

Original Article

Over-expression of long noncoding RNA LOWEG inhibits cell migration in human bladder cancer

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Received March 2, 2017; Accepted May 3, 2017; Epub June 1, 2017; Published June 15, 2017

Abstract: Accumulating evidences demonstrated that lncRNAs function as potential oncogenes or tumor suppressor genes to play vital regulatory roles in tumorigenesis and tumor progression. lncRNA-LOWEG, a 331-bp lncRNA encoded on human chromosome 5, acts as a tumor suppressor gene which inhibits tumor cells invasion by up-regulating the LIFR expression at the translational level in gastric cancer. However, the significance of lncRNA-LOWEG in bladder cancer is largely unknown. The relative expression levels of lncRNA-LOWEG in BC and BC cell lines were determined by quantitative RT-PCR (qRT-PCR). The relationships between lncRNA-LOWEG expression levels and the clinical characteristics were assessed. lncRNA-LOWEG expression was increased by transfecting a pcDNA-LOWEG vector. We used both wound healing assay and transwell assay to detect cell migration. The relative expression levels of lncRNA-LOWEG was remarkably down-regulated in bladder cancer tissues compared with adjacent noncancerous tissues ($P < 0.05$). lncRNA-LOWEG expression was also significantly down-regulated in four bladder cancer cell lines ($P < 0.01$). Compared with the negative control group, pcDNA-LOWEG mediated over-expression of lncRNA-LOWEG significantly inhibited cell migration capability of bladder cancer T24 and 5637 cells, suggesting that up-regulated lncRNA-LOWEG expression could suppress the metastasis capability of bladder cancer. These findings reveal lncRNA-LOWEG acts as a tumor suppressor in bladder cancer, contributing to tumor migration, and may function as a novel candidate biomarker and a potential therapeutic target for patients with BC.

Keywords: LOWEG, bladder cancer, lncRNAs

Introduction

Human bladder cancer, as one of the most common genitourinary tumors, has captured our attention in recent decades because of its incidence and mortality [1, 2]. Despite traditional therapies for patients with bladder cancer are mainly surgery, chemotherapy and radiation therapy, the specificity, efficiency and 5-year survival rate remain frustrated [2, 3]. Therefore, in order to developing an effective diagnosis and targeted therapy, there is indispensable to reveal the underlying pathogenetic mechanism of bladder cancer as well as find new candidate specific biomarkers and potential therapeutic targets which can be applied to precision medicine of BC.

With the fast development of sequencing technology, a tremendous amount of long noncod-

ing RNAs (lncRNAs) have been identified and involved in a great deal of human diseases, particularly in cancers [4, 5]. Lots of studies demonstrated that lncRNAs function as vital regulatory roles in a wide range of biological processes, such as transcriptional and posttranscriptional regulation, carcinogenesis and activity regulation of protein [6, 7]. lncRNA-LOWEG, also known as lncRNA-CTD-210809.1 (ENST-00000508986), a newly identified lncRNA, has been found to be down-regulated in gastric cancer [8]. However, the significance of lncRNA-LOWEG in bladder cancer is largely unknown.

Therefore, in this research, we explored the clinical significance of lncRNA-LOWEG in bladder cancer tissues and investigated the associations between its expression and clinicopathologic characteristics of patients with bladder cancer. Moreover, we also investigated

the effects of lncRNA-LOWEG expression on bladder cancer cells *in vitro*. Further experiments demonstrated that the overexpression of lncRNA-LOWEG could inhibit migration of the bladder cancer cell lines.

Materials and methods

Patient samples

In the present research, 55 patients with urothelial neoplasms of the bladder who received radical cystectomy were included. The bladder cancer tissues and their adjacent normal tissues were snap-frozen in liquid nitrogen rapidly after resection. The study was approved by the institutional research ethics committee of Shenzhen Second People's Hospital. Moreover, written consent was received from each patient.

