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LncRNA ZNFX1-AS1 targeting miR-193a-3p/SDC1 regulates cell proliferation, migration and invasion of bladder cancer cells

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Abstract. – OBJECTIVE: Long non-coding RNA (lncRNA) is closely associated with cancer occurrence and tumor development. However, the biological function of lncRNA ZNFX1-AS1 has not yet been reported in bladder cancer. The present study aimed to study the function of ZNFX1-AS1 in bladder cancer cells and the mechanism involved.

PATIENTS AND METHODS: The expression of ZNFX1-AS1 in bladder cancer tumor tissues and cell lines was examined by qRT-PCR. The effects of ZNFX1-AS1 knockdown on cell proliferation, cell cycle, cell migration, and invasion were assessed by Cell Counting Kit-8, flow cytometry (FCM), and transwell assays. Bioinformatics analyses and Luciferase reporter assays were performed to explore the mechanism by which ZNFX1-AS1 exerted its oncogenesis role in bladder cancer. The anti-tumor effect of ZNFX1-AS1 silencing on bladder cancer in vivo was also evaluated.

RESULTS: ZNFX1-AS1 was over-expressed in bladder cancer tumor tissues and cell lines. ZNFX1-AS1 expression was found to be associated with tumor size and advanced clinical stage in patients with bladder cancer. Downregulation of ZNFX1-AS1 inhibited cell proliferation, cell clone formation, migration, and invasion of bladder cancer cells. ZNFX1-AS1 was found to interact with miR-193a-3p/Syndecan 1 (SDC1). ZNFX1-AS1 expression was negatively correlated with miR-193a-3p expression, but positively correlated with SDC1 expression in bladder cancer samples. ZNFX1-AS1 knockdown also effectively suppressed tumor growth in an *in vivo* xenograft model.

CONCLUSIONS: ZNFX1-AS1 regulated bladder cancer progression by targeting the miR-193a-3p/SDC1 axis. Our study may provide novel insights for bladder cancer prognosis and therapy.

Key Words:
ZNFX1-AS1, Bladder carcinoma, Cell migration, Invasion, MiR-193a-3p, SDC1.

Abbreviations

Long non-coding RNA (lncRNA); Low cytometry (FCM); Syndecan 1 (SDC1); Quantitative Real Time-Polymerase Chain Reaction (qRT-PCR); microRNA (miRNA).

Introduction

Bladder cancer is one of the most common urological cancers in the world. With the increasing morbidity and mortality, bladder cancer has become the ninth and fourteenth leading cause of death in the world[1]. The highest incidence was found in Europe, North America, Western Asia, and North Africa. Although bladder cancer patients can be treated by radiotherapy, surgery, and chemotherapy, the 5-year survival rate is still unsatisfactory[2,3]. Therefore, it is very important to explore the molecular mechanism of the occurrence and development of bladder cancer.

It is well known that long non-coding RNA (lncRNA) is a transcript of RNA with a length of more than 200 nucleotides. Biological processes regulated by lncRNA[4,5] include DNA damage, angiogenesis, microRNA (miRNA) silencing, cell invasion, and cell apoptosis in cancer cells. At present, it has been found that lncRNA has become a participant in cancer research. Many lncRNAs have been reported to exert a crucial role in the occurrence, prognosis, and development of bladder cancer[6,7]. Previous studies reported that lncRNA ZNFX1-AS1 was overexpressed in colorectal cancer[8], hepatocellular carcinoma[9], and gastric cancer[9]. The biological function of ZNFX1-AS1 in bladder cancer is rarely known. We firstly found that the expression of ZNFX1-AS1 was abnormally expressed in bladder cancer samples.

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In this work, we explored the ZNFX1-AS1 expression in clinical bladder cancer samples and analyzed the relationship between ZNFX1-AS1 expression and clinical features of patients diagnosed with bladder cancer. The effects of ZNFX1-AS1 on cell proliferation, cell cycle, cell migration, and invasion of bladder cancer cells *in vitro* and in *vivo* were then evaluated. We subsequently reported the molecular mechanism by which ZNFX1-AS1 exerted the oncogenesis role in bladder cancer.

Patients and Methods

Patient and Tissue Samples

Sixty-seven bladder cancer tissues and corresponding adjacent non-tumor tissues were collected from the Drum Tower Hospital, Medical School of Nanjing University (Nanjing, China) from May 2011 to September 2017. After the patients signed the informed consent, bladder cancer tissues and adjacent tissues were removed surgically and immediately put into liquid nitrogen stored at -80°C for RNA extraction and expression detection. The collection of clinical data and human bladder cancer tissue specimens is approved by the Ethics Committee of the Drum Tower Hospital, Medical School of Nanjing University, and the whole study was performed in accordance with the Ethical Guidelines of the 1975 Declaration of Helsinki. All subjects provided written informed consent.

