



Effect of long noncoding RNA NONHSAT070806 on the apoptosis, proliferation, migration, invasion and tumorigenesis of bladder cancer

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Background: Bladder cancer (BC) is the most common malignancy of the urinary tract in China, and the extent of tumor invasion negatively correlates with prognosis. The mechanism of tumor invasion in BC has been unclear until recent studies revealed the critical role of long noncoding RNAs (lncRNAs) in the proliferation and invasion of tumors. Several lncRNAs have been reported to be associated with pathogenesis in BC, but not specifically.

Methods: We used a microarray to screen the candidate lncRNAs with different expressions in BC. The expression of the lncRNAs in BC tissues or cells was identified by reverse transcription polymerase chain reaction (RT-PCR) or quantitative real-time PCR (qRT-PCR), and their ectopic expressions were measured via transfection experiment. The function of the lncRNAs was investigated by flow cytometry, caspase-3 enzyme linked immunosorbent assay (ELISA), Cell Counting Kit-8 (CCK-8), wound healing, transwell and colony formation experiments *in vitro* and xenograft experiments *in vivo*.

Results: We identified a novel sense lncRNA, NONHSAT070806, that was downregulated in BC tissues and cells and negatively correlated with level of tumor invasion in patients. Furthermore, overexpression of NONHSAT070806 induced apoptosis of T24 and 5637 cells, inhibited the proliferation, migration and invasion of BC cells, and attenuated the tumorigenesis of BC cells both *in vitro* and *in vivo*.

Conclusions: NONHSAT070806 may act as a suppressor of BC and is a potential indicator of the invasiveness of BC.

Keywords: Bladder cancer (BC); long noncoding RNA (lncRNA); NONHSAT070806

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Introduction

Bladder cancer (BC) is one of the most common malignancies of the urinary tract in China, and its incidence and mortality rates have significantly increased in the past decade (1,2). Compared with other urinary tract tumors, BC has a higher recurrence rate (~40%), which adversely affects patient prognosis (3). It is a heterogeneous disease that can be divided into two types: muscle-invasive bladder cancer (MIBC) and non-muscle-invasive bladder cancer (NMIBC). MIBC represents ~20% of newly diagnosed cases of BC, and is relatively more aggressive with a significantly higher mortality rate than NMIBC (4). At present, the pathogenesis of BC is still unclear. Inactivation of tumor suppressor genes and the activation of oncogenes have been proved to be related to the development of BC, and several biomarkers have been clinically used for early diagnosis, including bladder tumor antigen, nuclear matrix protein 22 (NPM22) and urinary fibrin degradation product (FDP) (5). However, each of them has its limitations and cannot replace cystoscopy for definitive diagnosis. Therefore, the molecular mechanisms involved in the development of BC and effective biomarkers for early diagnosis of BC is urgently need to be elucidated.

Long noncoding RNAs (lncRNAs) are RNAs that contain >200 nucleotides but do not have a protein-coding region and were previously thought to be transcriptional noise (6). However, emerging evidence demonstrates that lncRNAs are involved in multiple biological process, affecting transcription, microRNA (miRNA) expression, chromatin remodeling and protein localization (6-10). Moreover, recent research has revealed that lncRNAs may have critical regulatory functions in multiple processes of malignancies, including invasion and proliferation, by acting as oncogenes or tumor suppressors (8). Meanwhile, several lncRNAs, including ADAMTS9-AS1, RP11-89, H19, MALAT1 and TUG1, have been revealed to promote BC cell invasion and migration, negatively regulating cell apoptosis and autophagy, facilitating tumorigenesis and ferroptosis, or promoting glutamine-driven anaplerosis of BC cells (11-16). However, none of them has been used clinically, and the specific lncRNAs involved in BC tumorigenesis have not been fully elucidated.

