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LncRNA AFAP1-AS1 promotes proliferation ability and invasiveness of bladder cancer cells

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Abstract. – OBJECTIVE: It was the aim of this study to explore the role and mechanism of long non-coding RNA (lncRNA) AFAP1-AS1 in the progression of bladder cancer (BCa) by *in vitro* experiments.

PATIENTS AND METHODS: AFAP1-AS1 levels in 40 pairs of clinical BCa tissue samples and normal ones collected from BCa patients were determined, and paired sample *t*-test was applied to compare the differences between groups. The prognosis data of patients with BCa were collected, and survival analysis and *t*-test were performed to specify the interplay between AFAP1-AS1 and the prognosis of BCa patients. Subsequently, AFAP1-AS1 expression level in BCa and normal cells were further confirmed by quantitative Real Time-Polymerase Chain Reaction (qRT-PCR), and Cell Counting Kit-8 (CCK-8), 5-Ethynyl-2'-deoxyuridine (EdU), and transwell assays were performed to figure out the influence of this lncRNA on the proliferation ability and invasiveness of BCa cells. Meanwhile, the interaction between AFAP1-AS1 and its sense mRNA was analyzed. We used co-transfection technology to simultaneously transfect si-AFAP1-AS1 and pcDNA3.1-AFAP1 or their corresponding negative controls into BCa cells, and cell proliferation and invasion ability in different subgroups were determined to explore the underlying mechanism through which AFAP1-AS1 plays a role in BCa progression.

RESULTS: No matter in BCa tissues or in cell samples, compared to the corresponding normal controls, AFAP1-AS1 was found highly expressed; at the same time, in invasive bladder cancer tissues, the expression level of AFAP1-AS1 was also higher than that in non-invasive tissues. Meanwhile, survival analysis revealed that patients with BCa with high expression of AFAP1-AS1 owned a shorter overall survival rate than those with low expression, indicating a negative interplay between AFAP1-AS1 expression and patients' prognosis. In addition, in BCa cell lines, according to the results of CCK-8, EDU, and transwell assays, the proliferative capacity, as well as the invasive ability of BCa cells, were found weakened after downregulation of AFAP1-AS1. Meanwhile, a negative interplay was discovered between AFAP1-AS1 and its sense mRNA. Finally, the results of cell reversal experiment using co-transfection technique revealed that overexpression of AFAP1 can reverse the inhibitory impact of lncRNAAFAP1-AS1 on the malignant ability of BCa cells.

CONCLUSIONS: AFAP1-AS1 may enhance the proliferation ability as well as the invasiveness of BCa cells so as to aggravate the degree of BCa malignancy.

Key Words:
AFAP1-AS1, BCa, Antisense RNA, Biological markers.

Introduction

Bladder cancer (BCa) is a common malignant tumor in the urinary system, accounting for a large proportion of all urinary tumors[1,2]. Among male patients, BCa ranks the fourth most common cancer, and the incidence rate of which is nearly 5 times that of female patients worldwide[3]. Due to the high incidence and mortality of BCa, the research of BCa is always in the hot field of tumor research. Although there are various treatment methods for BCa, such as surgery, chemotherapy, and radiotherapy, which have made great progress in recent years, the 5-year tumor-specific survival rate of BCa patients is still low[4,5]. The lack of in-depth understanding of the etiology of BCa and research on its pathogenesis have limited the further improvement of BCa treatment, which has become one of the most important reasons for the failure of BCa treatment. Therefore, it is urgent to find a more accurate and effective target for BCa treatment to help solve the huge social public health burden induced by BCa.

Li et al[6] have demonstrated that the occurrence of BCa is closely related to genetic and epigenetic changes. The mechanisms, including Akt, growth factor receptor (EGFR), nuclear factor kappa B (NF-κB), and other mechanisms, are engaged in the BCa progression and even the pathogenesis of tumor resistance; however, these classical pathways are still insufficient to explain the continuous progression and metastasis of BCa[7]. In re-

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cent years, long non-coding RNA (lncRNA), with transcript length greater than 200 nucleotides, has become a hot topic in tumor research, and some studies[8,9] have demonstrated that they can participate in the normal biological process and the development of human diseases under pathological conditions *via* regulating gene expression. Ramnarine et al[9] have demonstrated that LncRNA can be used to diagnose and predict the prognosis of prostate cancer, and can also be used to guide the clinical treatment of prostate cancer. In rectal cancer, a large number of lncRNA expression disorders were also found to be associated with epigenetic changes and gene splicing mutations in tumor cells[10]. The emergence of liquid biopsy technology has accelerated the clinical application of lncRNA, and the production of a large number of clinical data has provided great help for the discovery and exploration of more and more novel lncRNAs[11]. Similarly, the role of lncRNA in BCa has been widely studied, and some studies have confirmed that the expression of long non-coding RNA PART1 is abnormally highly expressed in patients with BCa, and the proliferation and apoptosis of BCa cells are affected by a series of factors after knocking down the expression of PART1[12]. Bioinformatics analysis also revealed a large number of abnormally expressed long non-coding RNAs in BCa tissues, including some that can be used as new prognostic markers for BCa[13]. Therefore, the new discoveries and studies on lncRNAs may provide new directions for the diagnosis and treatment of cancer. Among them, more attention has been paid to the mechanism of anti-sense RNA. LncRNA sox21-AS1 plays a pivotal role in the progression of cervical cancer[14], and lncRNA TTN-AS1 is closely relevant to the epithelial mesenchymal transformation of lung adenocarcinoma and other pathological processes[15]. Previously, we found that AFAP1-AS1, as a new long non-coding RNA, is of great significance in the development of various tumors[16]; however, there have been no studies on the relationship between AFAP1-AS1 and pathogenesis of BCa. The purpose of this study was to explore the specific role and related mechanisms of this oncogene in BCa.