Cell Cultures and Transfection

Bladder cancer cell lines (T24, SW780, J82, HT1379) and bladder epidermal cells (HCV-29) were obtained from Chinese Academy of Sciences Cell Bank (Shanghai, China). Bladder cancer cells and HCV-29 were cultured in Roswell Memorial Park Institute-1640 (RPMI-1640; Gibco, Grand Island, NY, USA) with 10% FBS (Gibco, Rockville, MD, USA) in an incubator with a volume fraction of 5% CO_2 at 37°C.

For cell transfection, miR-193a-3p mimic, small interfering RNA (siRNA) targeting ZNFX1-AS1 or SDC1 and relative control was obtained from GenePharma (Shanghai, China). The transfection was conducted by Lipofectamine® 2000 (Thermo Fisher Scientific, Waltham, MA, USA) in bladder cancer cells. Further experiments were carried out at 48 h after transfection. For ZNFX1-AS1 overexpression, bladder cancer cells were transfected with pCDNA3-ZNFX1-AS1 using Lipofectamine® 2000 (Thermo Fisher Scientific, Waltham, MA, USA). After transfection for 24 h, transfection efficiency was examined by quantitative Real Time-Polymerase Chain Reaction (qRT-PCR).

qRT-PCR

Total RNAs from cells and tissues were obtained using TRIzol reagent (Invitrogen; Thermo Fisher Scientific, Waltham, MA, USA), and then, reverse-transcribed by using TransScript Green qRT-PCR SuperMix (TransGen Biotechnology, Beijing, China). The qRT-PCR analysis was detected by SYBR-Green qRT-PCR assay (Bio-Rad, Hercules, CA, USA). β-actin was employed as endogenous control for lncRNA ZNFX1-AS1 and SDC1. U6 was used as the endogenous control for miRNA-193a-3p. The $2^{-\Delta\Delta Ct}$ method was used. The primer sequences used were as follows: ZNFX1-AS1 (forward, 5'-AGCCATTCGTTCTTTCGC-3' and reverse, 5'-GTGGTGACTCCCTCTTCCA-3'), SDC1 (forward, 5'-GGGACTCAGCCTTCAGACA-3' and reverse, 5'-CCCAAGACACCCCTCGT-3') and β-actin (forward, 5'-TCCTCTGACTTCAACAGCGACAC-3' and reverse, 5'-CACCCTGTTGCTGTAGCCAAATTC-3'), miRNA-193a-3p (forward, 5'-TGGGTCTTTGCGGGCG-3' and reverse, 5'-TGTGTTGTGCTGTGTCTGTG-3'), and U6 (forward, 5'-CTCGCTTCGGCAGCACA-3' and reverse, 5'-AACGCTTCACGAATTTGCGT-3').

Cell Proliferation

After incubation in incubator for 24, 48, 72 and 96 h, 100 μL CCK-8 solution (Dojindo Laboratories, Kumamoto, Japan) was added to the pore and mixed. Cells were cultured at 37°C for 2 hours. The absorbance (OD) at 450 nm was measured by enzyme-labelled instrument (Biotek, Winooski, VT, USA), and three compound pores were set in each group.

Cell Cycle Detection

Cells of each group were collected after 48 h of treatment under the corresponding conditions. All cells were fixed in 70% ethanol for 12 h, followed by washing twice by PBS. Then, PBS containing RNase and PI was added to these cells for 30 min incubation at room temperature. FACS Calibre flow cytometer (Becton-Dickinson, BD Biosciences, San Jose, CA, USA) was employed to detect the cell cycle distribution, and Cell Quest software (BD Biosciences, San Jose, CA, USA) was selected for data analysis.

Cell Invasion and Migration

For cell migration, the transfected cells were collected and re-suspended in serum-free RPMI-1640 medium. The cells were added into the upper chamber (Corning Costar, Cambridge, MA, USA) of Transwell chamber with 5×10^4 cell density per pore, and 600 mL complete culture medium was added into the lower chamber. The cells were cultured in the incubator for 24 hours and stained with 0.01% crystal violet after 4% polyformaldehyde treatment. The number of transmembrane cells was counted under 200-fold optical microscope. Five visual fields were taken, and the mean value was taken. Each group had three multiple holes, and the experiment was repeated three times. For cell invasion assay, the upper chamber was precoated with 1 mg/ml Matrigel (BD Biosciences, San Jose, CA, USA). The following steps were performed as previously described.

Dual-Luciferase Reporter Assay

The wild type miR-193a-3p binding sites in ZNFX1-AS1 or SDC1 3'-UTR and the mutant sites were constructed into psi-CHECK-2 Luciferase vector (Promega, Madison, WI, USA). HEK293T cells were inoculated into 24-well plates at a density of 1×10^5 cells per well. On the second day, when about 70% of the cells fused, they were co-transfected with Lipofectamine 2000. After 48 hours of routine culture, the fluorescence intensity was measured and analyzed by Promega (Promega, Madison, WI, USA).