In this study, we identified a novel sense lncRNA, NONHSAT070806, which was downregulated in BC patients and negatively correlated with tumor invasion. Further experiments demonstrated that ectopic expression of NONHSAT070806 in T24 and 5637 BC cells

induced apoptosis via the caspase-3 pathway. Moreover, the upregulation of NONHSAT070806 promoted the migration and invasion of BC cells. Additionally, overexpression of NONHSAT070806 inhibited the tumorigenesis of T24 and 5637 cells both *in vitro* and *in vivo*. Our findings suggest that NONHSAT070806 may act as a suppressor of BC and may be a potential indicator of the invasiveness of BC. We present the following article in accordance with the MDAR and ARRIVE reporting checklists (available at <https://tau.amegroups.com/article/view/10.21037/tau-22-644/rc>).

Methods

Clinical samples

A total of 35 patients with BC, 24 with MIBC, who underwent surgery between 2013 and 2016 at the PLA General Hospital were included in this research. Cancer tissues and pair-matched adjacent normal tissues were snap-frozen in liquid nitrogen immediately after resection by the surgeon. This research was performed with the approval of the Ethics Committee of the PLA General Hospital (No. 20170039), and informed consent was given by all subjects. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

Cell lines and cell culture

Two BC cell lines (T24 and 5637) were purchased from the China Infrastructure of Cell Line Resources (Beijing, China). The cells were cultured in RPMI-1640 medium (Gibco, Gaithersburg, MD, USA) supplemented with 10% fetal bovine serum (FBS) and 1% antibiotics (100 U/mL penicillin and 100 µg/mL streptomycin sulfate) in a humidified 5% CO₂ atmosphere at 37 °C.

lncRNA microarray

Total RNA of either tumor tissue or adjacent normal tissue from 5 MIBC patients was purified by the RNeasy Mini Kit (Agilent, Santa Clara, CA, USA) and then labeled by the Quick Amp Labeling Kit, One-Color (Agilent) following the manufacturer's instructions. Labeled cDNA was purified by the RNeasy Mini Kit (Agilent) and then hybridized with an Agilent Gene Expression Hybridization Kit (Agilent) for 17 h. After hybridization, the slides were washed with a Gene Expression Wash Buffer Kit (Agilent) and scanned

by an Agilent Microarray Scanner (Agilent). Data were extracted with Agilent Feature Extraction (Agilent).

Plasmid DNA transfection

The NONHSAT070806 sequence was synthesized and subcloned into the pENTER vector (Vigene, Rockville, MD, USA). Next, either pENTER-NONHSAT070806 (pLnc) or empty vector was transfected into T24 or 5637 cells using Lipofectamine 2000 Transfection Reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol.

RT-PCR and qRT-PCR

RNA was extracted from BC tissue, normal tissue or cells using TransZol Up reagent (TransGene, Beijing, China) according to the manufacturer's protocol and treated with RNase-free DNase. RNA was reverse transcribed to cDNA using a Prime Script RT Reagent Kit with gDNA Eraser (TOYOBO, Osaka, Japan) following the manufacturer's instructions. Reverse transcription polymerase chain reaction (RT-PCR) was performed with an RT-PCR SuperMix Kit (TransGene) according to the manufacturer's protocol with an ETC-811 PCR Instrument (Eastwin, Beijing, China). Quantitative real-time PCR (qRT-PCR) was performed with a Top Green qPCR SuperMix kit (TransGene) according to the manufacturer's protocol, with an IQ5 Multicolor Real-time PCR Detection System (Hercules, CA, USA), and each group was tested in triplicate. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as the normalization control. The primer sequences are listed below.

NONHSAT070806 primers:

5'-TTCTTGCCCTTCTGCT-3' (forward)

5'-GAACAACCTGCTCCTG-3' (reverse)

GAPDH primers:

5'-CTGACCTGCCGTCTAGAAAAAC-3' (forward)

5'-GTCTCTCTCTTCCCTCTTGTGCTCT-3' (reverse)

Flow cytometry assay

Cell apoptosis was evaluated by flow cytometry. After transfection with either pLnc or empty vector for 24 or 48 h, T24 and 5637 cells were collected and double stained with FITC-Annexin V and PI using the Annexin V-FITC/PI Cell Apoptosis Detection Kit (TransGene) according to

the manufacturer's protocol. The results were analyzed by FlowJo 7.6.1 software.

