

Inhibiting malignant phenotypes of the bladder cancer cells by silencing long noncoding RNA SChLAP1

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Abstract

Background and objectives Long noncoding RNAs (lncRNAs) play key roles in process of cancer cell growth and apoptosis and have received increasing attention. SChLAP1 is a novel lncRNA that is required for development and progression of prostate cancer. We hypothesized that SChLAP1 also has important biological functions in human bladder cancer which is another type of urological cancer.

Methods The expression of SChLAP1 in bladder cancer was determined using real-time qPCR. Bladder cancer T24 and 5637 cells were transfected with SChLAP1 siRNA or negative control siRNA. Cell proliferation, apoptosis and migration were determined using CCK-8 assay, flow cytometry analysis and wound healing assay, respectively.

Results SChLAP1 was overexpressed in bladder cancer tissues compared to paired normal bladder tissues. Cell growth arrest, apoptosis induction and migration inhibition were also observed in bladder cancer T24 and 5637 cells after transfection with SChLAP1 siRNA.

Conclusions Our data suggest that SChLAP1 plays oncogenic roles and can be used as a therapeutic target for treating human bladder cancer.

Keywords Bladder cancer · SChLAP1 · Cell proliferation · Cell apoptosis · Cell migration

Introduction

Bladder cancer is one of the most common types of genitourinary tumors, and the major subtype of this cancer is urothelial carcinoma. Although many genes and pathways are found to be correlated with bladder cancer, its carcinogenesis is still far from clear [1, 2].

Long noncoding RNAs (lncRNAs) are a family of nonprotein coding RNAs with length more than 200 nucleotides. With the completion of human genome project, long noncoding RNAs (lncRNAs) have increasingly been discovered to be deregulated in many human cancers, including bladder cancer [3]. For example, MALAT1 [4], TUG1 [5], UCA1 [6] and HOTAIR [7] were reported to have affected the development of bladder cancer. These lncRNAs play critical roles in carcinogenesis and act as potential tumor promoters or tumor suppressors [8]. lncRNA SChLAP1 is a novel biomarker that predicts poor clinical outcome for prostate cancer [9–12]. However, the relationship between SChLAP1 and bladder cancer is largely unknown.

In this study, we found that SChLAP1 was overexpressed in bladder cancer tissues compared to matched normal bladder tissues. Silencing SChLAP1 inhibited bladder cancer growth or migration and induced apoptosis.

Materials and methods

Patient samples

We included 36 bladder cancer patients who received partial or radical cystectomy in the expression analyses, and

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the Institutional Review Board of The People's Hospital of Xinjiang Uyghur autonomous region approved this study. We also got written informed consents from all patients. Bladder cancer tissues and paired normal bladder tissues from patients were snap-frozen in liquid nitrogen immediately after resection.

Cell culture

Bladder cancer T24 and 5637 cells were purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA). T24 cells were cultured in Dulbecco's Modified Eagle's Medium (Invitrogen, Carlsbad, CA, USA) supplemented with 10 % fetal bovine serum (Invitrogen, Carlsbad, CA, USA) at 37 °C in a 5 % CO₂ atmosphere. 5637 cells were cultured in RPMI-1640 Medium (Invitrogen, Carlsbad, CA, USA) supplemented with 10 % fetal bovine serum (Invitrogen, Carlsbad, CA, USA) at 37 °C in a 5 % CO₂ atmosphere.

siRNA transfection

SChLAP1 siRNA and negative control siRNA were purchased from Sigma-Aldrich. The sequence was 5'-CCAAUG AUGAGGAGCGGGA-3' [13]. The cells were incubated with either SChLAP1 siRNA or negative control siRNA using Lipofectamine 2000 Transfection Reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocols.

Real-time quantitative PCR

Total RNA was extracted from the tissue samples through using the TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the protocols. cDNA was synthesized from total RNA through using the M-MLV Reverse Transcriptase (Promega, USA) in a 25- μ l volume. The primer sequences for SChLAP1 primers and GAPDH were described in the previous works [13]. qPCR was performed in a total reaction volume of 20 μ l, which includes 1 μ l of first-strand cDNA, 0.4 μ l of forward primer, 0.4 μ l of reverse primer, 10 μ l of 2 \times All-in-One™ qPCR Mix (GeneCopoeia Inc, Rockville, MD, USA), 0.4 μ l of 50 \times ROX reference dye and 7.8 μ l of double-distilled water. The reactions were performed in triplicate by the ABI PRISM 7000 Fluorescent Quantitative PCR System (Applied Biosystems, Foster City, CA, USA). GAPDH was chosen as the internal control. Relative expression fold changes of SChLAP1 were calculated by using the comparative ΔC_t method (value of $2^{-\Delta C_t}$ (SChLAP1-GAPDH)) [13]. These experiments were repeated three times.