Cell lines and cell culture

Human bladder cancer T24, 5637, SW780 and UM-UC-3 cells and SV-40-immortalized human uroepithelial cell line (SV-HUC-1) were obtained from the Institute of Cell Biology, Chinese Academy of Sciences (Shanghai, China). The T24, SW780 and UM-UC-3 cells were incubated in Dulbecco's Modified Eagle Medium (Invitrogen, Carlsbad, CA, USA) plus 1% antibiotics (100 U/ml penicillin and 100 µg/ml streptomycin sulfates) and 10% fetal bovine serum. The 5637 cells were grown in RPMI-1640 Medium (Invitrogen, Carlsbad, CA, USA) plus 10% fetal bovine serum (FBS). The SV-HUC-1 cells were cultured in F12K (Invitrogen, Carlsbad, CA, USA) plus 1% antibiotics (100 U/ml penicillin and 100 µg/ml streptomycin sulfates) and 10% fetal bovine serum (FBS). All cells were cultured at 37°C, in a 5% CO₂ atmosphere.

Plasmid DNA transfection

The lncRNA-LOWEG sequence and negative control were synthesized and subcloned into pCDNA3.1 (GenePharma, Suzhou, China) vector. The pCDNA-LOWEG or negative control was transfected into T24 and 5637 cells cultured in six-well plates by utilizing Lipofectamine 3000 Transfection Reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. The expression level of lncRNA-LOWEG was determined by qRT-PCR.

RNA extraction and qRT-PCR

Total RNA was isolated from bladder cancer tissues and their adjacent normal tissues or cells after transfection using the Trizol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. cDNAs were synthesized by utilizing Prime Script RT Reagent Kit with gDNA Eraser (Takara, Dalian, China). The quantitative real-time polymerase chain reaction (qRT-PCR) was executed by using SYBR Green PCR kit (Takara, Dalian, China) according to the manufacturer's instructions. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was measured as an internal control. The primer sequences were as follows: lncRNA-LOWEG primers: 5'-CCCAGAAGTTTTTCAGCCCTCA-3' (forward) and 5'-GACACTTTCCTTCATGGAATTTG-3' (reverse), and GAPDH primers: 5'-CGCTCTGCTCCTCCTGTTC-3' (forward), 5'-ATCCGTTGACTCCGACCTTCAC-3' (reverse). The reactions were executed using an ABI PRISM 7300 Fluorescent Quantitative PCR System (Applied Biosystems, Foster City, CA, USA) in triplicate. The average value in each triplicate was used to calculate the relative amount of lncRNA-LOWEG by using 2^{-ΔΔCt} methods.

Wound healing assay

The cell motility assay was detected by wound healing assay. At 24 h post transfection, a wound field was created using a sterile 200 µl pipette tip in about 90% confluent cells. The cells were incubated for 20 h at 37°C, and then the migration of cells was monitored with a digital camera system. The software program HMIAS-2000 was applied to calculate the cell migration distance (µm).

Transwell assay

The cell motility assay were also performed using a transwell insert (8 µm, Corning). 24 h after transfection, 1×10⁵ cells were first starved in 200 µl serum-free medium and then put in the uncoated dishes. The lower chambers were filled with 700 µl medium containing 10% FBS. The cells were cultured for 48 h at 37°C in a 5% CO₂ atmosphere and then the cells that had migrated to the bottom surface of the filter membrane were stained with 0.5% crystal violet solution and photographed in five preset fields per insert.

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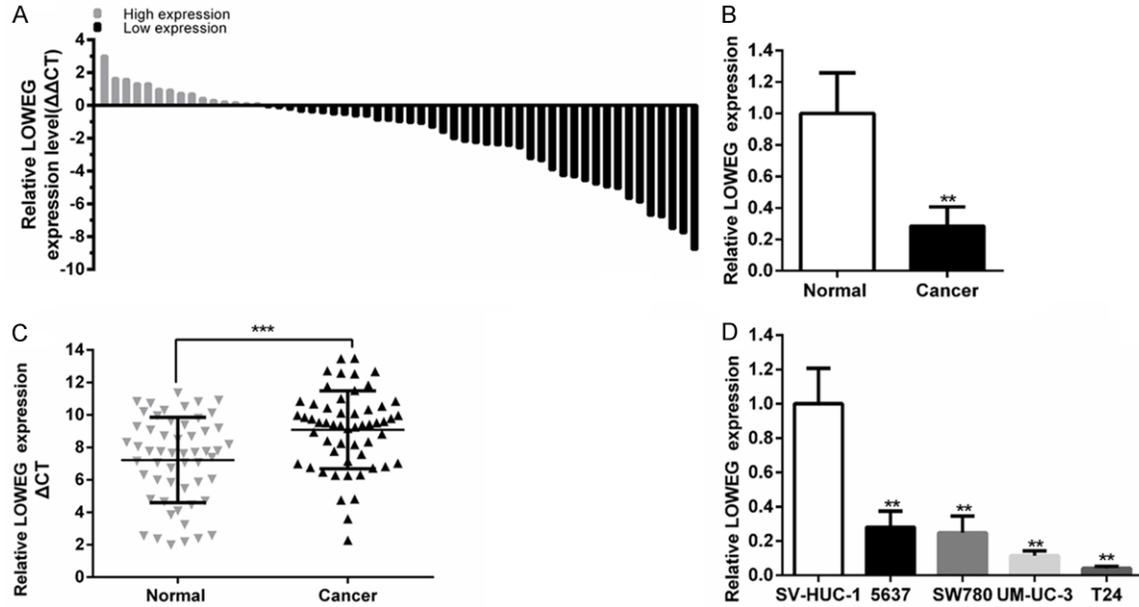


