit mAb (CST, USA) (dilution: 1:1,000); Secondary Antibody: Goat Anti-Rabbit IgG H&L (HRP) (CST, USA) (dilution: 1:4,000).

Statistics

Statistical analysis and graphs were conducted using

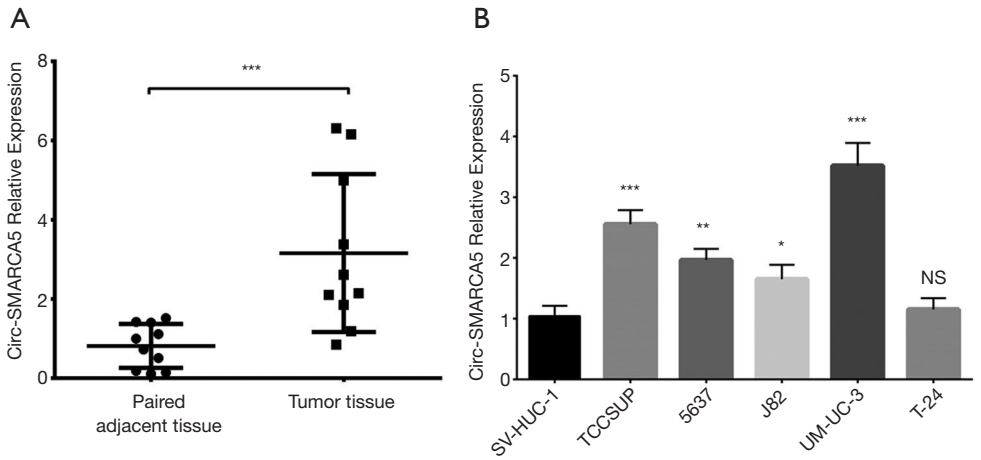

Figure 1 Detection of circ-SMARCA5 expression in tissue and cell lines. Compared to paired adjacent tissue, circ-SMARCA5 expression was increased in tumor tissue (A). Compared to normal human urothelial cell line SV-HUC-1, circ-SMARCA5 expression was elevated in several bladder cancer cell lines (TCCSUP, 5637, J82 and UM-UC-3) (B). Circ-SMARCA5, circular RNA-SMARVA5; comparison between two groups were determined by *t* test and One-Way ANOVA followed by Dunnett's multiple comparisons test. P<0.05 was considered significant. ***, P<0.001; **, P<0.01; *, P<0.05.

SPSS 22.0 Software (IBM, USA) and GraphPad 6.01 Software (GraphPad Int., USA). Data were presented as mean ± standard deviation, comparison among groups was determined by One-way ANOVA followed by Dunnett's multiple comparison test, comparison between two groups was determined by *t* test. P<0.05 was considered as significant.

Results

Circ-SMARCA5 expression in tissue samples and cells

Circ-SMARCA5 expression was remarkably higher in tumor tissue than that in paired adjacent tissue (P<0.001) (*Figure 1A*). In cell lines, circ-SMARCA5 expression was elevated in various bladder cancer cells including TCCSUP (P<0.001), 5637 (P<0.01), J82 (P<0.05) and UM-UC-3 (P<0.001) cells compared to normal human urothelial cell line SV-HUC-1, while no difference of circ-SMARCA5 expression was found between T-24 cells and SV-HUC-1 cells (P>0.05) (*Figure 1B*).