In Vivo Xenograft Experiments

All experimental procedures were performed in accordance with guidelines of the Animal Care and Use Committees at Drum Tower Hospital, Medical School of Nanjing University, and also approved by the Committee of the Drum Tower Hospital, Medical School of Nanjing University. Twelve BALB/c nude mice (6-week-old) were purchased from Beijing HFK Bioscience Co. Ltd. (Beijing, China) were divided for two groups (n=6/group): shNC group and shZNFX1-AS1 group, and all mice were maintained in pathogen-free conditions. SW780 cells with siNC or shZNFX1-AS1 transfection were subcutaneously injected into BALB/c nude mice. Tumor length (a) and width (b) were recorded every 7 day for 28 days. Tumor volume was measured as formula: volume = $\pi ab^2/6$. After 35 days, mice were sacrificed, and the tumors were isolated and weighed.

Western Blot

After transfection, the cells were added to cell lysate (Beyotime, Shanghai, China). Total protein (40 mg) was separated by 10% SDS-polyacrylamide gel electrophoresis, 300 mA voltage was transferred to 100 min, 5% skim milk powder at room temperature was closed for 90 min. The primary antibody anti-SDC1 (cat. No. ab ab128936; 1:1,000) was purchased from Abcam (Cambridge, MA, USA) was incubated overnight at 4°C, and then, incubated with the HRP labeled mouse anti-rabbit IgG secondary antibody (cat. No. 7074; Cell Signaling, Danvers, MA, USA; 1:3000 dilution). Incubation condition was room temperature for 30 minutes, and then, we performed ECL luminescence (Promega, Madison, WI, USA) detection. Quantity One image analysis software scanned and analyzed the protein bands. β-actin was used as the reference.

Statistical Analysis

Data were shown as mean ± SD of at least three replicates using the SPSS17.0 software (SPSS Inc., Chicago, IL, USA). Student's *t*-test was used to analyze the differences between two groups. Differences among multiple groups were determined by one-way ANOVA followed by Tukey's post-hoc test. Pearson's χ^2 test was employed to analyze the correlation between clinical parameters and ZNFX1-AS1 expression in bladder cancer patients. Spearman's correlation analysis was used to measure the correlations between the expression levels of miR-193a-3p and ZNFX1-AS1/SDC1 in bladder cancer tissues. $p<0.05$ showed significant difference.

Results

The Expression of LncRNA ZNFX1-AS1 Was Increased in Bladder Cancer

We first identified the expression of ZNFX1-AS1 in bladder cancer tissues. Sixty-seven paired bladder cancer tumor tissues and non-tumor tissues were collected for ZNFX1-AS1 expression analysis by qRT-PCR. As shown in Figure 1A, ZNFX1-AS1 was overexpressed in bladder cancer tissues, and bladder cancer patients of TNM III-VI stage exhibited a higher ZNFX1-AS1 expression than that in bladder cancer patients of lower stage ($p<0.01$, Figure 1B). Subsequently, the expressions of ZNFX1-AS1 in bladder cancer cell lines (T24, SW780, J82, HT1379) and bladder epidermal

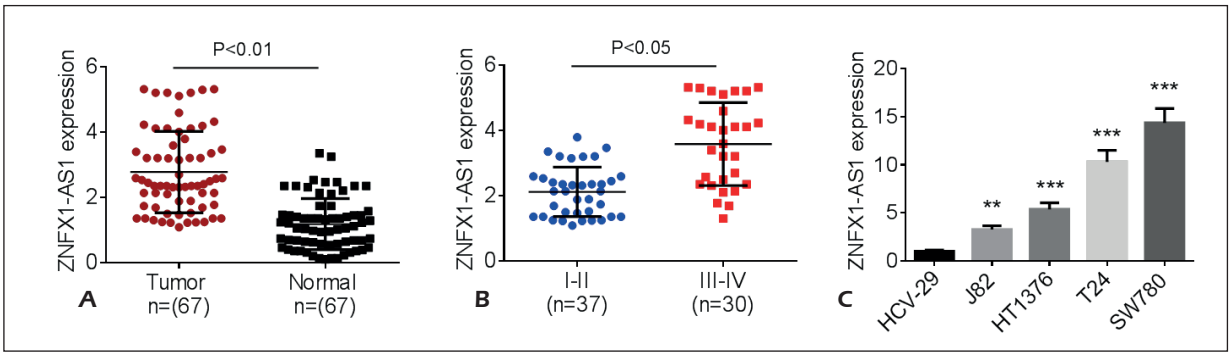

Figure 1. The expression of ZNFX1-AS1 in bladder cancer tissues and cell lines. **A**, The ZNFX1-AS1 expression in 67 paired clinical bladder cancer samples was identified by qRT-PCR analysis. **B**, The ZNFX1-AS1 expression in different stages of bladder cancer samples was examined. **C**, ZNFX1-AS1 expression in HCV-29 and bladder cancer cell lines (J82, HT1376, T24, and SW780) was detected by qRT-PCR analysis. Data are shown as mean ± SD. **p<0.01, and ***p<0.001 *vs.* HCV-29 cells.

cells (HCV-29) were examined by qRT-PCR. The ZNFX1-AS1 expression was increased notably in bladder cancer cell lines in comparison with that in bladder epidermal cells (p<0.01, Figure 1C). The clinical characteristics of ZNFX1-AS1 expression in bladder cancer patients was then explored. As shown in Table I, high ZNFX1-AS1 expression level was associated with advanced clinical stages and tumor size (p<0.05).