Caspase-3 ELISA

Caspase-3 activity was measured using the Caspase-3 Colorimetric Assay kit (Beyotime, Nanjing, China) according to the manufacturer's protocol. Briefly, cells transfected with pLnc, empty vector or negative control were lysed and centrifuged. The supernatant was transferred to a 96-well plate and mixed with buffer containing the substrate peptides for Caspase-3 conjugated to p-nitroanilide (pNA). After incubation at 37 °C for 2 h, the samples were assayed using ELISA at an optical density (OD) of 405 nm. Caspase-3 activity was expressed as a percentage compared with the negative control.

Cell Counting Kit-8 (CCK-8) assay

Cell migration was determined by a CCK-8 assay kit (DOJINDO, Shanghai, China). T24 cells (1×10^4) or 5637 cells (2×10^4) transfected with either pLnc or empty vector were seeded in 96-well plates and incubated for 24 h at 37 °C. Next, 10 μ L of the CCK-8 solution was added to each well at 0, 1, 2 and 3 days after transfection. Absorbance was read at an OD of 450 nm with 595 nm as a reference. Each group was tested in triplicate.

Wound healing assay

Cell motility was determined by the wound healing assay. Cells transfected with either pLnc or empty vector were seeded in 6-well plates at a density of 4×10^5 cells/well. At 24 h, wounds in each well of cells were created with a sterile 10- μ L pipette tip. After 12 or 24 h, wound closure was imaged using a digital camera system.

Transwell assay

Cell migration and invasion were determined by the transwell assay with a transwell insert (8 μ m, Corning, New York, USA). The upper chamber was precoated (for invasion detection) or not (for migration detection) with 1:6 diluted Matrigel (Corning). A total of 2×10^5 cells transfected with either pLnc or empty vector was resuspended in 10% FBS plus RPMI-1640 medium and added to the upper chamber. The lower chamber was filled with 500 mL of complete medium. After incubation for 24 h at 37 °C, the

medium was removed and the chambers were washed twice with phosphate-buffered saline (PBS). The cells in the lower chamber were fixed with formaldehyde, stained with 0.5% crystal violet solution, and counted in five randomly selected fields.

Clonogenic assay

A clonogenic assay was designed to determine the tumorigenesis of BC cell lines *in vitro*. The 200 T24 or 5637 cells were seeded in 6-well plates after transfection and incubated for 2 weeks. The cells were washed with PBS and stained with 0.5% crystal violet in 20% methanol for 5 min. The number of colonies (>50 cells) was counted under a microscope and the clone formation rate was calculated as follows: (clone number/number of incubated cells) × 100%.

Xenograft experiments

A subcutaneous xenograft mouse model was established to assess the inhibitory effect of overexpression of A on the tumorigenicity of BC cells *in vivo*. A total of 10 Balb/c nude mice (male, 18–20 g, 5 weeks old) were purchased from Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China) and randomly divided into two groups (5 mice/group): a treatment group (pLnc) and a control group (empty vector), using a random number method. A total of 2×10^6 5637 cells transfected with either pLnc or empty vector were subcutaneously injected into the right flank of the nude mouse in different group, respectively (n=5). After 21 days, tumor volume (V) was examined every week for 6 weeks using the formula $V = (L \times W^2)/2$ (where L is the length and W is the width of the tumor). Mice were humanely killed when the tumor volume exceeded 1,500 mm³ or at the 63rd day after the start of treatment. Once the mice were dead, the tumor tissue was separated and weighed. For each animal, three different investigators were involved: the first investigator administered the treatment procedure; the second investigator was responsible for the injections the third investigator recorded data and conducted the statistical analysis. All animal experiments were performed in the Animal center of Beijing Institute of Basic Medical Sciences in accordance with the national guidelines for the care and use of animals, and were approved by the Animal Care and Use Committee of the Beijing Institute of Basic Medical Sciences (No. IACUC-DWZX-2017-057). A protocol was prepared before the

study without registration.