Effect of circ-SMARCA5 on cell proliferation and cell apoptosis in UM-UC-3 cells

In order to evaluate the function of circ-SMARCA5 in bladder cancer, we performed upregulation and downregulation of circ-SMARCA5 by transfection with corresponding plasmids into UM-UC-3 cells. After 24 h after transfection, circ-SMARCA5 expression was increased in Circ-SMARCA5(+) group compared to Control(+) group (P<0.001), while it was decreased in Circ-SMARCA5(–) group compared to Control(–) group (P<0.001), indicating the plasmids were successfully transfected (*Figure 2*). Moreover, cell proliferation was raised in Circ-SMARCA5(+) group compared to Control(+) group at 72 h (P<0.05), while it was reduced in Circ-SMARCA5(–) group compared to Control(–) group at 48 h (P<0.05) and 72 h (P<0.01) (*Figure 3A*). Regarding the cell apoptosis rate at 48 h, it was lower in Circ-SMARCA5(+) group than that in Control(+) group (P<0.01) but higher in Circ-SMARCA5(–) group compared to Control(–) group (P<0.01) (*Figure 3B*). Meanwhile, expressions of apoptosis-related proteins were detected by Western Blot assay, which displayed that apoptotic protein C-Caspase 3 expression was decreased in Circ-SMARCA5(+) group but increased in Circ-SMARCA5(–) group compared to the corresponding groups, while anti-apoptotic protein Bcl-2 expression was elevated in Circ-SMARCA5(+) group but reduced in Circ-SMARCA5(–) group compared to the corresponding groups (*Figure 3C*). Besides, the results displayed by Flow Cytometry also showed that cell apoptosis rate was decreased in Circ-SMARCA5(+) group than that in Control(+) group but increased in Circ-SMARCA5(–) group compared to Control(–) group at 48

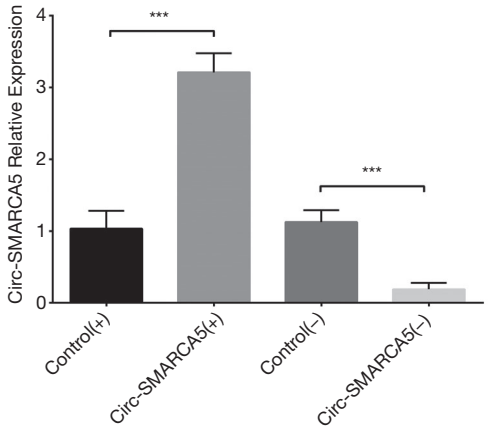

Figure 2 Detection of circ-SMARCA5 expression after upregulation and downregulation of circ-SMARCA5 in UM-UC-3 cells. Circ-SMARCA5 expression was greatly higher in Circ-SMARCA5(+) group compared to Control(+) group, while it was lower in Circ-SMARCA5(–) group compared to Control(–) group. Circ-SMARCA5, circular RNA-SMARVA5. Comparison between groups were determined by *t* test. P<0.05 was considered significant. ***, P<0.001.

h (*Figure 3D*). These results indicated that circ-SMARCA5 enhanced cell proliferation but repressed cell apoptosis in UM-UC-3 cells. Additionally, we found that SMARCA5 expression was elevated in Circ-SMARCA5(+) group compared to Control(+) group (P<0.01) but decreased in Circ-SMARCA5(–) group compared to Control(–) group (P<0.05) (*Figure 4*).

Effect of circ-SMARCA5 on cell migration in UM-UC-3 cells

Cell migration was measured by wound healing assay, which showed that migration rate was raised in Circ-SMARCA5(+) group compared to Control(+) group (P<0.001), while it was reduced in Circ-SMARCA5(–) group compared to Control(–) group (P<0.01) (*Figure 5*). These data indicated that circ-SMARCA5 promoted cell migration in UM-UC-3 cells.

Effect of circ-SMARCA5 on cell invasion in UM-UC-3 cells

Cell invasion was detected by Matrigel invasion assay, and we found increased invasive cell count in the Circ-SMARCA5(+) group compared to Control(+) group (P<0.01) and decreased invasive cell count in the Circ-SMARCA5(–) group compared to Control(–) group (P<0.01). These data suggested that circ-SMARCA5 enhanced cell invasion in UM-UC-3 cells (*Figure 6*).

Discussion

In this study, we found that circ-SMARCA5 was overexpressed in tumor tissues and tumor cells compared to the paired adjacent tissues and human urothelial cell line respectively. Furthermore, *in vitro* experiments displayed that circ-SMARCA5 promoted cell proliferation, repressed cell apoptosis, facilitates cell migration and enhanced cell invasion in UM-UC-3 cells.