Downregulation of ZNFX1-AS1 Inhibited Cell Proliferation, Induced Cell Cycle Arrest, and Suppressed Migration and Invasion

To identify the effect of ZNFX1-AS1 on malignant tumor cell phenotype of bladder cancer, we transfected T24 and SW780 cells with siZNFX1-AS1-1, siZNFX1-AS1-2, siZNFX1-AS1-3 and siNC, respectively. After transfection, the expression of ZNFX1-AS1 in cells was detected by qRT-PCR. The results revealed that siZNFX1-AS1-3 showed a better effect and was then carried out for further studies (p<0.001, Figure 2A). The effect of siZNFX1-AS1 on cell proliferation and cell cycle was detected by CCK-8 and flow cytometry. Cell migration and invasion of T24 and SW780 cells with siZNFX1-AS1 or siNC transfection were identified by transwell assay. The results presented that the downregulation of ZNFX1-AS1 significantly suppressed cell proliferation of T24 and SW780 cells compared with the cells with siNC transfection (p<0.001, Figure 2B). ZNFX1-AS1 knockdown effectively induced G0/G1 cell cycle arrest of T24 and SW780 cells (p<0.01, Figure 2C). Moreover, cell migration and invasion of bladder cancer cells transfected with siZNFX1-AS1 were significantly suppressed compared with that of bladder cancer cells transfected with siNC (p<0.01, Figure 2D). Moreover, we evaluated the anti-tumor effect of ZNFX1-AS1 knockdown on tumor growth *in vivo*. SW780 cells were transfected with shNC or shZNFX1-AS1, and then, subcutaneously injected into BALB/c nude mice. Tumor volume and weight were also measured. As shown in Figure 2E, tumor growth of shZNFX1-AS1 group was significantly delayed

Table I. Correlation between ZNFX1-AS1 expression and clinicopathological characteristics of bladder cancer patients.

Features	ZNFX1-AS1 expression		*p*-value
	Low (45)	High (22)	
Age			0.578
≤65	16	8	
>65	29	14	
Gender			0.567
Male	24	12	
Female	21	10	
Tumor multiplicity			0.426
Unifocal	26	14	
Multifocal	19	8	
Tumor size (cm)			0.036*
≤3.0	31	9	
>3.0	14	13	
Histological grade			0.028*
G1	21	5	
G2	11	6	
G3	13	11	
pT classification			0.228
pTa/pTis	19	7	
pT1	17	6	
pT2-pT4	9	9	
pN classification			0.189
pN−	31	12	
pN+	14	10	