Statistical analysis

The statistical significance of differences was determined using analysis of variance (ANOVA), *t*-test or χ^2 test. Differences were considered statistically significant at P values <0.05. All quantitative data are presented as the mean ± standard deviation. The percentage (%) was used to express enumeration data. Each data point was analyzed in triplicate using SPSS 17.0 software (IBM, Chicago, IL, USA).

Results

Downregulation of lncRNA NONHSAT070806 in BC tissues and cells, and association of its expression with level of tumor invasion in patients

To discover lncRNAs relationship to BC, a microarray analysis was performed to identify lncRNAs with ectopic expression in BC tissue compared with normal bladder tissue. A total of 9,653 lncRNAs were found to be differentially regulated in tumor tissues, 4,699 of which were downregulated (P<0.05, fold change >2) (Figure 1A). Among them, 4 lncRNAs with significant differential expression (fold change >25) in the tumor compared with the paired normal tissue were selected for further investigation (Table 1). The expression levels of the four lncRNAs were first detected using qRT-PCR in 10 patient tumor tissues compared with adjacent normal tissues. Notably, the 900-nt lncRNA NONHSAT070806 was found to be decreased in 7 of the 10 tumor samples, whereas the levels of the other three candidates varied (Figure 1B). The expression profile of NONHSAT070806 was further measured in another 24 patient samples, of which 17 (70.8%) cancer tissues exhibited downregulation of NONHSAT070806 compared with corresponding normal tissues (P<0.05) (Figure 1C,1D). In addition, the expression level of NONHSAT070806 was significantly lower in the MIBC group than in the NMIBC group (P<0.05) (Figure 1E). Next, the RT-PCR results confirmed that NONHSAT070806 was not expressed in either T24 or 5637 cells (Figure 1F). Subsequently, we investigated the correlation between NONHSAT070806 expression and clinical features (i.e., sex, age, smoking history, tumor size and tumor invasion) in the patients with BC. Our results indicated that the expression level of NONHSAT070806 negatively correlated with the tumor