Our previous work has indicated that lncRNA AFAP1-AS1 in BCa tissue along with cell specimens was remarkably upregulated compared with that in normal tissues and cells. In addition, comprehensive clinical analysis revealed that AFAP1-AS1 was correlated with poor prognosis of BCa patients. Therefore, in order to confirm our hypothesis, we designed corresponding *in vitro* tests and further explored the possible mechanism of AFAP1-AS1's role *via* biological test techniques.

Patients and Methods

Clinical Sample

Our tissue sample includes 40 BCa tissues and 40 corresponding normal adjacent tissues harvested from eligible BCa patients. They were primarily diagnosed and did not receive bladder perfusion therapy nor preoperative neoadjuvant therapy. Clinical data of BCa patients were recorded, including tumor staging, histological grade, metastatic state, etc. The excised tissue specimens were pathologically confirmed by two experienced independently pathologists, and they were immediately stored in a liquid nitrogen tank (Ambion, Austin, TX, USA). This study was approved by the Ethics Committee of Qujing No. 1 Hospital. Signed written informed consents were obtained from all participants before the study. This study was conducted in accordance with the Declaration of Helsinki.

Cell Culture

The BCa cell lines (T24, RT4, UMUC-3, J82) and the normal bladder epithelial cell line (SV-HUC-1) used in the experiment were purchased from the American Type Culture Collection (ATCC; Manassas, VA, USA). T24, RT4, J82 cell lines were cultured in Dulbecco's Modified Eagle's Medium (DMEM; Gibco, Rockville, MD, USA), UMUC-3 cell line was cultured in Roswell Park Memorial Institute-1640 (RPMI-1640) medium (HyClone, South Logan, UT, USA), and SV-HUC-1 cell line was cultured in F-12 medium. All cell culture medium used for cell culture was supplemented with 10% fetal bovine serum (FBS; Gibco, Rockville, MD, USA) and antibiotics, and the cells were cultured in an incubator with 5% CO_2 at 37°C.

Cell Transfection and quantitative Real Time-Polymerase Chain Reaction (qRT-PCR)

Small interfering RNA for cell transfection was purchased from Shanghai GM GenePharma (Shanghai, China) at a concentration of 50 nM. The reagent used for transfection in the BCa cell line was HilyMax Transfection Reagent (Dojindo Molecular Technologies, Kumamoto, Japan). The following is the small interfering RNAs for transfection, siRNA1, 5'-CCTATCTGGTCAACACGTATT-3';

siRNA2, 5'-GGGCTTCAATTTACAAGCATT-3'. Corresponding negative controls: 5'-UUCUCCGAACGUGUCACGUTT-3' and 5'-ACGUGACACGUUCGGAGAATT-3'. Total RNA was extracted using TRIzol reagent (TaKaRa, Dalian, China), and complementary deoxyribose nucleic acid (cDNA) was synthesized using the reverse transcription kit (Invitrogen, Carlsbad, CA, USA), which followed by qRT-PCR detection using SYBR Premix EX Taq™ (TaKaRa, Dalian, China) with ABI. For each sample, the expression levels of the genes were calculated using glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and $2^{-\Delta\Delta Ct}$ method. The primer sequences used are as follows: AFAP1-AS1, F: 5'-CACACAGGGGAATGAAGAGG-3', R: 5'-AATGGTGGTAGGAGGGAGGA-3'; AFAP1, F: 5'-CCGTGCATCAACGGCTCGCTC-3'; R: 5'-TTCACAACAGCCGCGGGATCC-3'; GAPDH, F: 5'-GAAGGTGAAGGTCGGAGTC-3'; R: 5'-GAAGATGGTGATGGGATTTC-3'.

Cell Counting Kit-8 (CCK-8) Assay

Two bladder tumor cell lines, T24 and UMUC-3, were separately digested to prepare a single cell suspension. After the two groups of cells were grown to a concentration of about 2 x 10^4 cells/ml, 100 μL of cells per well, i.e., 2000 cells/well, were plated. After incubated for 16 h, transfection was performed according to the instructions (0.2 μg plasmid + 0.5 μL transfection reagent, lipofectamine 2000). After transfected cells were cultured for 6, 24, 48, and 72 h, 10 μL of CCK-8 (Dojindo Molecular Technologies, Kumamoto, Japan) was added to the wells at each detection time point. After 3 h of culture, the optical density (OD) value was measured at 490 nm.

5-Ethynyl-2'-Deoxyuridine (EdU) Assay

Tumor cells in logarithmic growth phase were seeded in 96-well plates at 1×10^5 cells per well and cultured to normal growth stage. After adding the transfection reagent, we used the cell culture medium to dilute the EdU solution (Thermo Fisher Scientific, Waltham, MA, USA) in a ratio of 1000:1 to prepare an appropriate amount of 50 μm EDU medium; cell fixation and Apollo staining were performed according to the product specifications. Finally, image acquisition and analysis were performed under a fluorescence microscope.

Transwell Experiment

Cell invasiveness assays were performed using a transwell chamber (Corning, Corning, NY, USA). Approximately 2×10^5 cells were seeded in the upper compartment (8 mm pore size, Corning, Corning, NY, USA) and 100 μL of serum-free medium was added. The medium containing 10% FBS was added to the lower layer of the chamber for directional attraction. The upper and lower culture solutions are separated by a polycarbonate membrane. After cultured for 48 hours in a standard environment, the cells penetrating the membrane were stained with 0.5% crystal violet, and finally photographed with a microscope and counted.