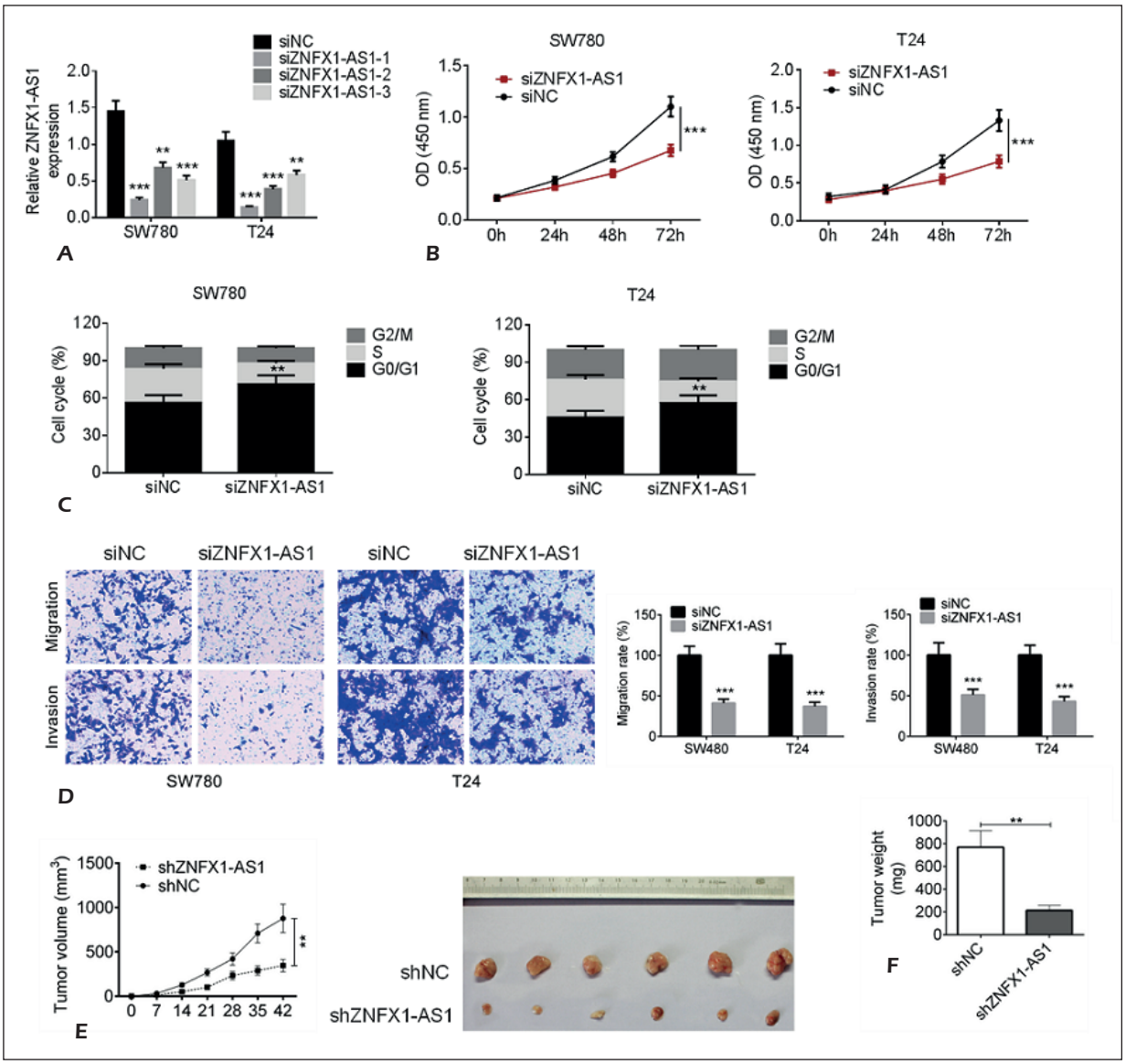

Figure 2. ZNFX1-AS1 knockdown inhibited cell proliferation, migration and invasion. **A**, SW780 and T24 cells were transfected with siZNFX1-AS1-1, siZNFX1-AS1-2 and siZNFX1-AS1-3, and the transfection efficiency was confirmed by qRT-PCR analysis. **B**, Cell proliferation was identified by CCK8 assay. **C**, Cell cycle was identified by FCM analysis. **D**, Cell migration and invasion were examined by transwell assay (magnification, x100). **E**, Tumor volume was measured every 7 for 42 days after injection. **F**, Tumor weights isolated from nude mice in each treatment group were determined on day 42 after injection. Data are shown as mean ± SD. **p<0.01, and ***p<0.001 *vs.* siNC or shNC group.

compared with that of shNC group (p<0.01). The ZNFX1-AS1 knockdown also significatly decreased the tumor weight compared with shNC treated group (p<0.01, Figure 2F).

ZNFX1-AS1 Directly Interacted with MiR-193a-3p/SDC1

We then identified the targeted miRNA of ZNFX1-AS1 by bioinformatics analysis and then proved this by Dual-Luciferase reporter. As shown in Figure 3A, miR-193a-3p was predicted to combine with ZNFX1-AS1. Luciferase reporters containing the wt-ZNFX1-AS1 and mut-ZNFX1-AS1 were conducted. As exhibited in Figure 3B, co-transfection of miR-193a-3p and wt-ZNFX1-AS1 notably reduced the Luciferase activity; while, co-transfection with miR-193a-3p and mut-ZNFX1-AS1 exhibited no significant change on the Luciferase activity. The expression of miR-193a-3p in bladder cancer cells with siNC or

