European Review for Medical and Pharmacological Sciences 2018; 22: 5471-5480

UPK1B promotes the invasion and metastasis of bladder cancer via regulating the Wnt/β-catenin pathway

F.-H. WANG[1], X.-J. MA[2], D. XU[3], J. LUO[1]

[1]Department of Oncology, Fourth People's Hospital of Zibo, Zibo, China
[2]Department of Surgery, Zibo Prevention and Treatment Center for Occupational Diseases, Zibo, China
[3]Department of Pharmacy, Fourth People's Hospital of Zibo, Zibo, China

Abstract. – OBJECTIVE: The aim of this study was to investigate the expression of UPK1B in bladder cancer (BCa), and to further explore the correlation between UPK1B expression and pathological parameters as well as the prognosis of BCa.

PATIENTS AND METHODS: Quantitative real-time polymerase chain reaction (qRT-PCR) was used to detect the expression of UPK1B in 92 pairs of BCa tissues and adjacent normal tissues. The relationship between UPK1B expression and pathological features as well as the prognosis of BCa patients was further analyzed. For in vitro experiments, the mRNA expression level of UPK1B in BCa cell lines (EJ and T-24) was detected by qRT-PCR. In addition, knockdown of UPK1B in BCa cells was constructed using small interfering RNA. Effects of UPK1B knockdown on biological functions of BCa cells were analyzed by Cell Counting Kit-8 (CCK-8), colony formation assay and transwell assay, respectively. Furthermore, the underlying mechanism of UPK1B in regulating BCa was evaluated by Western blot and qRT-PCR, respectively.

RESULTS: The expression of UPK1B in BCa tissues was remarkably higher than that of adjacent normal tissues ($p<0.05$). Compared with BCa patients with lower UPK1B expression, those with higher UPK1B expression exhibited higher tumor stage, lymph node metastasis and distant metastasis. In vitro experiments indicated that cell proliferation, invasion and metastasis were remarkably decreased in cells transfected with si-UPK1B when compared with those transfected with negative controls. Western blot showed that the expression of key proteins in the Wnt/β-catenin signaling pathway in cells transfected with si-UPK1B was significantly down-regulated compared with those transfected with negative controls, including β-catenin, c-myc and cyclinD1. In addition, rescue experiments found that UPK1B was regulated by β-catenin.

CONCLUSIONS: UPK1B is upregulated in BCa, and is significantly correlated with tumor stage, lymph node metastasis, distant metastasis and poor prognosis of BCa. Moreover, UPK1B promotes the proliferation, invasion and migration of BCa via regulating the Wnt/β-catenin signaling pathway.

Key Words:
UPK1B, β-catenin, Bladder cancer, Invasion, Metastasis.

Introduction

Bladder cancer (BCa) is one of the most common malignant tumors in the urinary system, which seriously affects physical and mental health[1,2]. In the United States, BCa is the fourth most common type of cancer in men and the ninth in women. The number of new cases and deaths of BCa in 2017 was 76,960 and 31,540, respectively[1-4]. In China, the incidence of BCa has risen annually and it has also increased with age. It's reported that males are more often affected than females. Meanwhile, the incidence of BCa in an urban area is higher than that of a rural area[5]. Risk factors for BCa include environmental pollution, smoking and exposure to some chemicals[5,6]. With the rapid development of molecular biology and gene diagnosis technology, BCa is believed to be an outcome of a long-term interaction between genetic and environmental factors. Uncontrolled biological functions, including proliferation, apoptosis, differentiation and others, all contribute to the development and progression of BCa[6-8]. Currently, the treatment of BCa depends on the depth of tumor invading the bladder wall, which is expensive and insufficient. Early diagnosis and treatment are urgently needed to improve the

Corresponding Author: Xuejun Ma, BM; e-mail: saisai10@sina.com

survival rate of BCa patients[8]. Therefore, it is of great significance in clarifying the molecular mechanism of BCa, thereby providing solid basis for the prediction, diagnosis and treatment of BCa. Studies have found that Uroplakin 1B (UPK1B) is closely related to the occurrence and development of malignant tumors[9,10]. UPK1B can promote the proliferation, invasion and metastasis of tumor cells and regulate the development and progression of tumors[11,12]. It has been shown that UPK1B is differentially expressed in multiple tumors with tissue specificity, including colorectal cancer, hepatocellular carcinoma, breast cancer and non-small cell lung cancer[13]. However, the specific mechanism of UPK1B in tumors has not been fully elucidated. Generally, UPK1B participates in various biological processes, such as chromosomal recombination, gene imprinting, nuclear transport, mRNA splicing and translation[9, 10]. However, the role of UPK1B in BCa has not been explored. Therefore, we investigated the effect of UPK1B on the Wnt pathway, EGFR pathway, TGF-β inhibition, β-catenin mutation and epithelial-mesenchymal transition (EMT) in BCa[14,15].

In this work, we analyzed the expression of UPK1B in 92 pairs of BCa tissues and adjacent normal tissues, and explored its underlying mechanism. Previous researches have shown that UPK1B can inhibit the invasion and metastasis of tumor cells, thus regulating the development of tumors. Therefore, our study aimed at investigating the relationship between UPK1B expression and pathological parameters as well as the prognosis of BCa.

Patients and Methods

Patients and BCa Samples

92 pairs of surgically resected BCa tissues and adjacent normal tissues were collected. All the enrolled patients were pathologically diagnosed as BCa based on the 8th Edition of UICC/AJCC TNM Staging Criteria. BCa patients did not receive any preoperative radiotherapy, chemotherapy or other anti-cancer treatments. This study was approved by the Ethics Committee of our hospital. Informed consent was obtained from all the patients.