CircRNAs, are recently recognized as a class of abundant endogenous non-coding RNAs with great regulatory potency in human mammalian cells owing to the development of next generation sequencing technology and bioinformatics. CircRNAs possess some prominent features as follows: (I) the stability due to the covalently closed loop structure (8,13); (II) the universality in human cells (14,15); (III) the specificity varying in tissues and developmental stages (13,16); (IV) the conservatism in different species (17). These features make circRNAs serve as potential biomarkers for human diseases (8,13,14,17-20). Furthermore, according to previous data, some major functions of circRNAs are as follows: (I) acting as miRNA sponges: circRNAs, which have many miRNA response elements, competitively bind to miRNAs and result in decrease of the functional miRNAs (8,13,21); (II) regulators of parental gene transcription: circRNAs are able to regulate the transcription of their parental genes (8); (III) circRNA-protein interaction: circRNAs could interact with proteins, and the interaction contributes to regulating transcription of parental genes, promoting interaction of multiple proteins, and altering subcellular localization of proteins (22). Based on these understandings of circRNAs, the investigation of circRNAs in cancer pathology and development has become a hotspot in the field of cancer research, including bladder cancer (8). For instance, an investigation using microarray assay finds 469 dysregulated circRNAs in bladder cancer, among which 285 were up-regulated (9). Moreover, a previous study shows that circular RNA Centrosomal protein 128 (circ-CEP128) enhances cell proliferation and represses cell apoptosis in bladder cancer through upregulating SRY-box-containing gene 11 (SOX11) (6). Also, an interesting study displays