Cell growth assay

Cell growth was detected using Cell Counting Kit-8, CCK-8 (Beyotime Institute of Biotechnology, Shanghai, China) according to instructions. Cells were grown in a 96-well plate for 24 h and cultured in normal medium. Then, cells were transfected with SChLAP1 siRNA or negative control siRNA. At 0, 24, 48 and 72 h after transfection, the absorbance in each well was measured at 490 nm with a microplate reader. These experiments were repeated three times.

Flow cytometry analysis of cell apoptosis

Cells were transfected with SChLAP1 siRNA or negative control siRNA and cultured in normal medium. At 48 h post-transfection, cells were harvested and resuspended in fixation fluid. Five microliters of AnnexinV-FITC and 2 μ l propidium iodide were added to 500 μ l cell suspension. Cell apoptosis was then determined by using flow cytometry analysis (BD, USA). These experiments were repeated three times.

Wound healing assay

Cell motility was determined by wound healing assay. At 24 h post-transfection, a wound field was created using a sterile 200 μ l pipette tip in about 90 % confluent cells. The cells were incubated for 16 h at 37 °C, and then the migration of cells was monitored with a digital camera system. The cell migration distance (μ m) was calculated by the software program HMIAS-2000. These experiments were repeated at least three times.

Statistical analysis

The SChLAP1 RNA expression changes between bladder cancer tissues and matched normal tissues were analyzed using paired samples *t* test. In the CCK-8 assay, flow cytometry analysis and wound healing assay, the differences between cells transfected with SChLAP1 siRNA and cells transfected with negative control siRNA were analyzed using ANOVA. All these statistical analyses were performed by using SPSS (version 17.0 SPSS Inc.). A *p* value of <0.05 was considered to be statistically significant.

Results

SChLAP1 was overexpressed in bladder cancer

The relative expression level of SChLAP1 was determined by using real-time qPCR in a total of 36 patients with

bladder cancer. The SChLAP1 expression change between bladder cancer tissues and matched normal tissues is shown in Fig. 1. SChLAP1 was overexpressed in bladder cancer tissues compared to paired normal tissues ($p = 0.002$). These results indicated that SChLAP1 should function as an oncogenic gene in bladder cancer. Patients' clinical parameters are listed in Table 1.

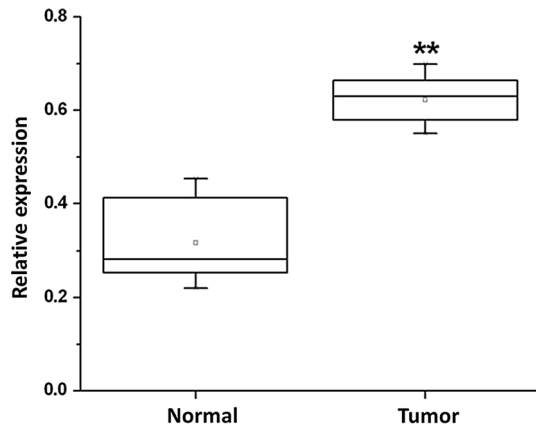


Fig. 1 SChLAP1 was overexpressed in bladder cancer. Relative SChLAP1 concentration was detected using real-time qPCR. **a** T represents tumor, N represents normal. SChLAP1 expression levels were higher in bladder cancer tissues than those in normal bladder tissues ($p < 0.01$)

Silencing SChLAP1 inhibited cell growth

We further detected whether SChLAP1 promotes cell growth in bladder cancer. Bladder cancer T24 and 5637 cells were transfected with SChLAP1 siRNA or negative control siRNA. The inhibitory rate (SChLAP1 siRNA/negative control siRNA) was $85.42 \pm 3.47\%$ in T24 cells and $86.12 \pm 4.33\%$ in 5637 cells, respectively. Data were shown as mean \pm SD. Then, the cell growth curves of bladder cells were determined by CCK-8 assay. Cell growth arrest was observed in both T24 cells (Fig. 2a) ($p < 0.001$) and 5637 cells (Fig. 2b) ($p < 0.001$) when transfected with SChLAP1 siRNA. These results demonstrated that SChLAP1 should increase cell growth in bladder cancer.