Cell Lines and Reagents

Four human BCa cell lines (EJ, T-24, 253j and J82) and one human normal urothelial cell line (SV-HUC-1) were purchased from ATCC (Manassas, VA, USA). Dulbecco's Modified Eagle Medium (DMEM) medium and fetal bovine serum (FBS) were purchased from the Life Technologies Corporation (Gaithersburg, MD, USA). Cells were cultured in DMEM medium supplemented with 10% fetal bovine serum and maintained in a 37°C, 5% CO_2 incubator.

Cell Transfection

Cell transfection was performed according to the manufacturer's instruction of Lipofectamine 2000. Si-NC and si-UPK1B were purchased from GenePharma (Shanghai, China). Briefly, cells were seeded into 6-well plates, and were then transfected with si-NC or si-UPK1B when cell confluence reached 70%.

Cell Counting Kit-8 (CCK-8) Assay

Transfected cells were collected and seeded into 96-well plates at a dose of 2×10^3/ml. 10 μL of CCK-8 solution (Dojindo, Kumamoto, Japan) was added into each well at 6 h, 24 h, 48 h and 72 h, respectively. The absorbance (OD) values at the wavelength of 450 nm were detected with a microplate reader.

Colony Formation Assay

Transfected cells were collected and seeded into 6-well plates at a dose of 200/mL, followed by culture in complete DMEM medium for 2 weeks. The medium was replaced 1 week later, and was then replaced twice in the second week. 2 mL 4% paraformaldehyde was applied to fix the colonies for 20 min, and 0.1% crystal violet solution was added for 20-min staining. After washing with phosphate-buffered saline (PBS) for 3 times, the colonies were pictured in a light environment.

Transwell Assay

48 h after transfection, the cells were digested with trypsin and re-suspended in serum-free medium. The cell density was diluted to 2.0×10^5/ml. Transwell chambers with or without matrigel were placed in 24-well plates, respectively. 200 μL of cell suspension were added to the upper chamber, and 500 μL of DMEM medium containing 10% FBS were added to the lower chamber. After inoculation for 48 h, cells were then fixed with 4% polyoxymethylene for 30 min at room temperature. After fixation, the colonies were stained with crystal violet for 15 min and washed with PBS twice. The inner surface of the basement membrane was carefully cleaned. The membranes were then dried, inverted, and mounted on microscope slides for analysis. Five fields were randomly selected from each well for cell counting.

Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR)

The mRNA of the cells was extracted by TRIzol reagent (Invitrogen, Carlsbad, CA, USA), and was then reversely transcribed to cDNA by using the Primescript RT Reagent. QRT-PCR was performed based on the instructions of SYBR®Premix Ex Taq™ and StepOne Plus Real-time PCR. Relative mRNA expression was calculated by the $2^{-\Delta\Delta Ct}$ method. Primers used in this study were as follows: UPK1B (F: 5'-CCAAAGACAACTCAACTGTTCGT-3', R: 5'-AATGCCGCAACAACCAATAATC-3'); β-catenin (F: 5'-GACCAGCTCTCTCTTCAGAACAGA-3', R: 5'-GTTCTCCCTGGGCACCAATA-3'); GAPDH (F: 5'-AGCCACATCGCTCAGACAC-3', R: 5'-GCCCAATACGACCAAATCC-3').

Western blot

The total proteins of transfected cells were extracted. The concentration of each protein sample was determined by the bicinchoninic acid (BCA) kit (Beyotime, Shanghai, China). Briefly, 50 μg of total protein were separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) under denaturing conditions and transferred to polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA, USA). Subsequently, the membranes were blocked with 5% skimmed milk, followed by the incubation with specific primary antibodies (β-catenin, c-myc, cyclinD1 and GAPDH) at 4°C overnight. The membranes were then incubated with secondary antibodies (anti-mouse and anti-rabbit) at room temperature for 1 h. Immuno-reactive bands were exposed by the enhanced chemiluminescence method.

Statistical Analysis

Statistical Product and Service Solutions SPSS 22.0 Software (IBM, Armonk, NY, USA) were used for all statistical analysis. Data were expressed as mean ± standard deviation (X ± SD). Comparison of continuous variables was conducted by using *t*-test. Classified variables were analyzed by using chi-square test. Kaplan-Meier curve was applied to assess the survival time and Log-rank test was used to compare the differences between the curves. $p<0.05$ was considered statistically significant.

Results

UPK1B Was Upregulated in BCa Tissues and Cell Lines

We first detected the expression of UPK1B in 92 pairs of BCa tissues and adjacent normal tissues, as well as BCa cells by qRT-PCR. The expression of UPK1B in BCa tissues was significantly higher than that of adjacent normal tissues (Figure 1A and 1B). Meanwhile, UPK1B was overexpressed in BCa cells compared with that of human normal urothelial cells (SV-HUC-1), and the difference was statistically significant ($p<0.05$, Figure 1C). Particularly, EJ and T-24 cells had the highest UPK1B expression, which were then selected for the following experiments.

UPK1B expression was correlated with tumor stage, lymph node metastasis, distant metastasis and overall survival of BCa patients

All the enrolled BCa patients were divided into high expression group and low expression group based on UPK1B expression in BCa tissues and adjacent normal tissues. The number of cases in each group was counted. The relationship between UPK1B expression and age, gender, tumor stage, lymph node metastasis and distant metastasis in BCa patients was analyzed by chi-square test. Our findings demonstrated that UPK1B expression was positively correlated with tumor stage, lymph node metastasis and distant metastasis in BCa. However, there was no correlation between UPK1B expression and age, gender as well as tumor location (Table I). In addition, follow-up data of 92 BCa patients were collected for exploring the relationship between UPK1B expression and the prognosis of BCa. The results of the Kaplan-Meier survival curves showed that higher UPK1B expression was significantly associated with worse prognosis of BCa ($p<0.05$, Figure 1D). These data all suggested that UPK1B might serve as a new biomarker for predicting the prognosis of BCa.