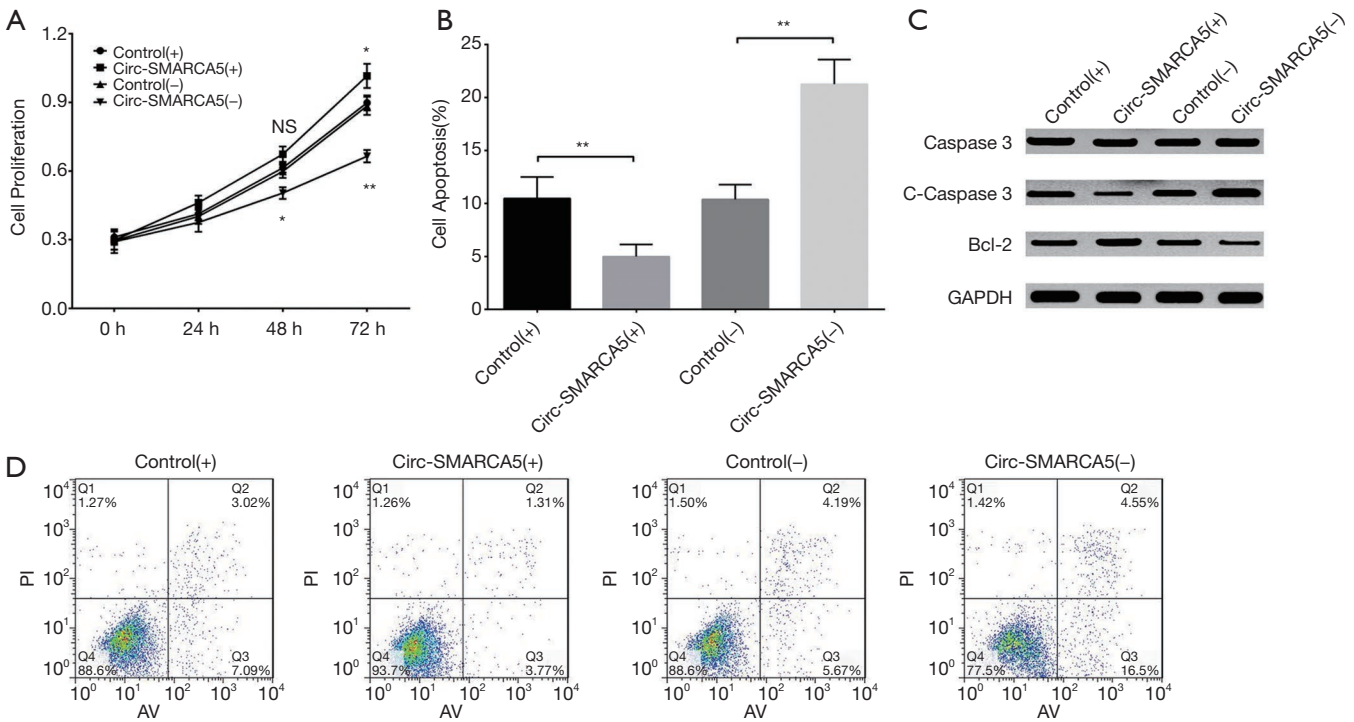

Figure 3 CCK-8, AV/PI and Western Blot assays. CCK-8 assay showed that cell proliferation was elevated in Circ-SMARCA5(+) group but reduced in the Circ-SMARCA5(–) group compared to the corresponding control groups (A). AV/PI assay revealed that cell apoptosis rate was deceased in Circ-SMARCA5(+) group but increased in Circ-SMARCA5(–) group compared to the corresponding control groups (B,D). Western Blot assay displayed that apoptotic protein C-Caspase expression was lower but anti-apoptosis protein Bcl-2 expression was higher in Circ-SMARCA5(+) group compared to Control(+) group, and C-Caspase expression was elevated in Circ-SMARCA5(–) group but Bcl-2 expression was reduced in Circ-SMARCA5(–) group compared to Control(–) group (C). Circ-SMARCA5, circular RNA-SMARVA5; CCK-8, Counting Kit-8; AV/PI, Annexin V Apoptosis Detection Kit II with propidium iodide; Bcl-2, B-cell lymphoma-2. Comparison between groups were determined by t test. P<0.05 was considered significant. **, P<0.01; *, P<0.05.

that circ_0000144 promotes cell proliferation and invasion through inhibiting miR-217 and upregulating runt-related transcription factor 2 (RUNX2) in bladder cancer cells (7). And another study discloses that circRNA Vang-like protein 1 (circ-VANGL1) is overexpressed in tumor tissues compared to adjacent normal tissues of bladder cancer, moreover, its promotive effects on cell proliferation, migration and invasion via suppressing miR-605-3p have been observed in *in vitro* assays (23). These previous data have revealed that circRNAs may serve as crucial regulators in the initiation and progression of bladder cancer through affecting the biological processes such as cell proliferation, apoptosis, migration and invasion.

Circ-SMARCA5, the circRNA deriving form exons 15 and 16 of SMARCA5 gene, have been reported to be involved in the pathology of some cancers (10,11). A previous study discloses that circ-SMARCA5 is upregulated in prostate cancer tissues compared to normal prostate tissues and its silencing inhibits prostate cancer cells proliferation but promotes cell apoptosis (10). As to the detailed mechanisms of how circ-SMARCA5 affects tumor cell apoptosis, only a previous study shows that downregulation of SMARCA5, which is the upstream gene of circ-SMARCA5, causes deficiencies in DNA repair and consequently promotes chromosomal instability, and then leads to increased cell apoptosis in bladder cancer. Meanwhile, downregulation of circ-SMARCA5 decreased SMARCA5 expression in our study. Taken together, we speculated that the influence on DNA repair and chromosomal instability might also underlay the mechanisms of circ-SMARCA5 in cell apoptosis, while the direct evidence of circ-SMARCA5 in affecting cell apoptosis was not reported (24). Whereas, another study reveals that circ-SMARCA5 is downregulated in