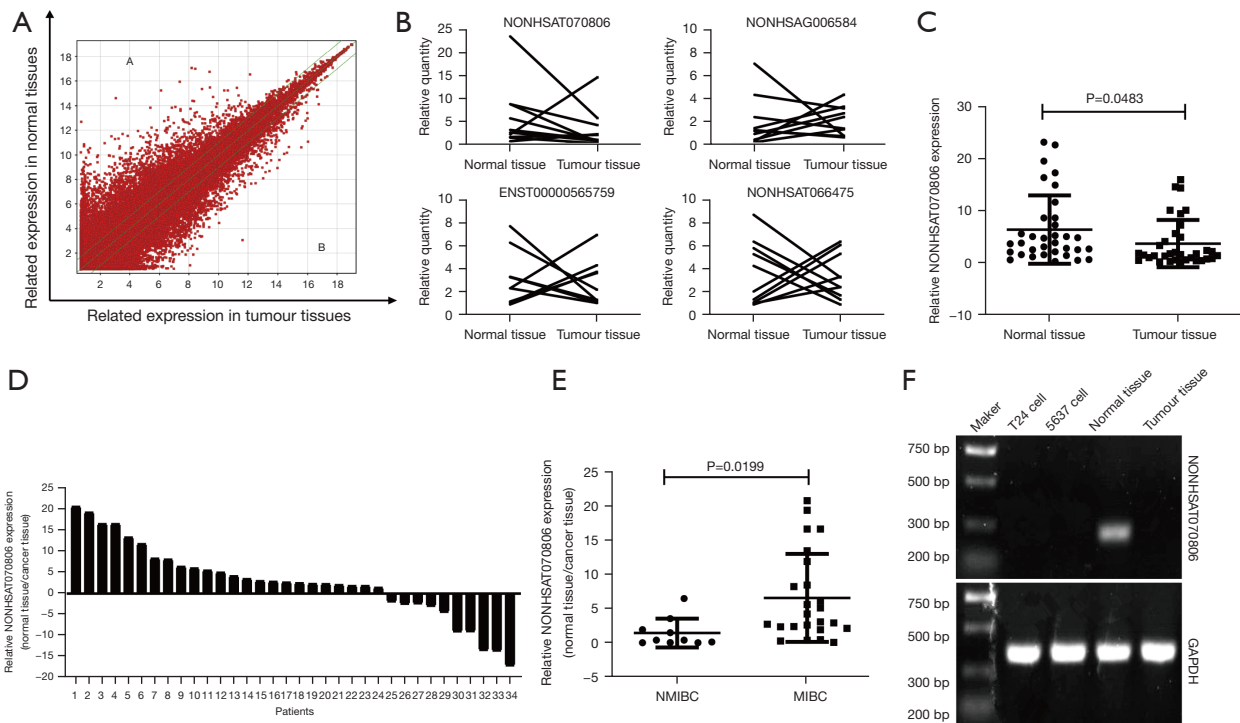


Figure 1 Downregulation of NONHSAT070806 in bladder cancer tissues and cells, and negative association of its expression with tumor invasion. (A) Microarray analysis results for lncRNAs were differentially expressed in cancer tissues compared with normal bladder tissues. Spots falling in area A represent lncRNAs upregulated in tumor tissues (>2-fold), while spots falling in area B represent lncRNAs upregulated in normal tissues (>2-fold). (B) Validation of the four lncRNA candidates in 10 patient samples using qRT-PCR. (C,D) Expression of NONHSAT070806 in tumor tissues and normal tissues from 34 patients with bladder cancer, as determined using qRT-PCR. (E) Expression of NONHSAT070806 in patients with MIBC and NMIBC. (F) Expression of NONHSAT070806 in bladder cancer cells and tissues, as determined using RT-PCR. GAPDH was used as internal control. The P values were analyzed by paired *t*-test (D) or unpaired *t*-test (E). Data are shown as the mean \pm SD. lncRNA, long noncoding RNA; qRT-PCR, quantitative real-time PCR; MIBC, muscle-invasive bladder cancer; NMIBC, non-muscle-invasive bladder cancer; RT-PCR, reverse transcription polymerase chain reaction; GAPDH, glyceraldehyde 3-phosphate dehydrogenase.

Table 1 Candidate lncRNAs identified by microarray analysis

No.	Fold change	Regulation: cancer vs. normal	Source	lncRNA name
1	47.933964	Up	Ensembl	ENST00000565759
2	45.415073	Up	NONCODE v4	NONHSAT066475
3	84.374916	Down	NONCODE v4	NONHSAT070806
4	63.21236	Down	NONCODE v4	NONHSAG006584

lncRNA, long noncoding RNA.

Table 2 Characteristics of bladder cancer patients

Characteristics	Patient, No. (%)	NONHSAT070806		F value	P value
		Downregulation	Upregulation		
Total	34 (88.2)	24	10		
Sex					
Male	30 (88.2)	21	9	0.04	0.843
Female	4 (11.8)	3	1		
Age (years)					
>60	18 (52.9)	12	6	0.269	0.608
≤60	16 (47.1)	12	4		
Smoking history					
Yes	15 (44.1)	12	3	1.115	0.299
No	19 (55.9)	12	7		
Tumor size					
>3 cm	15 (44.1)	10	5	0.199	0.656
≤3 cm	19 (55.9)	14	5		
Tumor invasion					
Muscle invasion	24 (70.6)	20	4	6.384	0.012*
Non-muscle invasion	10 (29.4)	4	6		

*, P<0.05 was considered to be significant.

invasion level (P<0.05) but was not related to the other four clinical features examined (Table 2).