Knockdown of UPK1B inhibited Cell Proliferation

To explore the effect of UPK1B on the proliferation of BCa cells, we successfully constructed a small interfering sequence of UPK1B and related negative control (si-UPK1B and si-NC) (Figure 2A and 2B). The proliferation of EJ and T-24 cells transfected with si-UPK1B and si-NC was detected by CCK-8 assay, respectively (Figure 2C and 2D). The results illustrated that the proliferation rate of BCa cells transfected with si-UPK1B was remarkably decreased when compared with those transfected with si-NC. Meanwhile, colony formation assay showed similar results (Figure 2E and 2F).

Table I. Association of UPK1B expression with clinicopathologic characteristics of bladder cancer.

Parameters	Number of cases	UPK1B expression Low (%)	High (%) *p*-value
Age (years)	0.343		
<60	40	24	16
≥60	52	28	24
Gender	0.146		
Male	45	29	16
Female	47	23	24
T stage	0.008		
T1-T2	51	34	17
T3-T4	41	18	23
Lymph node metastasis	0.033		
No	53	34	19
Yes	39	18	21
Distance metastasis	0.029		
No	68	42	26
Yes	24	10	14

Knockdown of UPK1B Inhibited Cell Migration and Invasion

The effect of UPK1B on the migration and invasion of BCa cells was detected by transwell assay. The number of migrated EJ cells transfected with si-UPK1B was remarkably lower than that of the cells transfected with si-NC (Figure 3A and 3B). We obtained the same migration and invasion results in T-24 cells (Figure 3C and 3D), indicating that the migration ca-

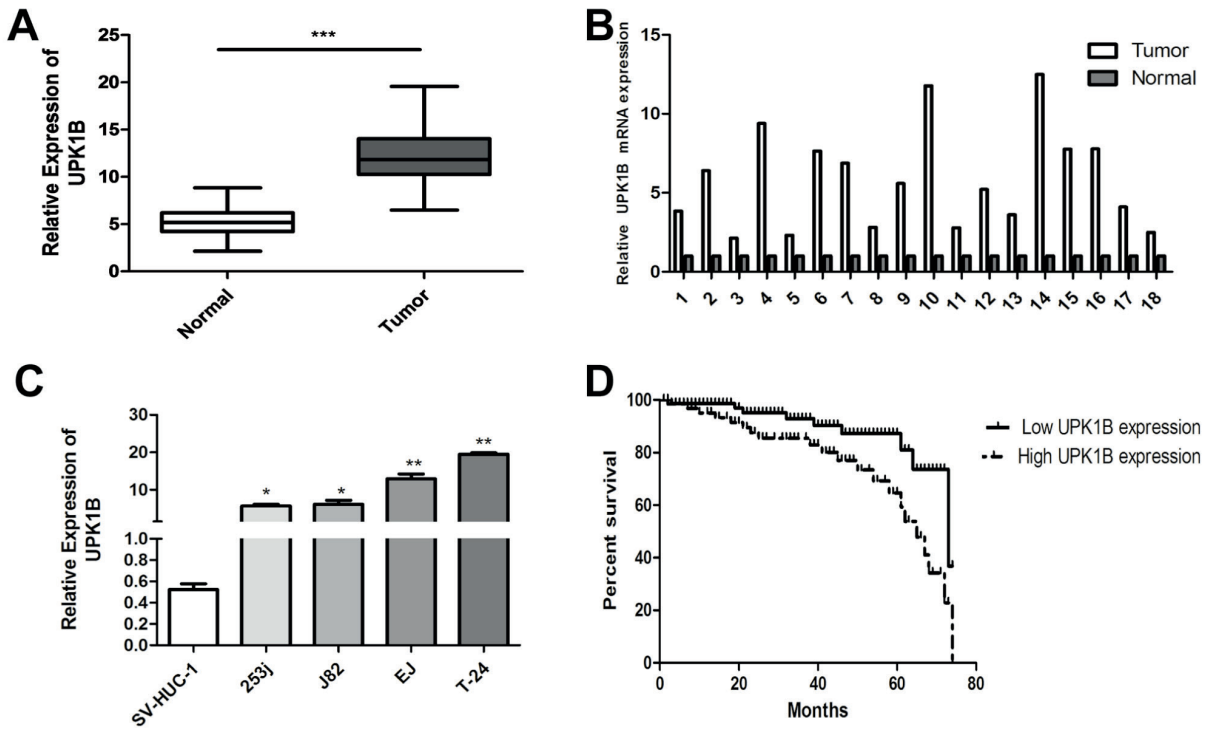

Figure 1. UPK1B was upregulated in BCa tissues and cell lines. ***A-B,*** The expression of UPK1B in 92 pairs of BCa tissues was significantly higher than that of adjacent normal tissues. ***C,*** Expression level of UPK1B in 4 BCa cell lines (EJ, T-24, 253j and J82) and 1 normal bladder cell line (SV-HUC-1). ***D,*** Kaplan-Meier survival curves of BCa patients based on UPK1B expression. Patients in the high expression group had a worse prognosis than those in low expression group. A representative data set was displayed as mean ± SD values (**p*<0.05, **p<0.01).