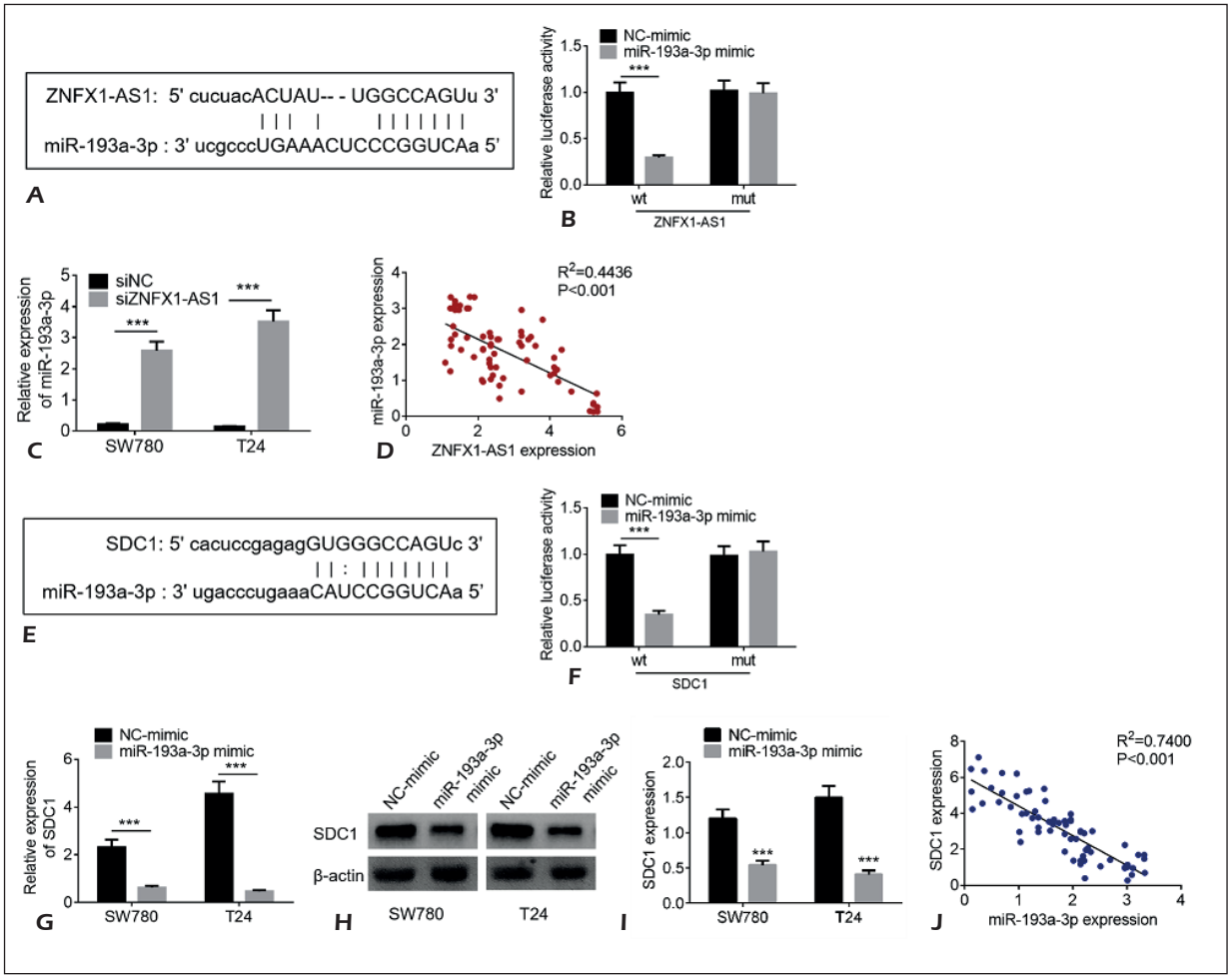

Figure 3. ZNFX1-AS1 directly interacted with miR-193a-3p/SDC1. **A**, The binding site of ZNFX1-AS1 and miR-193a-3p. **B**, Luciferase reporter assay was performed to verify the interaction of ZNFX1-AS1 and miR-193a-3p. **C**, The expression of miR-193a-3p in SW780 and T24 cells transfected with siZNFX1-AS1 was identified by qRT-PCR. **D**, ZNFX1-AS1 expression was negatively correlated with miR-193a-3p expression. **E**, The binding site of SDC1 and miR-193a-3p. **F**, Luciferase reporter assay was performed to verify the interaction of SDC1 and miR-193a-3p. **G-I**, SDC1 expression in in SW780 and T24 cells transfected with miR-193a-3p mimic was examined by qRT-PCR and western blot analysis. **J**, SDC1 expression was negatively correlated with miR-193a-3p expression. Data are shown as mean ± SD. ***p<0.001 *vs.* NC mimic group.

siZNFX1-AS1 was measured by qRT-PCR. The results presented that the miR-193a-3p expression was increased in bladder cancer cells with siZNFX1-AS1 transfection (p<0.001, Figure 3C). Furthermore, miR-193a-3p expression in bladder cancer samples was examined, which exerted a negative correlation with ZNFX1-AS1 expression (p<0.001, Figure 3D).

To further investigate the molecular mechanism by which ZNFX1-AS1 regulated cell phenotype in bladder cancer, the target mRNA of ZNFX1-AS1 was also examined. As shown in Figure 3E, SDC1 was predicted as the target of miR-193a-3p, and then, verified by Dual-Luciferase reporter assay. As exhibited in Figure 3F, co-transfection of miR-193a-3p and wt-SDC1 notably declined the Luciferase activity. However, co-transfection with miR-193a-3p and mut-SDC1 showed no significant effect on Luciferase activity. The SDC1 expression in bladder cancer cells with miR-193a-3p mimic transfection was dramatically decreased compared with that with NC-mimic treatment (p<0.001, Figure 3G-I). Furthermore, SDC1 expression in bladder cancer clinical samples was evaluated, and it negatively associated with miR-193a-3p expression (p<0.001, Figure 3J).