Figure 1. The lncRNA-LOWEG was down-regulated in bladder cancer. The relative expression levels of lncRNA-LOWEG were detected by using qRT-PCR. (A) lncRNA-LOWEG expression levels were lower in bladder cancer tissues. (B and C) The relative expression level of lncRNA-LOWEG was significantly lower in bladder cancer tissues compared with pair-matched adjacent normal bladder tissues. (D) The relative expression levels of lncRNA-LOWEG were lower in four bladder cancer cell lines (T24, 5637, SW780 and UM-UC-3) than those in SV-40-immortalized human uroepithelial cells (SV-HUC-1). Data (B) are shown as mean \pm SEM. Data (A, C and D) are shown as mean \pm SD. (** $P < 0.01$, *** $P < 0.001$).

Statistical analyses

All experimental data were analyzed by using SPSS 20.0 software (IBM, Chicago, IL, USA). Paired samples' t test was used to analyze the lncRNA-LOWEG expression difference between bladder cancer tissues and para-cancer tissues and independent samples' t test was used to analyze other data. The chi-square test was used to examine the relationship between lncRNA-LOWEG expression level and clinicopathologic characteristics. $P < 0.05$ were considered statistically significant.

Results

LOWEG was down-regulated in bladder cancer tissues and cells

The relative expression level of lncRNA-LOWEG was detected by using quantitative real-time PCR in a total of 55 patients with urothelial bladder cancer and different cell lines. The lncRNA-LOWEG expression was down-regulated in 72.7% (40 of 55) of cancer tissues compared to pair-matched adjacent normal tissues (Figure 1A-C). Compared to normal urothelial

cell line SV-HUC-1, the expression of lncRNA-LOWEG was significantly decreased in four bladder cancer cell lines (T24, 5637, SW780, UM-UC-1) (Figure 1D).

Correlations between LOWEG and clinicopathologic parameters of bladder cancer

Next, we analyzed the relationships between the relative expression level of lncRNA-LOWEG and clinicopathologic characteristics of patients with bladder cancer. As shown in Table 1, down-regulated lncRNA-LOWEG expression was positively correlated with male patients with urothelial bladder cancer. However, the expression level of lncRNA-LOWEG was not correlated with other clinicopathologic characteristics, including age, tumor size, histological grade, TNM stage and lymph nodes metastasis (Table 1).

Specific plasmid vectors up-regulated the expression of LOWEG

Two bladder cancer cell lines (T24, 5637) were cultivated and transfected with pCDNA-LOWEG or negative control. The related expression

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Table 1. Correlation between LOWEG expression and clinicopathological characteristics of bladder cancer patients

Characteristics	Group	Total	LOWEG expression		P value
			High	Low	
Gender	Male	38 (69%)	14 (25%)	24 (44%)	0.04*
	Female	17 (31%)	1 (2%)	16 (29.1%)	
Age (years)	<65	29 (52.7%)	7 (12.7%)	22 (40%)	0.58
	≥65	26 (47.3%)	8 (14.5%)	18 (32.7%)	
Tumor size (cm)	<3 cm	16 (29.1%)	5 (9.1%)	11 (20%)	0.97
	≥3 cm	39 (70.9%)	12 (21.8%)	27 (40.1%)	
Histological grade	L	19 (34.5%)	6 (10.9%)	13 (23.6%)	0.60
	H	36 (65.5%)	9 (16.4%)	27 (49.1%)	
TNM stage	0/I	18 (32.7%)	7 (12.7%)	11 (20%)	0.18
	II/III/IV	37 (67.3%)	8 (14.5%)	29 (52.7%)	
Lymph nodes metastasis	Negative	51 (92.7%)	13 (23.6%)	38 (69.1%)	0.63
	Positive	4 (7.3%)	2 (3.6%)	2 (3.6%)	
Distant metastasis	Negative	55 (100%)	15 (27.3%)	40 (72.7%)	
	Positive	0	0	0	