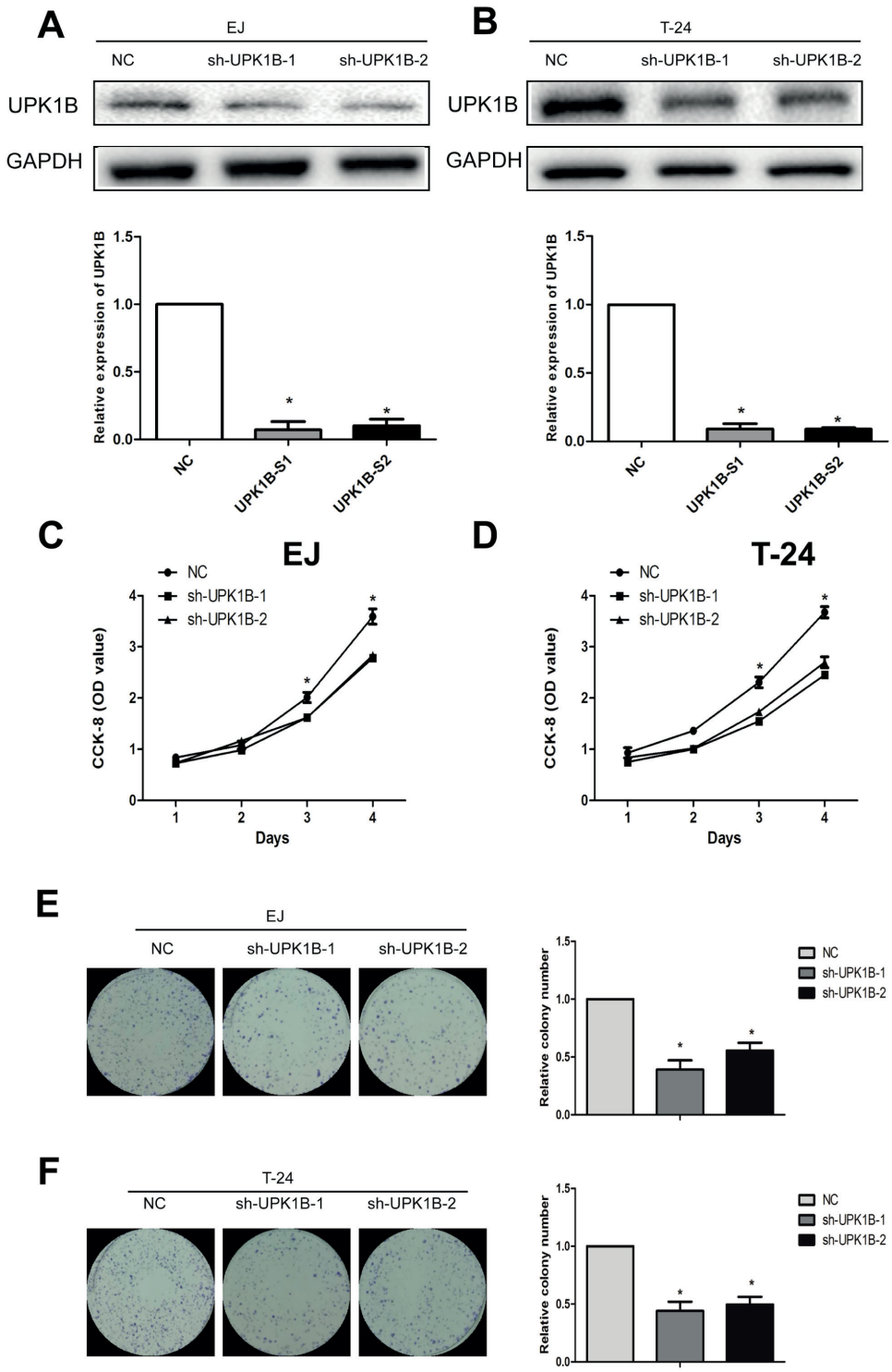

Figure 2. Knockdown of UPK1B inhibited cell proliferation. ***A-B,*** Western blot and qRT-PCR were used to verify the efficiency of UPK1B knockdown in EJ and T-24 cell lines. ***C-D,*** Growth curve analysis showed the cell growth of EJ and T-24 cells with UPK1B knockdown. ***E-F,*** The efficiency of colony formation in EJ and T-24 cells with UPK1B knockdown. A representative data set was displayed as mean ± SD values (*p<0.05).