Statistical Analysis

All data in this experiment were analyzed by Statistical Product and Service Solutions (SPSS; IBM Corp, Armonk, NY, USA) 20.0 and GraphPad Prism (Version X; La Jolla, CA, USA) software. Differences between two groups were analyzed by using the Student's *t*-test. Comparison between multiple groups was done using One-way ANOVA test followed by post-hoc test (Least Significant Difference). Survival analysis of BCa patients was performed using the Kaplan-Meier method. $p<0.05$ was considered statistically significant.

Results

AFAP1-AS1 is Highly Expressed in BCa

The results of gene expression analysis revealed that AFAP1-AS1 level in 40 BCa tissues was higher than that in normal adjacent bladder tissues (Figure 1A); and in bladder tumor cell lines (T24, RT4, UMUC-3, J82), although AFAP1-AS1 level was different, it was still higher than that in normal bladder cell line (SV-HUC-1) (Figure 1B). Subsequently, we collected the clinical information of patients, and then, divided the 40 BCa cases into two groups, invasive BCa group (17) and non-invasive BCa group (23), according to the clinical tumor grade of BCa. Statistical analysis revealed that AFAP1-AS1 gene expression in invasive BCa samples was remarkably higher than that in non-invasive ones (Figure 1C); in addition, BCa patients with highly-expressed AFAP1-AS1 had a shorter survival time (Figure 1D). These results suggested that the AFAP1-AS1 gene may act as a potential oncogene, which is closely relevant to the poor prognosis of BCa.

Knockdown of AFAP1-AS1 Expression Can Inhibit the Proliferation Ability and Invasiveness of BCa Cells

We selected T24 and UMUC-3 cells for subsequent experiments. Small interfering RNA