*P<0.05 was considered significant (Chi-square test between 2 groups).

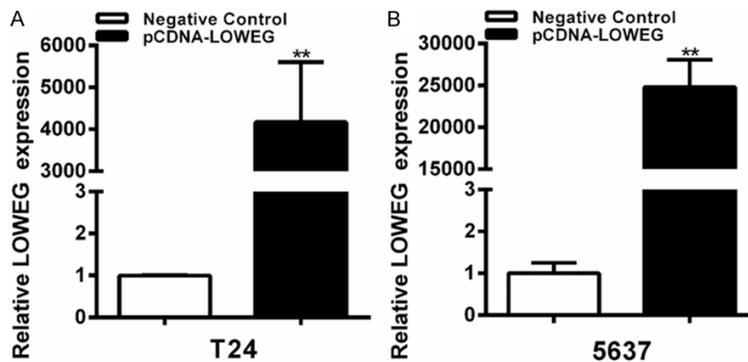


Figure 2. The pCDNA-LOWEG significantly overexpressed the expression level of lncRNA-LOWEG in T24 (A) and 5637 (B) cells. Data are shown as mean \pm SD. (**P<0.01).

level of lncRNA-LOWEG was tested by qRT-PCR at 48 hours after transfection. As shown in **Figure 2**, the relative expression level of lncRNA-LOWEG in bladder cancer cell line T24 (**Figure 2A**) and 5637 (**Figure 2B**) were significantly over-expressed by pCDNA-LOWEG.

Overexpression of LOWEG inhibited cell migration

In order to explore the possible impact of lncRNA-LOWEG on the migration of human bladder cancer cells. Bladder cancer cells lines T24 and 5637 were transfected with pCDNA-LOWEG or negative control and cell migration

ability of bladder cells were detected by both wound healing assay and transwell assay. Compared to the negative control group, cell migration ability was significantly inhibited by pCDNA-LOWEG in T24 cells (**Figures 3A, 3B** and **4A, 4B**) and 5637 cells (**Figures 3C, 3D** and **4C, 4D**). These results indicated that lncRNA-LOWEG inhibits the bladder cancer cell migration.

Discussion

Accumulating evidences demonstrated that lncRNAs function as potential oncogenes or tumor suppressor genes to play vital regulatory roles in tumorigenesis and tumor progression [5, 9]. Till now, some lncRNAs have been identified to involve in bladder cancer, contributing to tumor proliferation, apoptosis, and migration, such as BANCR [10], HOTAIR [11]. lncRNA-LOWEG, a 331-bp lncRNA encoded on human chromosome 5, has firstly been identified in gastric cancer [8]. lncRNA-LOWEG acts as a tumor suppressor gene which inhibits tumor cells invasion by up-regulating the LIFR expression at the translational level in gastric cancer [8]. LIFR gene, located on the same strand within 50 kbp downstream of lncRNA-LOWEG, is an integral component of the glycoprotein 130-LIFR signaling complex [12]. LIFR has been found to be down-regulated in several cancers, such as colorectal cancer [13], breast cancer [14], and hepatocellular carcinoma [15]. LIFR could act as a metastasis suppressor by involving in the Hippo-YAP pathway and the PTEN pathway [14, 16]. However, the functions of lncRNA-LOWEG in bladder cancer were completely unknown.