Silencing SChLAP1 induced apoptosis

After that, we asked the question of whether SChLAP1 can also suppress cell apoptosis in bladder cancer. Bladder cancer T24 and 5637 cells were transfected with SChLAP1 siRNA or negative control siRNA. At 48 h post-transfection, the cell apoptosis of both T24 (Fig. 3a) and 5637 cells (Fig. 3b) was determined by flow cytometry analysis. Induced cell apoptosis was observed in both T24 ($p = 0.038$) and 5637 cells ($p = 0.014$) transfected with SChLAP1 siRNA (Fig. 3c, d). These results confirmed that SChLAP1 inhibits cell apoptosis in bladder cancer.

Table 1 Summary of clinicopathological features of tissues of bladder cancer

Pt no.	Age	Sex	Grade	Stage	Pt no.	Age	Sex	Grade	Stage
1	63	M	L	T1N0M0	19	70	M	H	T2N0M0
2	53	M	H	T2N0M0	20	55	M	L	T2N0M0
3	38	M	H	T1N0M0	21	69	M	H	T2N0M0
4	63	M	H	T2N1M0	22	76	M	L	T1N0M0
5	58	M	H	T2N1M0	23	67	F	H	T3N0M0
6	47	M	H	T2N0M0	24	59	M	H	T3N0M0
7	61	F	H	T3N1M0	25	48	M	H	T2N0M0
8	52	M	H	T2N0M0	26	51	M	L	T1N0M0
9	78	M	L	T2N0M0	27	56	F	L	T2N0M0
10	48	M	H	T2N0M0	28	73	F	L	T1N0M0
11	58	M	H	T2N0M0	29	61	M	H	T2N0M0
12	64	M	L	T1N0M0	30	59	M	L	T2N0M0
13	67	M	H	T3N0M0	31	57	M	L	T2N0M0
14	54	M	L	T1N0M0	32	46	M	L	T2N0M0
15	44	M	H	T3N0M0	33	49	M	H	T3N1M0
16	49	F	H	T3N0M0	34	56	M	H	T2N0M0
17	52	M	H	T2N0M0	35	49	F	H	T3N1M0
18	61	M	H	T2N0M0	36	55	M	L	T1N0M0

Pt No. patient number, M male, F female, Grade the World Health Organization 2004 classification, H high, L low, Stage the American Joint Committee on Cancer TNM classification

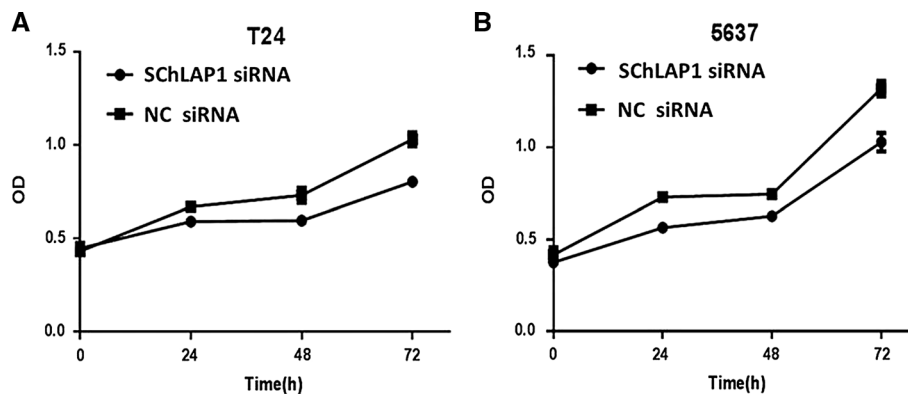


Fig. 2 Silencing SCHLAP1 inhibited cell growth. Cell proliferation was detected by CCK-8 assay. After transfection of SCHLAP1 siRNA or negative control siRNA, OD values were measured. ANOVA was used for the comparison of curves of cell growth. **a** Cell growth arrest

was observed in bladder cancer T24 cells ($p < 0.01$). **b** Cell growth arrest was observed in bladder cancer 5637 cells ($p < 0.01$). Data were shown as mean \pm SE. Each experiment in both cell lines was performed in triplicate for three independent times