Effect of overexpression of NONHSAT070806 on apoptosis of BC cells

To investigate whether overexpression of NONHSAT070806 could affect the biological activity of BC cells, we synthesized the pLnc expression vector and achieved ectopic expression of NONHSAT070806 in T24 and 5637 cells following transfection with the vector (Figure 2A). Cell apoptosis was first detected by flow cytometry assay. At 24 and 48 h after transfection, cells transfected with pLnc exhibited increased apoptosis rates compared with cells transfected with empty vector (P<0.05) (Figure 2B). Furthermore, the activity of caspase-3, which is a marker of cell apoptosis, was detected by ELISA at 24 h after transfection. The results showed that overexpression of NONHSAT070806 promoted the expression of caspase-3 (P<0.05), indicating that NONHSAT070806 may induce apoptosis of BC cells via

the caspase-3 pathway (Figure 2C,2D).

Effect of overexpression of NONHSAT070806 on proliferation, migration, and invasion of BC cells in vitro

Next, a series of arrays were analyzed to determine whether overexpression of NONHSAT070806 could alter the malignant behavior of BC cells. First, proliferation of T24 and 5637 cells was detected by CCK-8 assay. As shown in Figure 3A,3B, after overexpression of NONHSAT070806 for 48 and 72 h, the proliferative ability of T24 and 5637 cells was suppressed compared with the control group (P<0.05). Moreover, the wound healing assay results demonstrated that overexpression of NONHSAT070806 inhibited the migration of T24 and 5637 cells (Figure 3C,3D). Subsequently, transwell assays were performed to further measure the migration and invasion of cancer cells. As shown in Figure 3E-3H, overexpression of NONHSAT070806 significantly decreased the migration and invasion of T24 and 5637 cells compared with cells transfected with empty vector (P<0.05).