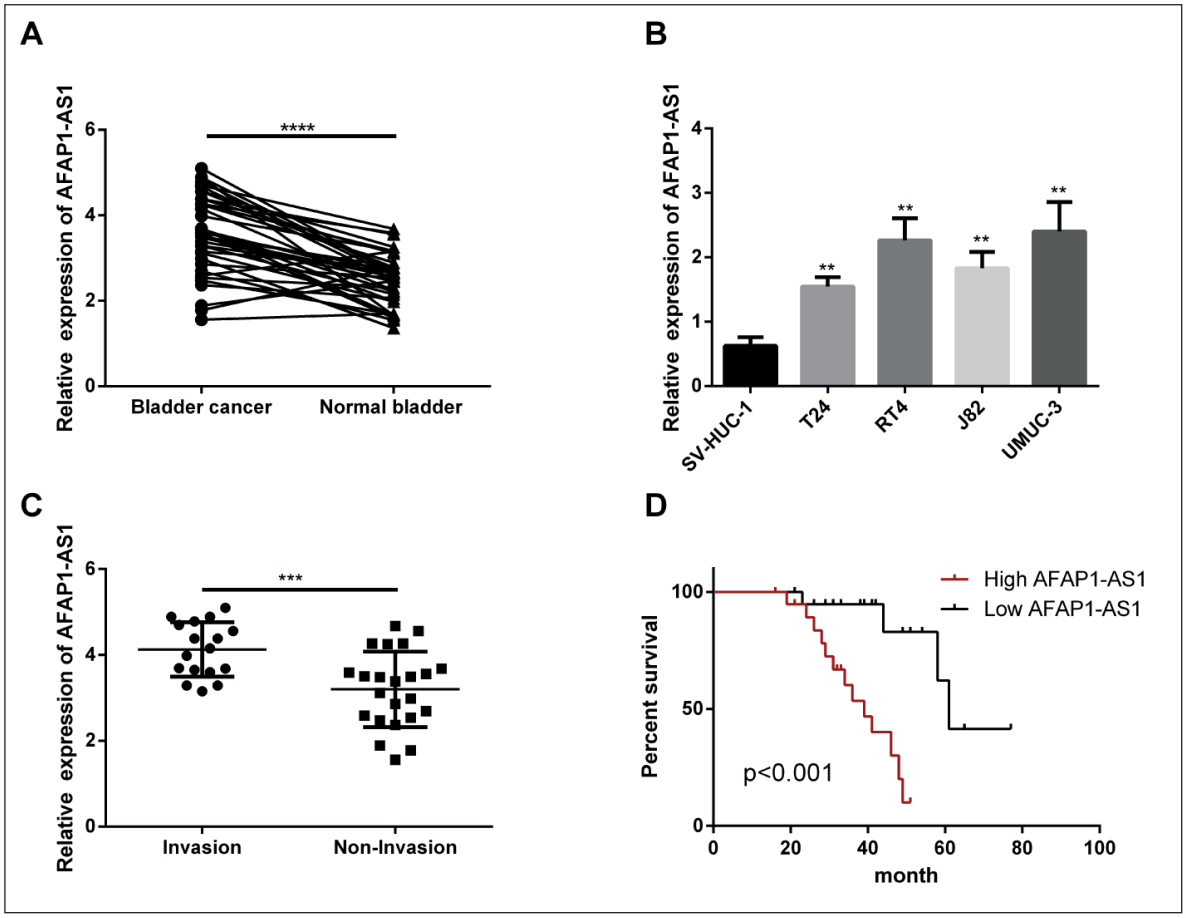

Figure 1. A, Paired sample t-test analysis of bladder cancer specimens showed that the expression level of AFAP1-AS1 in bladder cancer was higher than that of normal control bladder tissue. **B**, The expression level of AFAP1-AS1 was determined by qRT-PCR in different bladder cancer cell lines. The results showed that the expression of AFAP1-AS1 was higher than that of normal bladder epithelial cell line, SV-HUC-1. **C**, The expression level of AFAP1-AS1 was determined by qRT-PCR in different grades of bladder cancer tissue samples. The results showed that the expression of AFAP1-AS1 in the high-grade group was higher than that in the low-grade bladder cancer tissue group. **D**, Survival analysis showed that the overall survival of patients with high expression of AFAP1-AS1 was much lower than that of patients with low expression. (**p<0.01, ***p<0.001, ****p<0.0001).

was used to target knockdown AFAP1-AS1 level in BCa cells, and the transfection efficiency was determined by qRT-PCR 24 hours later; as a result, the knockdown efficiency was confirmed higher than 70% (Figure 2A). Subsequently, both the results of CCK-8 and EDU assays revealed that the cell proliferation activity of BCa cells in si-AFAP1-AS1 group was remarkably attenuated (Figure 2B, 2C); meanwhile, transwell invasion detection revealed that the migratory ability of BCa cells was also weakened after AFAP1-AS1 was downregulated (Figure 2D). The results of these *in vitro* experiments revealed an evident influence of AFAP1-AS1 expression on the proliferative and invasive activity of BCa cells.

AFAP1-AS1 Level in BCa is Negatively Correlated With AFAP1 mRNA Level

To investigate the interplay between AFAP1-AS1 and AFAP1, and its sense strand mRNA, we examined the expression level of AFAP1 in above-mentioned tissue samples. As a result, in BCa tissues, AFAP1 gene expression was lower than that in normal bladder tissue specimens (Figure 3A); meanwhile, AFAP1 revealed an increased gene level in invasive BCa tissues compared to the non-invasive ones (Figure 3B). Meanwhile, Pearson correlation analysis revealed a negative interplay between AFAP1-AS1 and AFAP1 expression in BCa tissues, and the correlation coefficient reached R^2=0.3470 (Figure 3C). In addition, the AFAP1 gene level was found