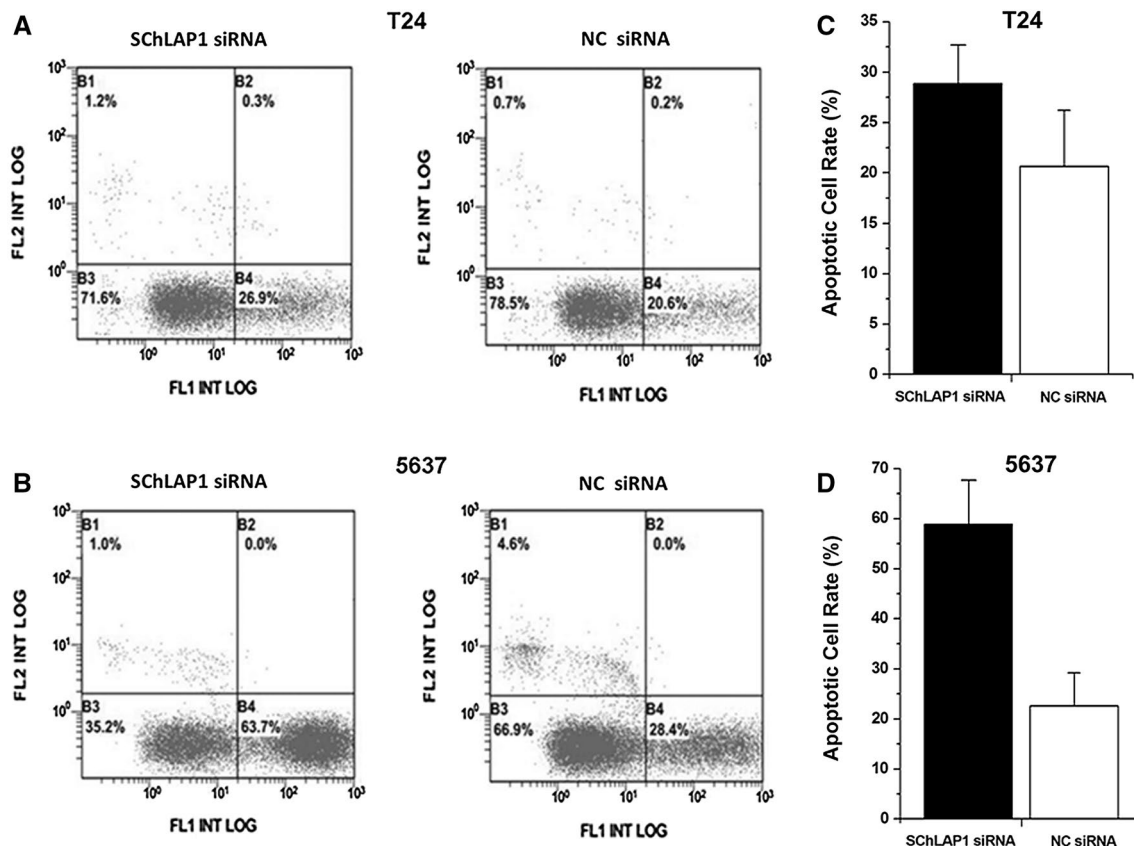


Fig. 3 Silencing SCHLAP1 induced apoptosis. Forty-eight hours after transfection of SCHLAP1 siRNA or negative control siRNA, the cell apoptosis changes were determined by flow cytometry analysis. **a** Representative images of flow cytometry analysis in T24 cells. **b**