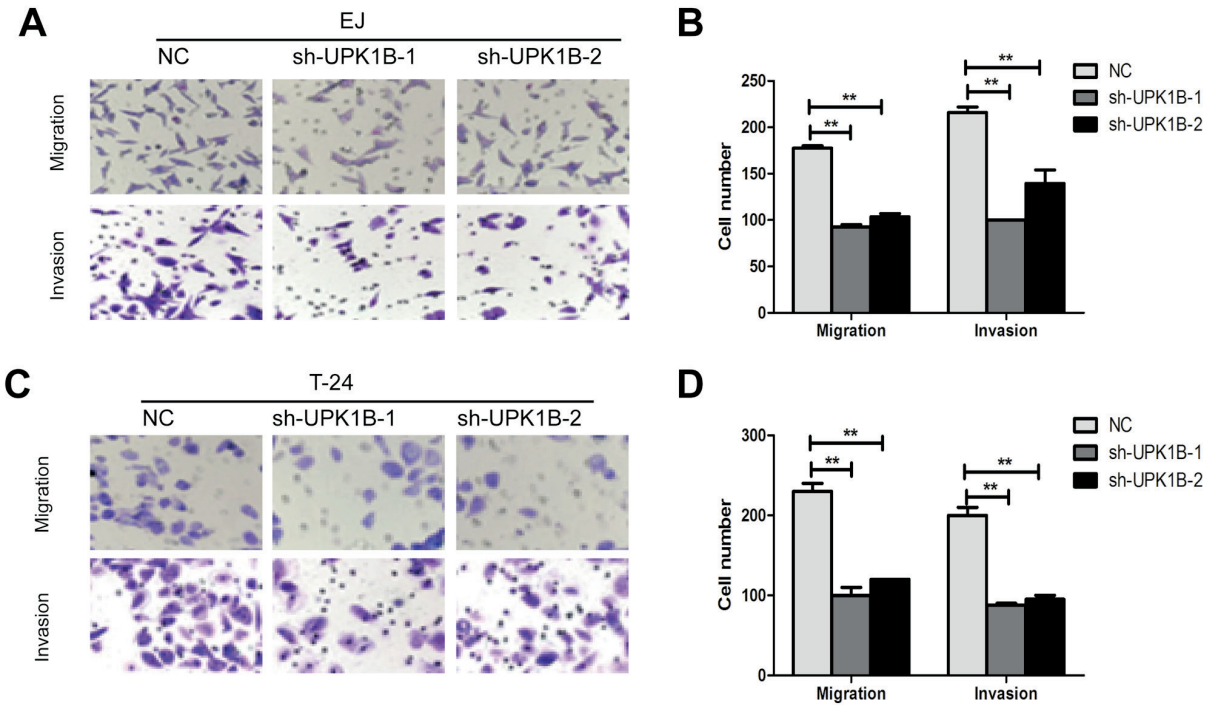

Figure 3. Knockdown of UPK1B inhibited cell migration and invasion. ***A-B,*** EJ cells transfected with si-UPK1B displayed significantly lower migration and invasion capacity. ***C-D,*** T-24 cell transfected with si-UPK1B displayed significantly lower migration and invasion capacity. A representative data set was displayed as mean ± SD values ($^{**}p<0.05$).

pacity of BCa cells was inhibited after UPK1B knockdown (Figure 3).

Knockdown of UPK1B Changed the Expression of the β-catenin Signaling Pathway

To analyze the underlying mechanism of UPK1B in promoting the proliferation, invasion and migration of BCa cells, we detected the protein expression of key genes in the β-catenin pathway after UPK1B knockdown by Western blot. The results demonstrated that UPK1B knockdown significantly decreased the protein expression of β-catenin, c-myc and cyclinD1 (Figure 4A). To further clarify the relationship between β-catenin and UPK1B, we then detected β-catenin expression in 92 pairs of BCa tissues and adjacent normal tissues, as well as BCa cells by qRT-PCR and Western blot, respectively. These data indicated that β-catenin expression was significantly lower in BCa tissues than that of adjacent normal tissues ($p<0.05$, Figure 4B). Moreover, lower β-catenin expression was found in BCa cells when compared with SV-HUC-1 cells ($p<0.05$, Figure 4C).

β-catenin Modulated UPK1B Expression in Human Bladder Cancer Cells

We further explored the interaction between UPK1B and β-catenin in BCa cells, and found that UPK1B was upregulated after the β-catenin knockdown in BCa cells. Therefore, we suggested that β-catenin could regulate UPK1B in BCa cells. Lower β-catenin expression was found in BCa cells when compared with SV-HUC-1 cells. Here, we constructed a small interfering sequence of β-catenin. The transfection efficacy of si-β-catenin was verified by qRT-PCR and Western blot respectively (Figure 5A and 5B). The proliferation, invasion and migration of EJ and T-24 cells co-transfected with si-UPK1B and si-β-catenin were significantly increased than those only transfected with si-UPK1B, indicating that UPK1B was regulated by β-catenin (Figure 5C and 5D).

Discussion

Globally, BCa is one of the most common malignant tumors in the urinary system. The incidence of BCa is the consequence of intracellu-

lar multi-molecular dysregulation[6]. At present, molecular diagnosis and prognostic markers for BCa have been well recognized[7,8]. UPK1B exerts a crucial role in the development and progression of BCa, which may serve as a target for clinical diagnosis. We believe that in-depth study of UPK1B associated with BCa can provide the basis for the prediction, diagnosis and treatment of BCa. In recent years, the incidence and mortality rate of BCa in our country have gradually risen. However, the early diagnosis rate of BCa in China is very low. Most of BCa patients have already developed to an advanced stage when first diagnosed[4,7]. It has been found that genetics, diet, unhealthy lifestyle and precancerous lesions are closely related to BCa development. Over 50% of BCa patients have experienced micro-metastasis before radical surgery, which is the direct cause of postoperative metastasis and recurrence of BCa[7,8]. Recent studies have found that UPK1B participates in multiple diseases, including malignant tumors[9,10,12]. Therefore, we investigated the effect of UPK1B on BCa patients in this study, thus aiming at improving the diagnosis and treatment of BCa. In the present study, we first detected UPK1B expression in 92 pairs of BCa tissues and adjacent normal tissues. The results showed that UPK1B expression was significantly upregulated in BCa tissues, which was positively correlated with tumor stage, lymph node metastasis, distant metastasis and poor prognosis of BCa. Therefore, we speculated that UPK1B might promote the occurrence and development of BCa. *In vitro* experiments demonstrated that UPK1B knockdown could inhibit the invasion and migration of BCa cells, indicating that UPK1B could promote the