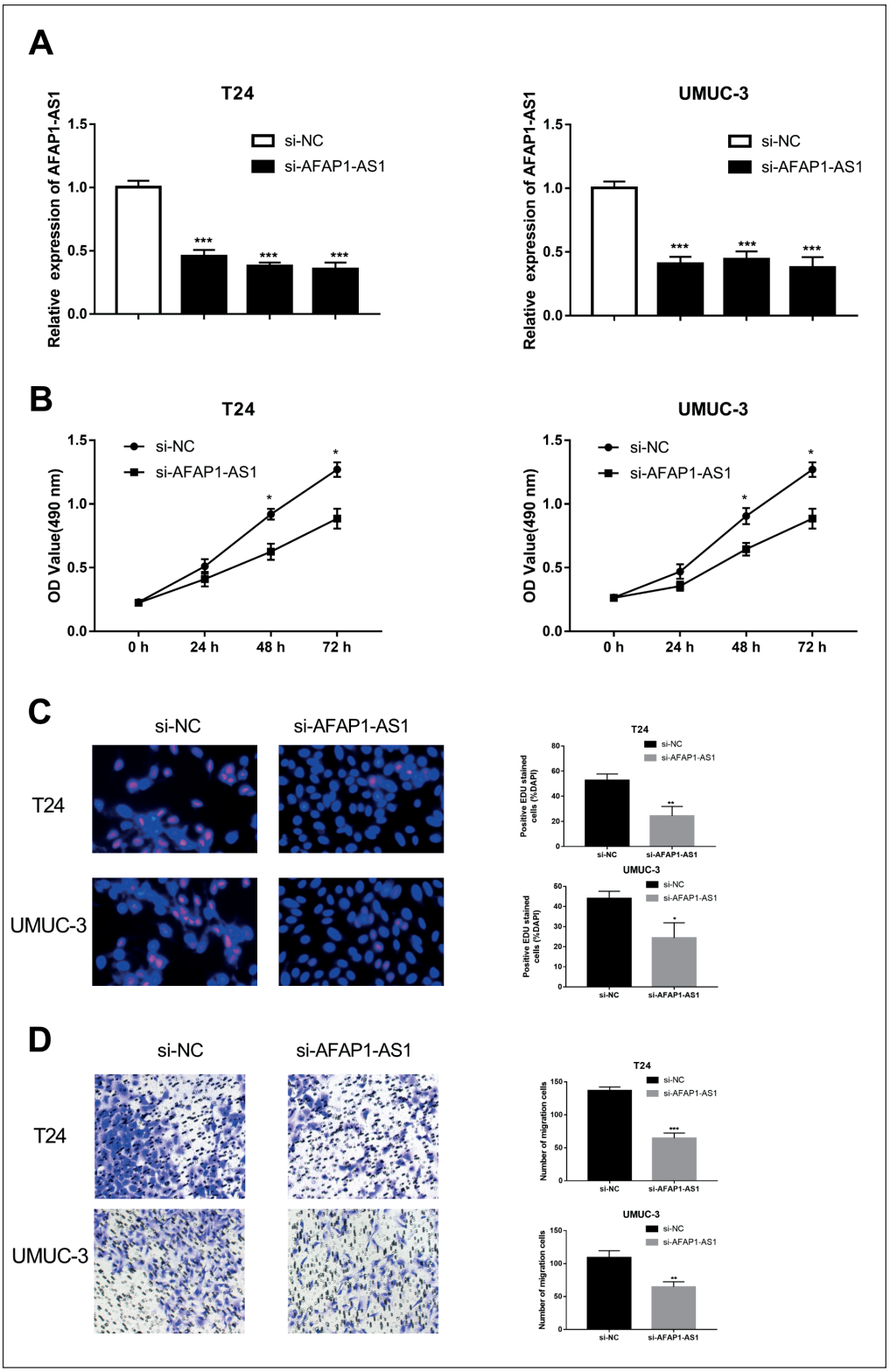

Figure 2. **A**, Transfection of si-AFAP1-AS1 in bladder cancer cell lines T24 and UMUC-3 can reduce the expression level of AFAP1-AS1 gene. **B**, CCK8 cell proliferation assay showed that knockdown of AFAP1-AS1 could inhibit the proliferation of bladder cancer cell lines T24 and UMUC-3. **C**, The results of EDU cell proliferation assay showed that knockdown of AFAP1-AS1 could inhibit the proliferation of bladder cancer cell lines T24 and UMUC-3 (magnification: 400×). **D**, transwell cell migration assay results showed that knockdown of AFAP1-AS1 could inhibit the invasion of bladder cancer cell lines T24 and UMUC-3 (magnification: 400×); (*p<0.05, **p<0.01, ***p<0.001; taking multiples of 200x).

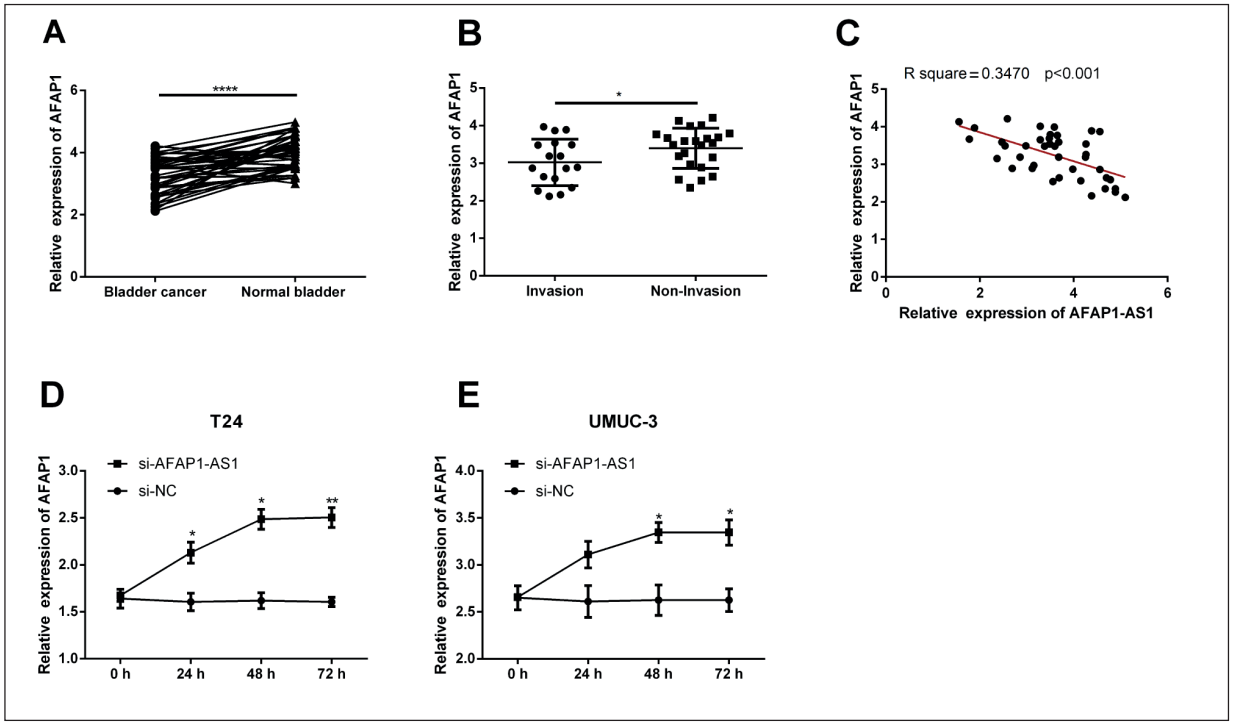

Figure 3. A, Paired sample t-test analysis of bladder cancer specimens showed that the expression level of AFAP1 in bladder cancer was lower than that of normal control bladder tissue. **B**, The expression level of AFAP1-AS1 was determined by qRT-PCR in different grades of bladder cancer tissue samples. The results showed that the expression of AFAP1 in the high-grade group was lower than that in the low-grade bladder cancer tissue group. **C**, qRT-PCR determination of AFAP1-AS1 and AFAP1 expression levels in 40 pairs of clinical samples showed that the expression of this two lncRNA in bladder cancer tissues was negatively correlated. **D**, **E**, After transfection of si-AFAP1-AS1 in bladder cancer cell lines T24 and UMUC-3, expression of AFAP1 was found up-regulated after 0, 1, 2, and 3 days; (*p<0.05, **p<0.01, ****p<0.0001).

upregulated at 24, 48, and 72 hours of transfection in T24 cells and UMUC-3 cells transfected with si-AFAP1-AS1, and the difference in gene expression was statistically significant (Figure 3D, 3E), suggesting that AFAP1 may be a potential target gene for the lncRNA AFAP1-AS1.