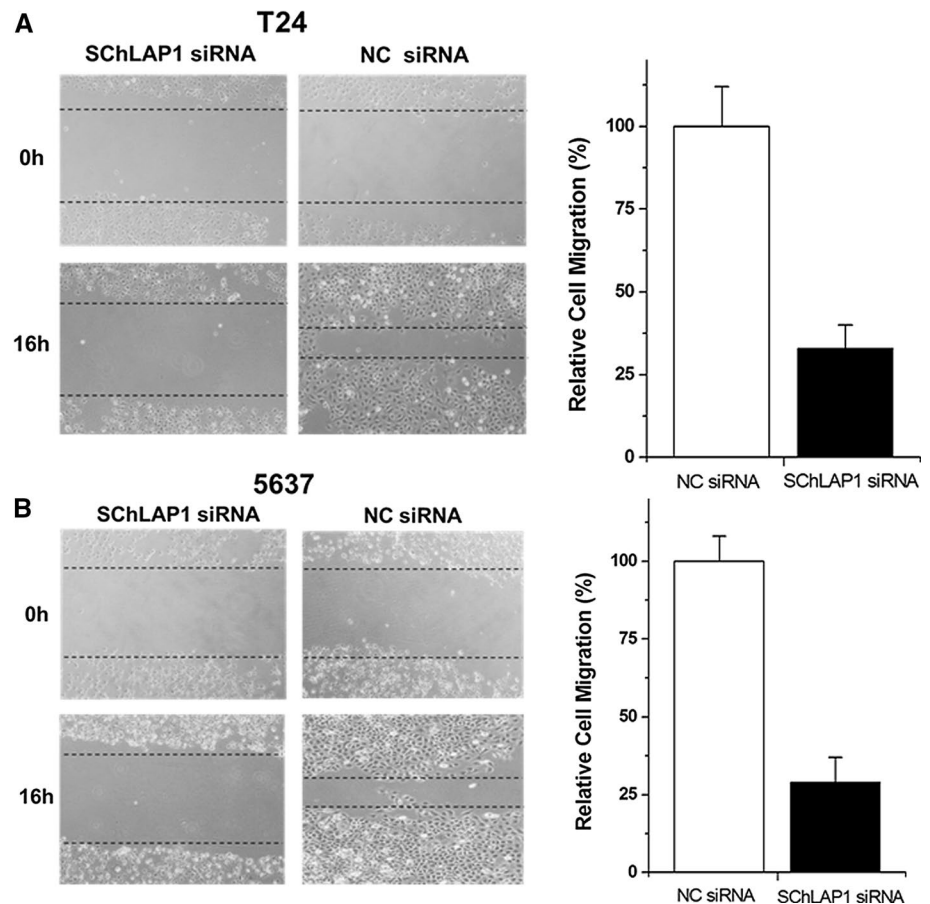
Representative images of flow cytometry analysis in 5637 cells. **c** Cell apoptosis induction was observed in bladder cancer T24 cells ($p < 0.01$). **d** Cell apoptosis induction was observed in bladder cancer 5637 cells ($p < 0.01$)

Silencing SCHLAP1 inhibited cell migration

Finally, we determined whether SCHLAP1 promotes cell migration in bladder cancer. Bladder cancer T24 and 5637

cells were transfected with SCHLAP1 siRNA or negative control siRNA, and the cell migration changes of bladder cells were determined by wound healing assay. Cell migration arrest was observed in both T24 cells (Fig. 4a)

Fig. 4 Silencing SChLAP1 inhibited cell migration. Cell migration was determined by wound healing assay. Cell migration inhibition was observed in bladder cancer T24 cells (**a**) ($p < 0.01$) and 5637 cells (**b**) ($p < 0.01$)



($p = 0.022$) and 5637 cells (Fig. 4b) ($p = 0.014$) as expected. These results confirmed that SChLAP1 increases cell migration in bladder cancer.

Discussion

As a noncoding gene, SChLAP1 promotes aggressive prostate cancer and antagonizes the SWI/SNF complex [13]. SChLAP1 impairs SNF5-mediated gene expression regulation and genomic binding [13]. However, the relationship between SChLAP1 and bladder cancer is still unclear and mysterious.

This study provided novel insights into the function of SChLAP1 in bladder cancer. The first objective of this study was to test the hypothesis that the expression levels of SChLAP1 were up-regulated in most bladder cancer tissue samples. This hypothesis was confirmed by the qPCR results. We found that SChLAP1 was overexpressed in bladder cancer. A deregulated lncRNA should play a vital role in regulating cancer cell biological behavior. So the second objective of the study was to test the hypothesis that down-regulation of SChLAP1 inhibited cell proliferation or

migration and promoted apoptosis. So we detected the cell growth, apoptosis and migration by silencing SChLAP1 in two bladder cancer cell lines. Cell growth arrest, increased apoptosis and suppressed migration were observed in bladder cells transfected with SChLAP1 siRNA. These findings suggested that SChLAP1 may play essential functions in the carcinogenesis of bladder cancer.

In summary, SChLAP1 plays a tumor promoter role in bladder cancer. More work will be needed to determine the molecular mechanisms of SChLAP1 as a candidate biomarker for bladder cancer in the clinic.

Compliance with ethical standards

Conflict of interest All authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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