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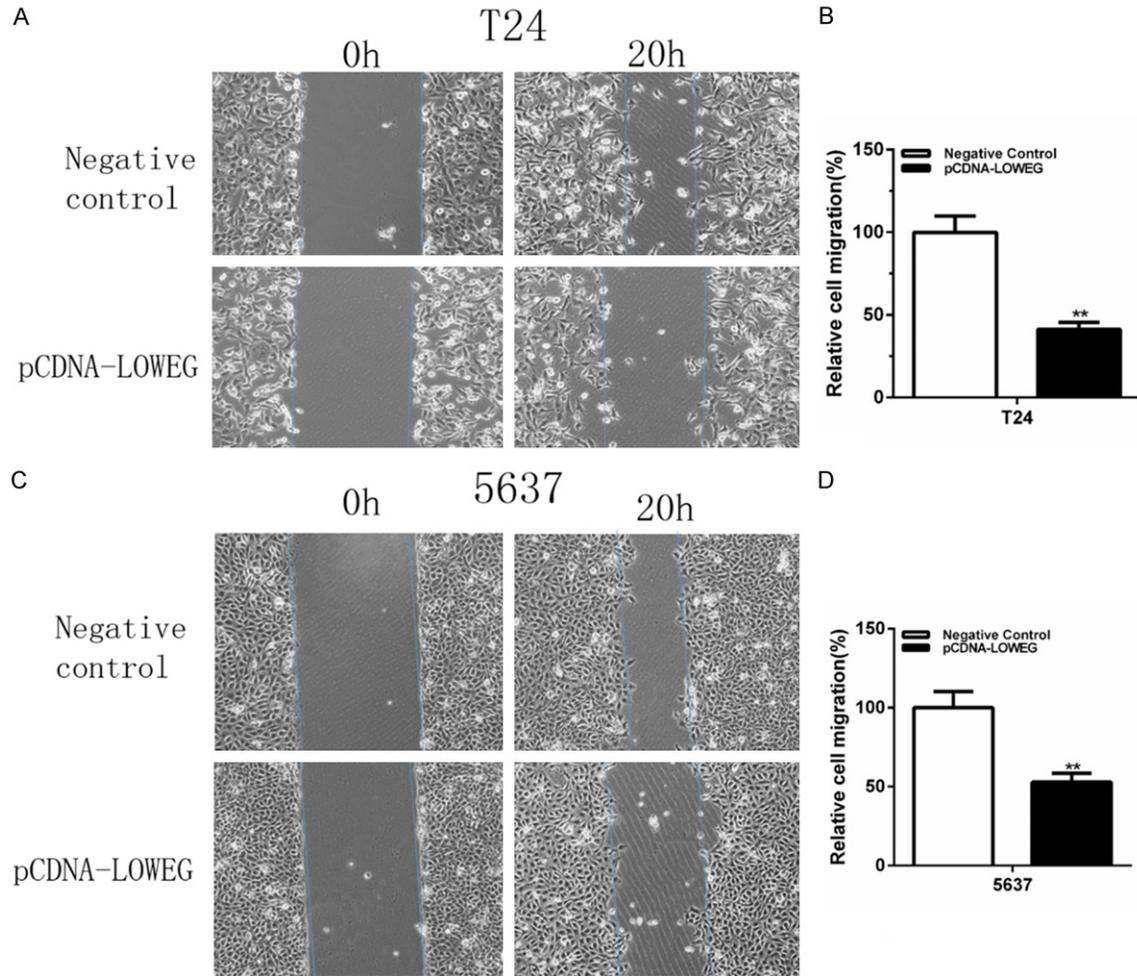


Figure 3. Overexpression of lncRNA-LOWEG inhibited cell migration in bladder cancer cells. Cell migration was detected by Wound healing assay. Cell migration inhibition was observed in bladder cancer T24 (A and B) and 5637 (C and D) cells. Data are shown as mean \pm SD. (** $P < 0.01$).

To our best knowledge, this is the first evidence that lncRNA-LOWEG was significantly down-regulated in bladder cancer tissues compared with paired-adjacent noncancerous tissues and down-regulated lncRNA-LOWEG expression was positively correlated with male patients with bladder cancer. Next, compared with the human uroepithelial cell line SV-HUC-1, the relative expression level of lncRNA-LOWEG in four bladder cancer cell lines (T24, 5637, SW780 and UM-UC-1) was also significantly downregulated. In order to investigate the biological function of lncRNA-LOWEG in bladder cancer, we detected the cell migration by up-regulating lncRNA-LOWEG in the bladder cancer cell lines T24 and 5637. Compared with the negative control group, pCDNA-LOWEG mediated overexpression of lncRNA-LOWEG significantly inhibited cell migration capability of bladder can-

cer T24 and 5637 cells, suggesting that up-regulated lncRNA-LOWEG expression could suppress the metastasis capability of bladder cancer.