ZNFX1-AS1 Targeting MiR-193a-3p/SDC1 Regulated Cell Proliferation, Cell Cycle, Migration and Invasion of Bladder Cancer Cells

To further explore the molecular mechanism by which ZNFX1-AS1 promoted the tumorigenesis of bladder cancer, SW780 cells first transfected with NC, or ZNFX1-AS1, or ZNFX1-AS1 + miR-193a-3p mimic plasmids. Cell proliferation, cell cycle, cell migration and invasion were then evaluated. As exhibited in Figure 4A-C, ZNFX1-AS1 overexpression effectively promoted cell proliferation ($p<0.05$), increased the cell number in S phase, and accelerate the migration and invasion ($p<0.001$). Nevertheless, miR-193a-3p mimic reversed the promoting effect of ZNFX1-AS1 on SW780 cells ($p<0.01$, Figure 4A-C). For the relation of ZNFX1-AS1 and SDC1, SW780 cells were transfected with NC, or ZNFX1-AS1, or ZNFX1-AS1 + siSDC1 plasmids. We then found that siSDC1 also reversed the promoting effect of ZNFX1-AS1 on cell proliferation, cell cycle, cell migration and invasion of SW780 cells ($p<0.01$, Figure 4D-F). The results indicated that ZNFX1-AS1 facilitated cell proliferation, cell cycle, migration and invasion of bladder cancer cells by directly targeting miR-193a-3p/SDC1 axes.

Discussion

In recent decades, the incidence of bladder cancer has shown an upward trend year by year, and the situation is worrying[10]. In China, bladder cancer ranks the seventh most common malignant cancer in men[11]. Because of its unfavorable prognosis, the 5-year survival rate of patients has been significantly reduced. LncRNA plays a role as signal marker in some biological process and exhibits abnormal expression under some pathological conditions[12,13]. Many lncRNAs are involved in different types of cancer, such as gastric cancer, liver cancer, breast cancer, and bladder cancer[14]. LncRNAs are also involved in a large number of malignant tumors and almost all stages of tumorigenesis[14,15]. Therefore, the discovery of lncRNAs provides a new way to study the diagnosis and treatment of bladder cancer.

Previous study investigated that ZNFX1-AS1 was abnormally expressed in some cancers. Low expression of ZNFX1-AS1 was seen in liver carcinoma, and ZNFX1-AS1 regulated cell viability and induced hepatocellular cancer (HCC) cell apoptosis by regulating the methylation of miR-9[16]. Nevertheless, Shi et al[8] investigated that ZNFX1-AS1 was overexpressed in colorectal cancer, and ZNFX1-AS1 knockdown suppressed cell proliferation and invasion, and tumor growth and metastasis. Moreover, Xian et al[9] reported that ZNFX1-AS1 could be considered as a potential biomarker in gastric cancer diagnosis. In this study, we first found that ZNFX1-AS1 was over-expressed in bladder cancer tissues and cell lines. Increased ZNFX1-AS1 expression was positively associated with tumor size and histological grade of patients with bladder cancer. The results implied that ZNFX1-AS1 might function as an oncogene in bladder cancer. Then, cell proliferation, cell cycle, migration and invasion of bladder cancer cells were examined, and the data presented that downregulation of ZNFX1-AS1 effectively inhibited cell proliferation, induced cell cycle arrest, and suppressed cell migration and invasion. The anti-tumor effect of ZNFX1-AS1 knockdown on bladder cancer *in vivo* was then evaluated by xenograft experiments. Downregulation of ZNFX1-AS1 remarkably inhibited the tumorgenesis of bladder cancer. The above results imply that increased ZNFX1-AS1 expression gives rise to the progression of bladder cancer.

Salmena et al[17] firstly investigated the crosstalk between RNAs and proposed that "competing endogenous RNA" (ceRNA) activity forms a large-scale regulatory network across the transcriptome. A lot of scholars[18] also investigate that lncRNA could act as miRNA sponges to regulate biological process. The underlying molecular mechanism by which ZNFX1-AS1 exerts oncogenesis role in bladder cancer was explored. Bioinformatics analysis revealed that miR-193a-3p could interact with ZNFX1-AS1, and this was validated by Luciferase reporter assay. The expression of miR-193a-3p was increased in T27 and SW780 cells with ZNFX1-AS1 silencing. Furthermore, miR-193a-3p expression was negatively correlated with ZNFX1-AS1 expression in bladder cancer samples. Previous studies[19-21] implied that miR-193a-3p expression was decreased in some kinds of tumors, and miR-193a-3p may be considered as tumor suppressor. The expression of miR-193a-3p is also decreased in bladder cancer and regulates cancer progression and multi-drug resistance[22,23]. We, then, explored the target mRNA of miR-193a-3p, and SDC1 was predicted and verified by Luciferase reporter assay. SDC1 is an oncogene in several cancers[24-27]. In our study, we found that SDC1 expression was decreased in bladder cancer cells with miR-193a-3p mimic transfection, and miR-193a-3p expression was negatively correlated