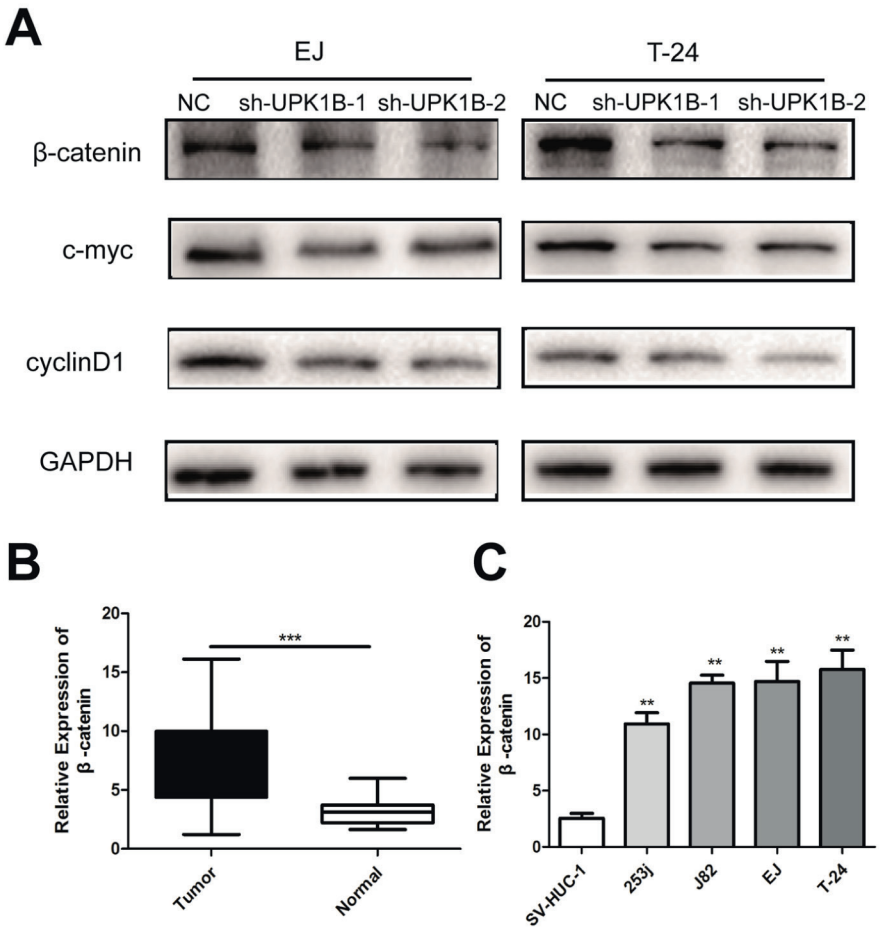

Figure 4. Knockdown of UPK1B changed the expression of the β-catenin signaling pathway. ***A,*** Knockdown of UPK1B significantly decreased the expression of molecules in the β-catenin signal pathway, including β-catenin, c-myc and cyclinD1. ***B, C,*** The mRNA expression level of β-catenin relative to GAPDH in human BCa tissues and corresponding adjacent tissues, as well as cell lines were detected by using qRT-PCR. A representative data set was displayed as mean ± SD values ($^{**}p<0.05$, $^{***}p<0.01$).