Conclusion

In conclusion, the expression level of the lncRNA-LOWEG is down-regulated in bladder cancer tissues compared with paired-adjacent nontumorous tissues. Moreover, we preliminarily revealed the significant function of lncRNA-LOWEG in regulating cell migration of BC cells. These findings demonstrated that lncRNA-LOWEG may play a tumor suppressor role in bladder cancer and may serve as a new candidate biomarker and potential therapeutic target of bladder cancer. However, the molecular mechanism by which lncRNA-LOWEG was

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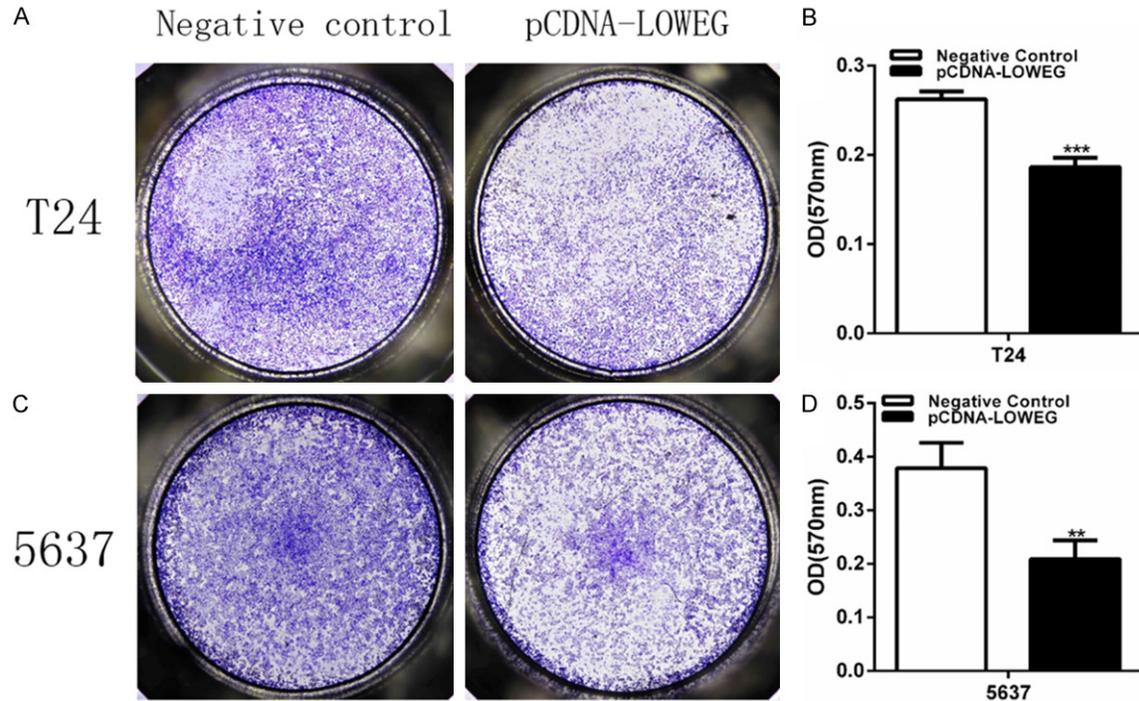


Figure 4. Overexpression of lncRNA-LOWEG inhibited cell migration in bladder cancer cells. Cell migration was determined by transwell assay. Cell migration inhibition was observed in bladder cancer T24 (A and B) and 5637 (C and D) cells. Data are shown as mean \pm SD. (** $P < 0.01$, *** $P < 0.001$).

down-regulated in bladder cancer also should be explored in the future works.

Acknowledgements

The authors are indebted to all the donors whose names were not included in the author list, but who participated in our study. This work was supported by Health Care 3F Project of Shenzhen (Peking University First Hospital-The Second People's Hospital of Shenzhen, Academician Yinglu Guo's Team), the Shenzhen Key Medical Discipline Fund, Special Support Funds of Shenzhen for Introduced High-Level Medical Team, Shenzhen Foundation of Science and Technology [JCYJ20150330102720182] and Shenzhen Health and Family Planning Commission Scientific Research Project [20160-1025].

Disclosure of conflict of interest

None.

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