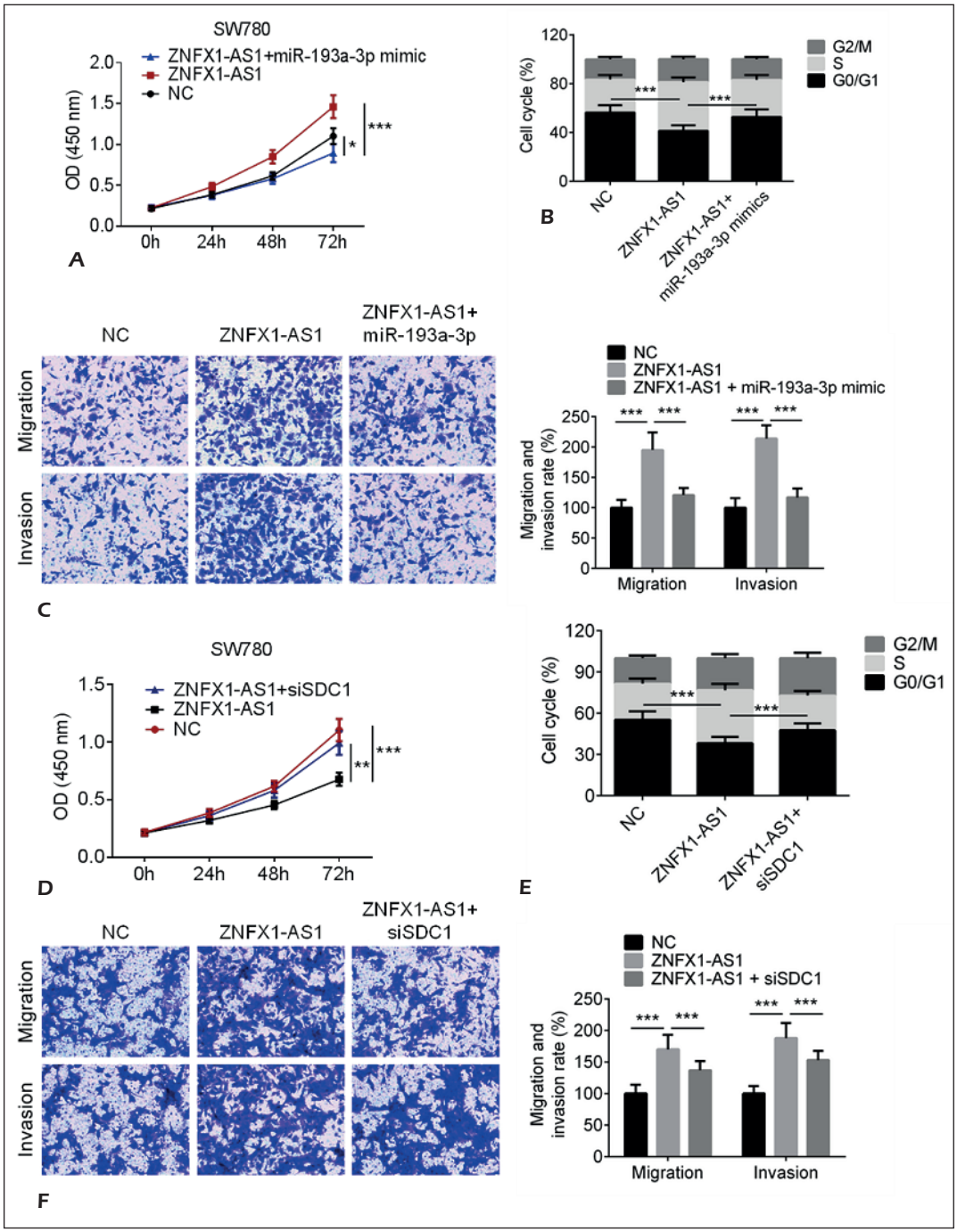

Figure 4. The miR-193a-3p mimic and siSDC1 could rescue the carcinogenic role of ZNFX1-AS1. SW780 cells were transfected with NC, ZNFX1-AS1, and ZNFX1-AS1 + miR-193a-3p, and cell proliferation (**A**), cell cycle (**B**), cell migration and invasion (**C**) were then examined (magnification, x100). SW780 cells were transfected with NC, ZNFX1-AS1, and ZNFX1-AS1 + siSDC1, and cell proliferation (**D**), cell cycle (**E**), cell migration and invasion (**F**) were then evaluated (magnification, x100). Data are shown as mean ± SD. *p<0.05, **p<0.01, and ***p<0.001.

with SDC1 expression in bladder cancer samples. Ultimately, rescue experiments were performed, and the results exhibited that ZNFX1-AS1 overexpression promoted cell proliferation, migration, and invasion of T24 and SW780 cells. While, miR-193a-3p mimic and SDC1 knockdown could reverse the carcinogenesis role of ZNFX1-AS1 on bladder cancer cells.

Conclusions

The present work indicates that ZNFX1-AS1 is overexpressed in bladder cancer clinical samples. ZNFX1-AS1 accelerates the occurrence and progression of bladder cancer by upregulating SDC1 expression *via* sponging miR-193a-3p. Moreover, the biological function and the underlying mechanism of SDC1 in bladder cancer need further clarification. ZNFX1-AS1 can function as a potential parameter for the diagnosis and molecular targeted therapy of bladder cancer.S

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Conflict of Interests

The authors declare that there are no competing interests associated with the manuscript.

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