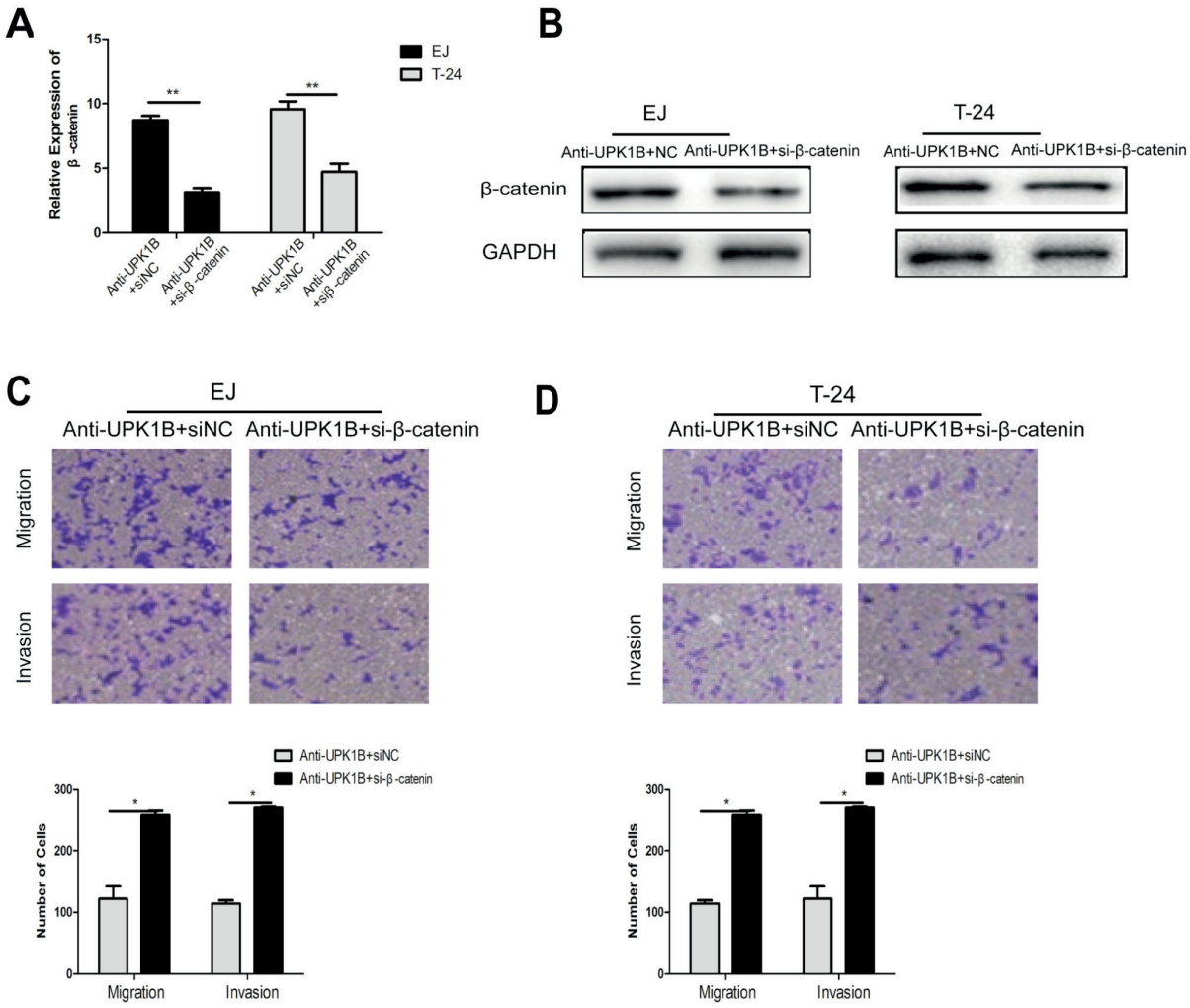

Figure 5. β-catenin modulated *UPK1B* expression in human bladder cancer cells. ***A,*** The expression of β-catenin in co-transfected cell lines was verified by qRT-PCR. ***B,*** Western blot was used to verify the protein expression of β-catenin in co-transfected cell lines. ***C-D,*** The role of *UPK1B* and β-catenin in the regulation of BCa cell migration and invasion were examined by transwell assay. A representative data set was displayed as mean ± SD values (**p*<0.05).

development and progression of BCa. However, the specific molecular mechanism of UPK1B was not fully elucidated.

The Wnt/β-catenin signaling pathway is an important pathway in tumorigenesis[15,16]. Wnt protein regulated by β-catenin binds to Frizzled and LRP family receptors, thus transmitting the signal to Dishevelled (Dsh). Activated Dsh, in turn, may inhibit the activity of a complex that is composed of Axin, APC and GSK-3β. Consequently, cytoplasmic β-catenin is accumulated and bound with TCF/LEF, thereby regulating the expression of target genes[17,18]. In the absence of the Wnt signaling pathway, cytoplasmic β-catenin is phosphorylated by GSK-3β and subsequently ubiquitinated, which is eventually degraded by proteasomes. Studies have confirmed that Wnt antagonists and dominant negative mutants of TCF are capable of blocking the Wnt signaling pathway[19,20]. It is reported that activated Wnt/β-catenin pathway is involved in various diseases[18-20]. For example, embryo line mutation of APC gene regulated by the Wnt signaling pathway can lead to familial adenomatous carcinomas[21]. The Notch signaling pathway is believed to be related with Wnt protein[22]. In addition, the Wnt/β-catenin signaling pathway is activated in most gastric cancers and promotes the proliferation of gastric cancer[23]. Wnt-1 overexpression is associated with the proliferation, progression and poor prognosis of non-small cell

lung cancer[24]. Besides, activated Wnt/β-catenin signaling pathway is observed in over 90% of patients with colorectal cancer[25]. When the Wnt signaling pathway is activated, aggregated nuclear β-catenin leads to the loss of epithelial structure, which is significantly associated with tumor invasion and metastasis[26]. Advanced researches on the Wnt signaling pathway are beneficial for the diagnosis and treatment of various tumors. Hence, our study showed that UPK1B knockdown remarkably decreased the expression of the key genes in the Wnt/β-catenin signaling pathway, including β-catenin, c-myc and cyclinD1 in BCa cells, indicating that UPK1B could regulate BCa via the Wnt/β-catenin signaling pathway.

Conclusions

We showed that UPK1B was upregulated in BCa and significantly correlated with tumor stage, lymph node metastasis, distant metastasis and poor prognosis of BCa. Moreover, UPK1B promoted the proliferation, invasion and migration of BCa via regulating the Wnt/β-catenin signaling pathway.

Conflict of Interest

The Authors declare that they have no conflict of interest.