Overexpression of AFAP1 Reverses the Reduced Proliferation Ability and Invasiveness of BCa Cells Induced by AFAP1-AS1

We designed an *in vitro* functional reversal experiment to verify that AFAP1-AS1 is engaged in the progression of BCa *via* modulating AFAP1. Three subgroups were set, namely, si-AFAP1-AS1 group, pcDNA3.1-AFAP1+si-AFAP1-AS1 group and negative control group. It was found by CCK-8 and EDU experiments that co-transfection of pcDNA3.1-AFAP1+si-AFAP1-AS1 in BCa cell lines partially restored the reduced proliferation rate and viability of BCa cells induced by knockdown of AFAP1-AS1 (Figure 4A, 4B); in addition, transwell assay indicated that compared to the si-AFAP1-AS1 group, the longitudinal invasion ability of tumor cells was enhanced by simultaneous transfection of pcDNA3.1-AFAP1 and si-AFAP1-AS1 (Figure 4C). These results implied that overexpression of AFAP1 could reverse the influence of AFAP1-AS1 on the proliferation ability and invasiveness of BCa cells.

Discussion

At present, the overall prognosis of bladder cancer patients still remains poor, mainly due to the advanced clinical stage at the time of initial diagnosis. Therefore, early detection and diagnosis of BCa has become an effective solution to the management problems of BCa patients[17]. At present, the diagnosis of BCa mainly relies on imaging features, which lacks of effective biomarkers for early detection[18]. At the same time, due to the low sensitivity and specificity of existing examination methods, the misdiagnosis rate is still high[19].