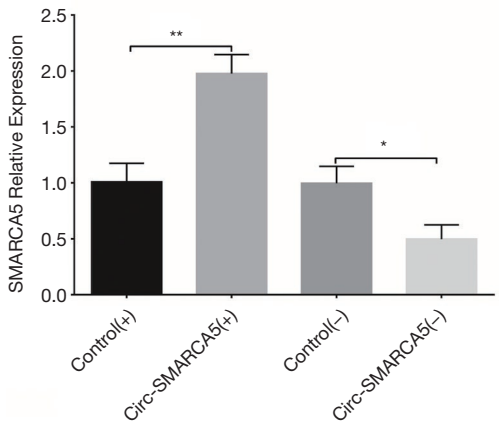

Figure 4 Detection of SMARCA5 expression after upregulation and downregulation of circ-SMARCA5 in UM-UC-3 cells. SMARCA5 expression was increased in Circ-SMARCA5(+) group compared to Control(+) group, while it was reduced in Circ-SMARCA5(–) group compared to Control(–) group. Comparison between groups were determined by *t* test. P<0.05 was considered significant. **, P<0.01; *, P<0.05.

hepatocellular carcinoma tissues compared to adjacent noncancerous liver tissues, moreover, circ-SMARCA5 protects tumor suppressors through the miR-17-3p/miR-181b-5p-TIMP3 pathway, thereby represses cell proliferation in hepatocellular carcinoma cells (11). Although these previous studies show different roles of circ-SMARCA5 in different cancers, they emphasize that circ-SMARCA5 may be a crucial regulator in the pathology of cancers. However, limited evidences reveal the function of circ-SMARCA5 in bladder cancer. Considering circ-SMARCA5 has shown critical regulatory influence in several carcinomas, we supposed that circ-SMARCA5 might also have effects in bladder cancer. To prove our hypothesis, in this study, we firstly compared the circ-SMARCA5 expression between tumor tissue and the paired adjacent tissue, as well as cancer cell lines and normal human urothelial cell line, which showed that circ-SMARCA5 was overexpressed in tumor tissue as well as tumor cells. Subsequently, we conducted several *in vitro* experiments including CCK-8, AV/PI, wound healing and Matrigel invasion assays, and we observed that circ-SMARCA5 promoted cell proliferation but repressed cell apoptosis in UM-UC-3 cells, furthermore, circ-SMARCA5 facilitated the migration and invasion abilities of UM-UC-3 cells. The possible reasons of these results were that: (I) circ-SMARCA5 might regulate SMARCA5, which is important for gene expression, DNA replication, DNA repair and the maintenance of chromatin structure, thereby affects the biological processes such as proliferation, apoptosis, migration and invasion of bladder cancer cells (25-27); (II) circ-SMARCA5 might sponge some target miRNAs to affect the proliferation, apoptosis, migration and invasion of bladder cells, while the related data of its target miRNAs was limited. Despite further study was needed to enrich the knowledge on circ-SMARCA5 in bladder cancer cells, these results provided supports to understand the mechanisms of circ-SMARCA5 in bladder cancer and shed a light on the application of circ-SMARCA5 as a treatment target in this