References

1) Wu J, Wang J. HMGN5 expression in bladder cancer tissue and its role on prognosis. Eur Rev Med Pharmacol Sci 2018; 22: 970-975.
2) Wang M, Guo C, Wang L, Luo G, Huang C, Li Y, Liu D, Zeng F, Jiang G, Xiao X. Long noncoding RNA GAS5 promotes bladder cancer cells apoptosis through inhibiting EZH2 transcription. Cell Death Dis 2018; 9: 238.
3) Siegel RL, Miller KD, Jemal A. Cancer Statistics, 2017. CA Cancer J Clin 2017; 67: 7-30.
4) Siegel RL, Miller KD, Jemal A. Cancer statistics, 2018. CA Cancer J Clin 2018; 68: 7-30.
5) Singla N, Fang D, Su X, Bao Z, Cao Z, Jafri SM, Xiong G, Zhang L, Hutchinson R, Sagalowsky A, Lotan Y, Li X, Zhou L, Raman JD, Margulis V. A multi-institutional comparison of clinicopathological characteristics and oncologic outcomes of upper tract urothelial carcinoma in China and the United States. J Urol 2017; 197: 1208-1213.
6) Radosavljevic V, Belojevic G. Shortcomings in bladder cancer etiology research and a model for its prevention. Tumori 2014; 100: 1-8.
7) [No authors listed]. Bladder cancer: diagnosis and management of bladder cancer: © NICE (2015) Bladder cancer: diagnosis and management of bladder cancer. BJU Int 2017; 120: 755-765.
8) DeGeorge KC, Holt HR, Hodges SC. Bladder cancer: diagnosis and treatment. Am Fam Physician 2017; 96: 507-514.
9) Webb GC, Finch JL, Cowled PA. Assignment of the Uroplakin 1b (Upk1b) gene to mouse chromosome 16 bands B5-C2 by in situ hybridization. Cytogenet Cell Genet 1999; 84: 37-38.
10) Finch JL, Webb GC, Evdokiou A, Cowled PA. Chromosomal localization of the human urothelial "tetraspan" gene, UPK1B, to 3q13.3-q21 and detection of a TaqI polymorphism. Genomics 1997; 40: 501-503.
11) Osman I, Kang M, Lee A, Deng FM, Polsky D, Mikhail M, Chang C, David DA, Mitra N, Wu XR, Sun TT, Bajorin DF. Detection of circulating cancer cells expressing uroplakins and epidermal growth factor receptor in bladder cancer patients. Int J Cancer 2004; 111: 934-939.
12) Olsburgh J, Harnden P, Weeks R, Smith B, Joyce A, Hall G, Poulsom R, Selby P, Southgate J. Uroplakin gene expression in normal human tissues and locally advanced bladder cancer. J Pathol 2003; 199: 41-49.
13) Su J, Zhang Y, Su H, Zhang C, Li W. A recurrence model for laryngeal cancer based on SVM and gene function clustering. Acta Otolaryngol 2017; 137: 557-562.
14) Wu C, Zhu X, Liu W, Ruan T, Tao K. Hedgehog signaling pathway in colorectal cancer: Function, mechanism, and therapy. Onco Targets Ther 2017; 10: 3249-3259.
15) Liang J, Tang J, Shi H, Li H, Zhen T, Duan J, Kang L, Zhang F, Dong Y, Han A. MiR-27a-3p targeting RXRα promotes colorectal cancer progression by activating Wnt/ β-catenin pathway. Oncotarget 2017; 8: 82991-83008.
16) Colini Baldeschi A, Pittaluga E, Andreola F, Rossi S, Cozzolino M, Nicotera G, Sferrazza G, Pierimarchi P, Serafino A. Atrial natriuretic peptide acts as a neuroprotective agent in in vitro models of Parkinson's disease via up-regulation of the Wnt/β-catenin pathway. Front Aging Neurosci 2018; 10: 20.
17) Maretto S, Cordenonsi M, Dupont S, Braghetta P, Broccoli V, Hassan AB, Volpin D, Bressan GM, Piccolo S. Mapping Wnt/beta-catenin signaling during mouse development and in colorectal tumors. Proc Natl Acad Sci USA 2003; 100: 3299-3304.
18) Yang Z, Balic A, Michon F, Juuri E, Thesleff I. Mesenchymal Wnt/β-catenin signaling controls epithelial stem cell homeostasis in teeth by inhibiting the antiapoptotic effect of fgf10. Stem Cells 2015; 33: 1670-1681.
19) Burke ZD, Reed KR, Yeh SW, Meniel V, Sansom OJ, Clarke AR, Tosh D. Spatiotemporal regulation of liver development by the Wnt/β-catenin pathway. Sci Rep 2018; 8: 2735.

20) Kim MS, Cho HI, Yoon HJ, Ahn YH, Park EJ, Jin YH, Jang YK. JIB-04, a small molecule histone demethylase inhibitor, selectively targets colorectal cancer stem cells by inhibiting the Wnt/β-catenin signaling pathway. Sci Rep 2018; 8: 6611.

21) Dubbink HJ, Hollink IHIM, Avenca VC, Wang W, Liu P, Doukas M, van Noesel MM, Dinjens WNM, Wagner A, Smits R. A novel tissue-based β-catenin gene and immunohistochemical analysis to exclude familial adenomatous polyposis among children with hepatoblastoma tumors. Pediatr Blood Cancer 2018; 65: e26991.

22) Kim W, Khan SK, Yang Y. Interacting network of Hippo, Wnt/β-catenin and Notch signaling represses liver tumor formation. BMB Rep 2017; 50: 1-2.

23) Song B, Lin HX, Dong LL, Ma JJ, Jiang ZG. MicroRNA-338 inhibits proliferation, migration, and invasion of gastric cancer cells by the Wnt/β-catenin signaling pathway. Eur Rev Med Pharmacol Sci 2018; 22: 1290-1296.

24) Ma R, Feng N, Yu X, Lin H, Zhang X, Shi O, Zhang H, Zhang S, Li L, Zheng M, Gao M, Yu H, Qian B. Promoter methylation of Wnt/β-catenin signal inhibitor TMEM88 is associated with unfavorable prognosis of non-small cell lung cancer. Cancer Biol Med 2017; 14: 377-386.

25) Qi H, Wang J, Wang F, Ma H. Long non-coding RNA SNHG1 promotes cell proliferation and tumorigenesis in colorectal cancer via Wnt/β-catenin signaling. Pharmazie 2017; 72: 395-401.

26) Yang G, Shen T, Yi X, Zhang Z, Tang C, Wang L, Zhou Y, Zhou W. Crosstalk between long non-coding RNAs and Wnt/β-catenin signaling in cancer. J Cell Mol Med 2018; 22: 2062-2070.