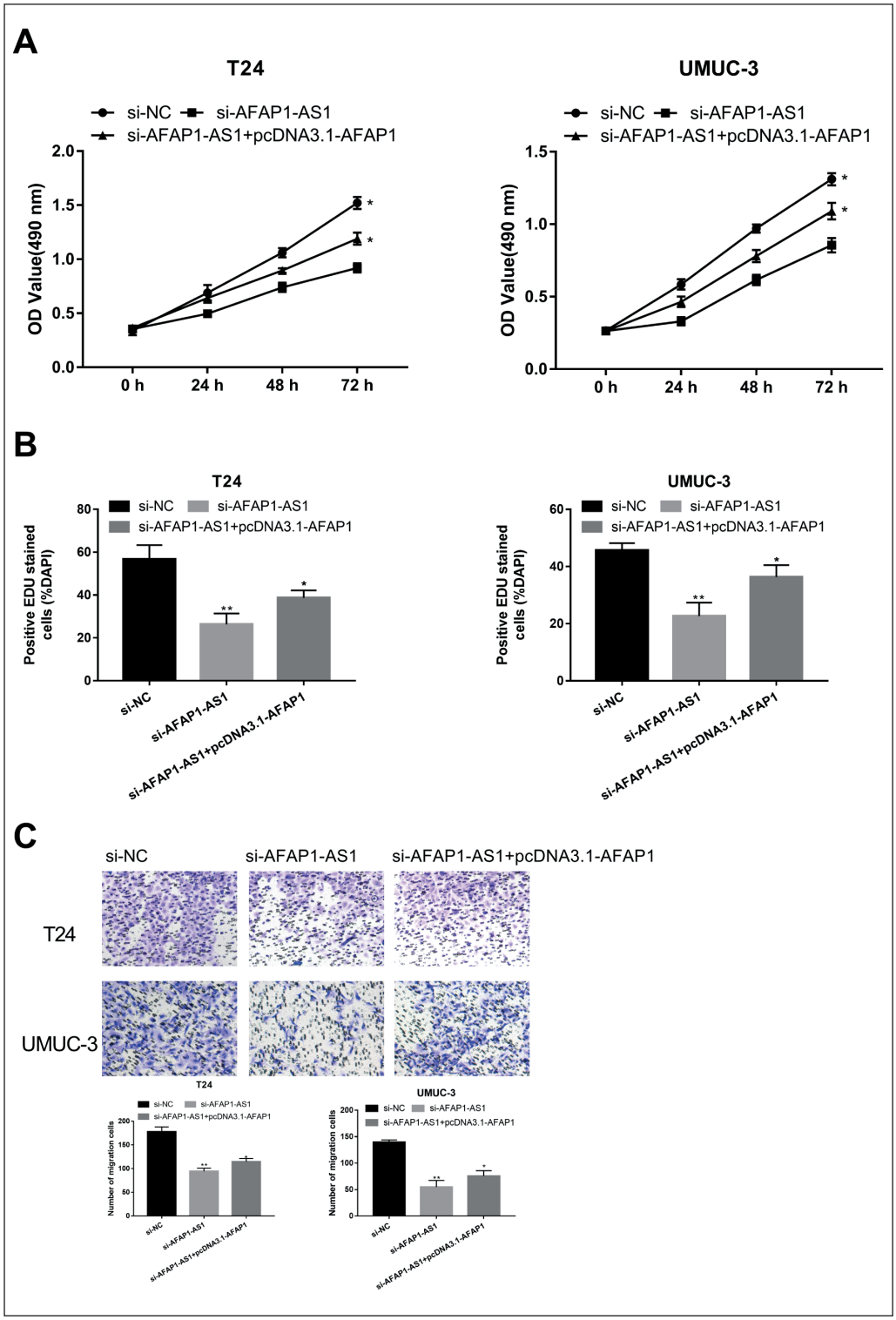

Figure 4. ***A***, The results of CCK8 cell proliferation assay showed that the proliferation of bladder cancer cell lines T24 and UMUC-3 was restored when pcDNA3.1-AFAP1+si-AFAP1-AS1 was co-transfected compared with transfection of si-AFAP1-AS1 alone. **B**, EDU cell proliferation assay showed that co-transfection of pcDNA3.1-AFAP1+si-AFAP1-AS1 restored the proliferative capacity of bladder cancer cell lines T24 and UMUC-3 compared with transfection of si-AFAP1-AS1 alone. **C**, Transwell cell invasion assay showed that the invasive ability of bladder cancer cell lines T24 and UMUC-3 was restored when co-transfected with pcDNA3.1-AFAP1+si-AFAP1-AS1 compared with transfection of si-AFAP1-AS1 alone (magnification:400×); (*p<0.05, **p<0.01, taking multiples of 200x).

Currently, the emergence of large-scale gene sequencing technology has made great progress in the research on long non-coding RNA, which has become a new marker for the diagnosis and treatment of clinical tumors and has advantages of stable expression and easy detection[20]. As one type of lncRNA, natural antisense RNA has been found to regulate the expression level of its sense mRNA by affecting its transcriptional activity and stability, and thus participate in the regulation of tumor progression[21]. AFAP1-AS1, a newly discovered natural antisense lncRNA, is located on the opposite strand of AFAP1, and has been found to be dysregulated in a variety of tumors, including gastric cancer, pancreatic cancer, cervical cancer, etc.[16,22,23]. Our previous study confirmed that AFAP1-AS1 also showed an abnormally high expression in BCa tissues. We analyzed the clinical sample data and found that AFAP1-AS1 was remarkably correlated with the prognosis of patients, including the total survival time, tumor grade, and malignancy degree.

To understand the specific impact of AFAP1-AS1 on BCa progression, we used CCK-8, EDU, transwell, and other techniques to confirm that AFAP1-AS1 can participate in regulating the proliferation ability and invasiveness ability of BCa cells, indicating that AFAP1-AS1, as an oncogene, is involved in the pathological progression of BCa. The mechanism of natural antisense RNA employs to regulate the malignant progression of tumors and includes various forms, among which, interfering with the mRNA expression of its sense strand cannot be ignored. For example, SPINT1-AS1 can affect the mRNA expression level of its corresponding sense strand SPINT1 and therefore participate in the occurrence and development of esophageal cancer[24]. Long non-coding RNA OIP5-AS1 was found to be capable of promoting tumor progression by increasing the stability of OIP5 gene, leading to a poor prognosis of tumor patients[25]. At present, there have also been studies on the role of natural antisense RNA in regulating the stability of tumor suppressor genes in breast cancer[26]. In this study, we found a significant negative correlation between AFAP1-AS1 and AFAP1 in BCa tissue samples and cell lines. In addition, it was found that high expression of AFAP1-AS1 may indicate a poor prognosis of BCa patients, and the dysregulation of AFAP1-AS1 expression could induce the development of BCa by affecting the gene transcription of AFAP1. We subsequently verified through co-transfection and biological cell function experiments that overexpression of AFAP1 could reverse the inhibitory effect of AFAP1-AS1 on BCa cells, suggesting that AFAP1 may be a potential target gene of AFAP1-AS1, which deserves further exploration and confirmation.

In summary, we revealed by qRT-PCR that AFAP1-AS1 was abnormally expressed no matter in BCa tissue samples or in cell lines. And knockdown of this lncRNA was detected by CCK-8, EDU, and transwell assays to be able to suppress the proliferative rate and invasive capacity of BCa cells.

We identified for the first time the role of lncRNA AFAP1-AS1 in the progression of BCa, which was verified in both clinical samples and BCa cell lines. In addition, a negative interplay between AFAP1-AS1 and AFAP1 was verified by *in vitro* reverse experiments; and both may be able to play a synergistic role in BCa progression, and thus become a new entry point for BCa diagnosis and treatment in the future.

Conclusions

We found for the first time that the synergistical role between AFAP1-AS1 and its synonymous mRNA, AFAP1, can promote the proliferation ability and invasiveness of BCa cells and aggravate the malignant degree of BCa cancer.

Conflict of Interests

The authors declare that they have no conflict of interest.

References

1) Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. CA Cancer J Clin 2015; 65: 87-108.
2) Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin 2011; 61: 69-90.
3) Siegel R, DeSantis C, Virgo K, Stein K, Mariotto A, Smith T, Cooper D, Gansler T, Lerro C, Fedewa S, Lin C, Leach C, Cannady RS, Cho H, Scoppa S, Hachey M, Kirch R, Jemal A, Ward E. Cancer treatment and survivorship statistics, 2012. CA Cancer J Clin 2012; 62: 220-241.
4) Rose TL, Milowsky MI. Improving systemic chemotherapy for bladder cancer. Curr Oncol Rep 2016; 18: 27.
5) Kamat AM, Hahn NM, Efstathiou JA, Lerner SP, Malmstrom PU, Choi W, Guo CC, Lotan Y, Kassouf W. Bladder cancer. Lancet 2016; 388: 2796-2810.
6) Li G, Zhang Y, Mao J, Hu P, Chen Q, Ding W, Pu R. LncRNA TUC338 is a potential diagnostic biomarker for bladder cancer. J Cell Biochem 2019; 120: 18014-18019.