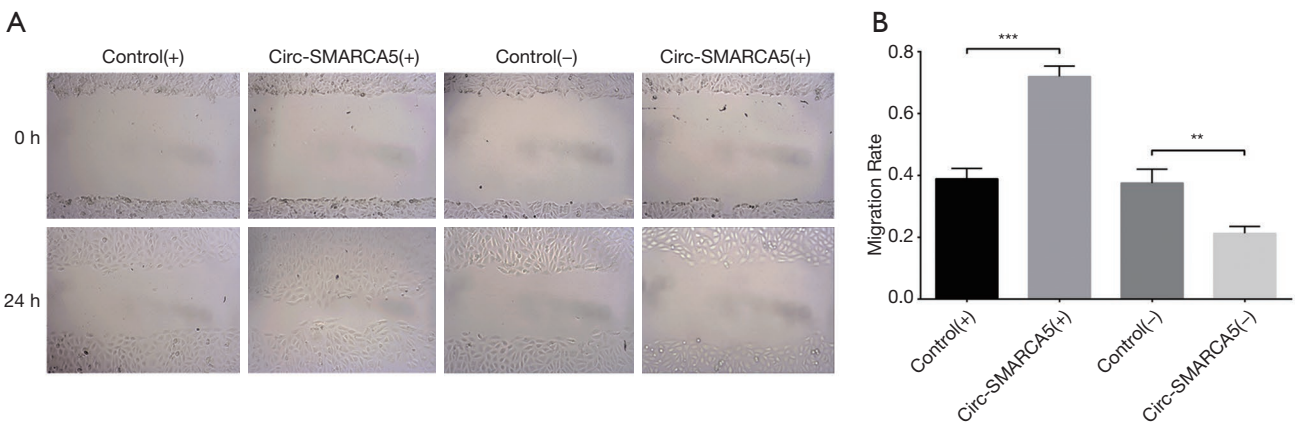

Figure 5 Wound healing assay. Wound healing assay disclosed that cell migration rate was increased in Circ-SMARCA5(+) group compared to Control(+) group but was decreased in Circ-SMARCA5(–) group compared to Control(–) group (A, B). Circ-SMARCA5, circular RNA-SMARVA5. Comparison between groups were determined by *t* test. P<0.05 was considered significant. ***, P<0.001; **, P<0.01.

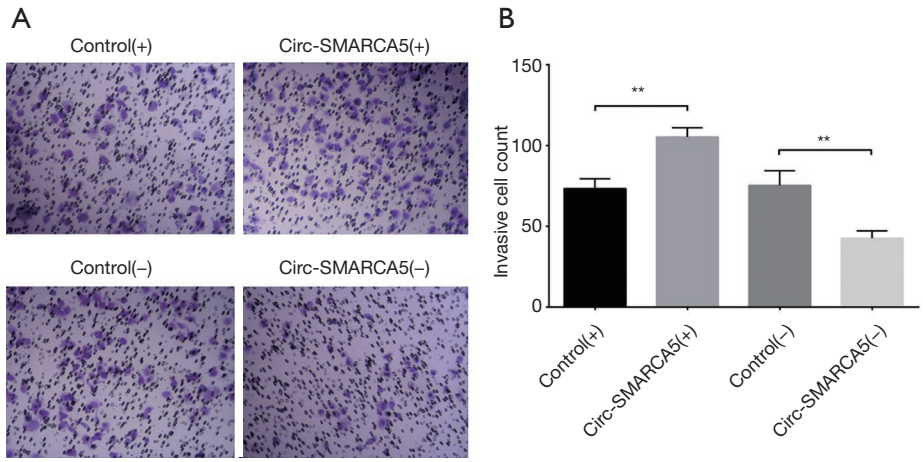

Figure 6 Matrigel invasion assay. Matrigel invasion assay (crystal violet staining, 200×) showed that invasive cell count was higher in Circ-SMARCA5(+) group compared to Control(+) group but was lower in Circ-SMARCA5(–) group compared to Control(–) group (A, B). Circ-SMARCA5, circular RNA-SMARVA5. Comparison between groups were determined by *t* test. P<0.05 was considered significant. **, P<0.01.

disease. In addition, there were some limitations existed in our study: (I) changes in cellular morphology induced by manipulating circ-SMARCA5 was not recorded, and further study investigating the effect of circ-SMARCA5 on EMT was needed, which would give important clues for the role of circ-SMARCA5 in malignant process; (II) since this study was not intended to do a detailed mechanistic study, circ-SMARCA5-mediated mechanisms of cell apoptosis, migration and invasion were not investigated. According to previous data, the possible reason for the influence of circ-SMARCA5 on cell activities might be account of that circ-SMARCA5 regulated some target genes (such as miR-103 or miR-202) to affect the cell apoptosis, migration and invasion, while further mechanistic study was needed; (III) it was better to validate the knock-down and overexpression results in another cell line, while this was a preliminary study to investigate the function of circ-SMARCA5 on cell activities, and further study validating these results with more cell lines was needed.

In conclusion, circ-SMARCA5 is overexpressed in bladder cancer tissues and cell lines, and it promotes cell proliferation, migration and invasion, but represses apoptosis in bladder cancer cells.

Acknowledgments

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Footnote

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/tcr.2019.08.08). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The Ethics Committee of our hospital approved this study before its initiation, and the number of the approval was 20170082. The study was carried out in accordance with the principles expressed in the Declaration of Helsinki. Besides, all subjects provided written informed consents before the initiation of our study.

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