7) Halim CE, Xinjing SL, Fan L, Bailey VJ, Arfuso F, Tan CH, Narula AS, Kumar AP, Sethi G, Ahn KS. Anti-cancer effects of oxymatrine are mediated through multiple molecular mechanism(s) in tumor models. Pharmacol Res 2019; 147: 104327.
8) Hu Y, Ma Z, He Y, Liu W, Su Y, Tang Z. PART-1 functions as a competitive endogenous RNA for promoting tumor progression by sponging miR-143 in colorectal cancer. Biochem Biophys Res Commun 2017; 490: 317-323.
9) Ramnarine VR, Kobelev M, Gibb EA, Nouri M, Lin D, Wang Y, Buttyan R, Davicioni E, Zoubeidi A, Collins CC. The evolution of long noncoding RNA acceptance in prostate cancer initiation, progression, and its clinical utility in disease management. Eur Urol 2019; 76: 546-559.
10) Amirkhah R, Naderi-Meshkin H, Shah JS, Dunne PD, Schmitz U. The intricate interplay between epigenetic events, alternative splicing and noncoding RNA deregulation in colorectal cancer. Cells-Basel 2019; 8: 929.
11) Pardini B, Sabo AA, Birolo G, Calin GA. Noncoding RNAs in extracellular fluids as cancer biomarkers: the new frontier of liquid biopsies. Cancers (Basel) 2019; 11: 1170.
12) Hu X, Feng H, Huang H, Gu W, Fang Q, Xie Y, Qin C, Hu X. Downregulated long noncoding RNA PART1 inhibits proliferation and promotes apoptosis in bladder cancer. Technol Cancer Res Treat 2019; 18: 1078114286.
13) Lyu L, Xiang W, Zhu JY, Huang T, Yuan JD, Zhang CH. Integrative analysis of the lncRNA-associated ceRNA network reveals lncRNAs as potential prognostic biomarkers in human muscle-invasive bladder cancer. Cancer Manag Res 2019; 11: 6061-6077.
14) Wang R, Li Y, Du P, Zhang X, Li X, Cheng G. Hypomethylation of the lncRNA SOX21-AS1 has clinical prognostic value in cervical cancer. Life Sci 2019; 233: 116708.
15) Jia Y, Duan Y, Liu T, Wang X, Lv W, Wang M, Wang J, Liu L. LncRNA TTN-AS1 promotes migration, invasion, and epithelial mesenchymal transition of lung adenocarcinoma via sponging miR-142-5p to regulate CDK5. Cell Death Dis 2019; 10: 573.
16) Li Z, Ding Z, Rong D, Tang W, Cao H. Overexpression of lncRNA AFAP1-AS1 promotes cell proliferation and invasion in gastric cancer. Oncol Lett 2019; 18: 3211-3217.
17) Cao HL, Liu ZJ, Huang PL, Yue YL, Xi JN. lncRNA-RMRP promotes proliferation, migration and invasion of bladder cancer via miR-206. Eur Rev Med Pharmacol Sci 2019; 23: 1012-1021.
18) Wu Z, Zhang Z, Xia W, Cai J, Li Y, Wu S. Extracellular vesicles in urologic malignancies-Implementations for future cancer care. Cell Prolif 2019; 52: e12659.
19) Bellmunt J, Orsola A, Leow JJ, Wiegel T, De Santis M, Horwich A. Bladder cancer: ESMO Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol 2014; 25 Suppl 3: i40-i48.
20) Chen F, Li Z, Deng C, Yan H. Integration analysis for novel lncRNA markers predicting tumor recurrence in human colon adenocarcinoma. J Transl Med 2019; 17: 299.
21) Zhang F, Li J, Xiao H, Zou Y, Liu Y, Huang W. AFAP1-AS1: a novel oncogenic long non-coding RNA in human cancers. Cell Prolif 2018; 51: e12397.
22) Lou S, Xu J, Wang B, Li S, Ren J, Hu Z, Xu B, Luo F. Downregulation of lncRNA AFAP1-AS1 by oridonin inhibits the epithelial-to-mesenchymal transition and proliferation of pancreatic cancer cells. Acta Biochim Biophys Sin (Shanghai) 2019; 51: 814-825.
23) Bo H, Fan L, Gong Z, Liu Z, Shi L, Guo C, Li X, Liao Q, Zhang W, Zhou M, Xiang B, Li X, Li G, Xiong W, Zeng Z, Cao K, Zhang S, Xiong F. Upregulation and hypomethylation of lncRNA AFAP1AS1 predicts a poor prognosis and promotes the migration and invasion of cervical cancer. Oncol Rep 2019; 41: 2431-2439.
24) Shen FF, Pan Y, Yang HJ, Li JK, Zhao F, Su JF, Li YY, Tian LQ, Yu PT, Cao YT, Zhang YW, Zhou FY. Decreased expression of SPINT1-AS1 and SPINT1 mRNA might be independent unfavorable prognostic indicators in esophageal squamous cell carcinoma. Onco Targets Ther 2019; 12: 4755-4763.
25) Wang Y, Shi F, Xia Y, Zhao H. LncRNA OIP5-AS1 predicts poor prognosis and regulates cell proliferation and apoptosis in bladder cancer. J Cell Biochem 2019; 120: 7499-7505.
26) Jadaliha M, Gholamalamdari O, Tang W, Zhang Y, Petracovici A, Hao Q, Tariq A, Kim TG, Holton SE, Singh DK, Li XL, Freier SM, Ambs S, Bhargava R, Lal A, Prasanth SG, Ma J, Prasanth KV. A natural antisense lncRNA controls breast cancer progression by promoting tumor suppressor gene mRNA stability. PLoS Genet 2018; 14: e1007802.