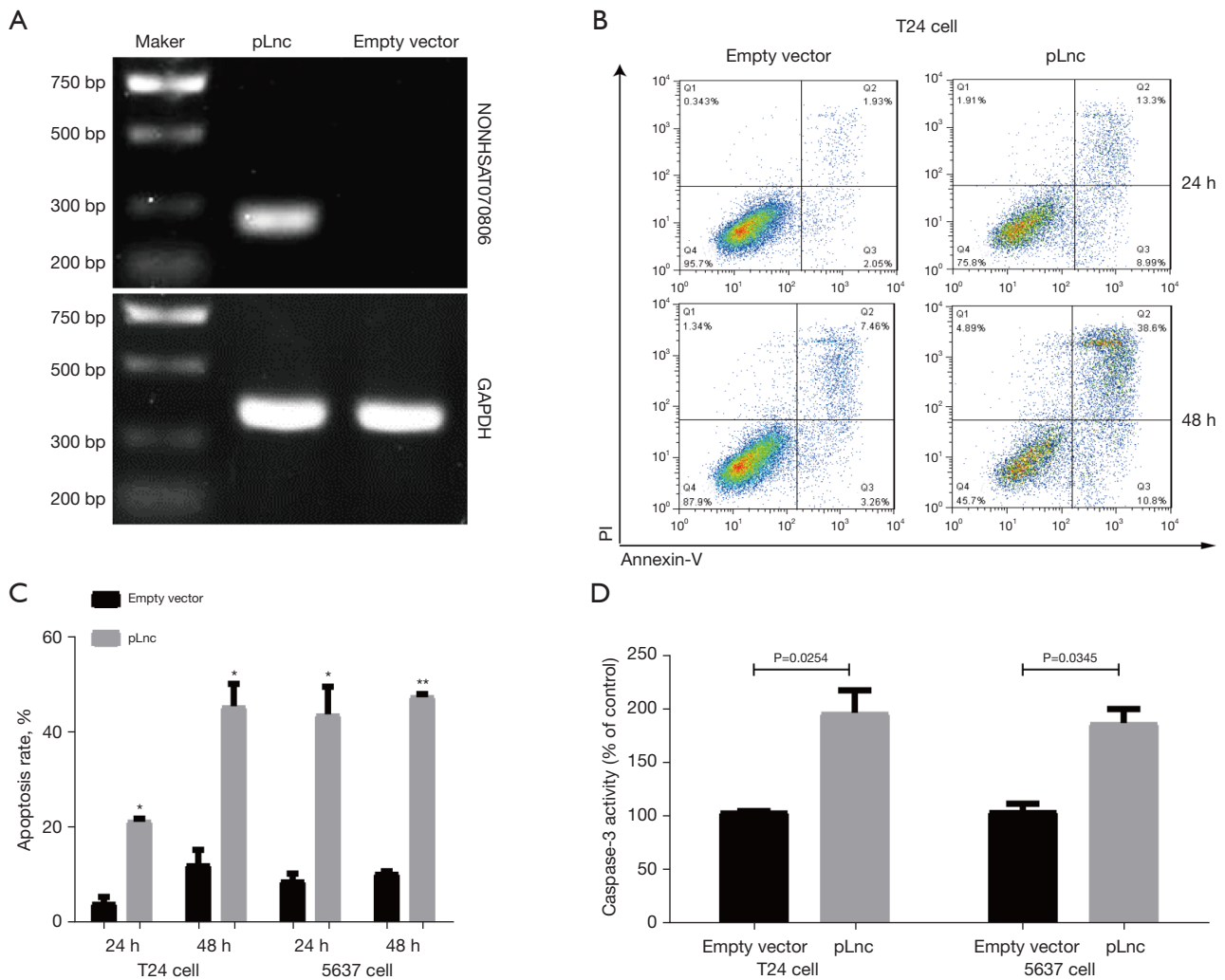


Figure 2 Overexpression of NONHSAT070806 inducing apoptosis of bladder cancer cells via the caspase-3 pathway. (A) Validation of the transfection of pLnc in T24 and 5637 cells using RT-PCR. GAPDH was the internal control. (B,C) Apoptosis of T24 and 5637 cells was determined using flow cytometry after transfection with pLnc or empty vector for 24 or 48 h. The Q2 and Q3 zones represent cells in late apoptosis and early apoptosis, respectively. (D) Caspase-3 activity measured via ELISA. The P values were analyzed by unpaired *t*-test (C,D) (*, $P < 0.05$; **, $P < 0.01$). Data are shown as the mean \pm SD. PI, propidium iodide; RT-PCR, reverse transcription polymerase chain reaction; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; ELISA, enzyme linked immunosorbent assay.

Effect of overexpression of NONHSAT070806 on tumorigenesis of BC cells *in vitro* and *in vivo*

To further evaluate the role of NONHSAT070806 in BC cell tumorigenesis, we first designed a plate clone formation assay. The results demonstrated a significant decrease in colony number in the pLnc group compared with the empty vector group after 2 weeks ($P < 0.05$) (Figure 4A,4B), indicating that overexpression of NONHSAT070806 attenuated the tumorigenesis of T24 and 5637 cells *in vitro*.

Moreover, in the xenograft nude mouse model established to evaluate the effect of NONHSAT070806 on BC cell tumorigenesis *in vivo*, the tumor volume was significantly smaller in the pLnc group than in the empty vector group ($P < 0.05$) (Figure 4C-4E). Additionally, 6 weeks after injection, the tumor weight in the pLnc group was obviously less than that in the empty vector group ($P < 0.05$). These results demonstrated that, consistent with the *in vitro* results, overexpression of NONHSAT070806 attenuated the tumorigenesis of BC cells *in vivo*.

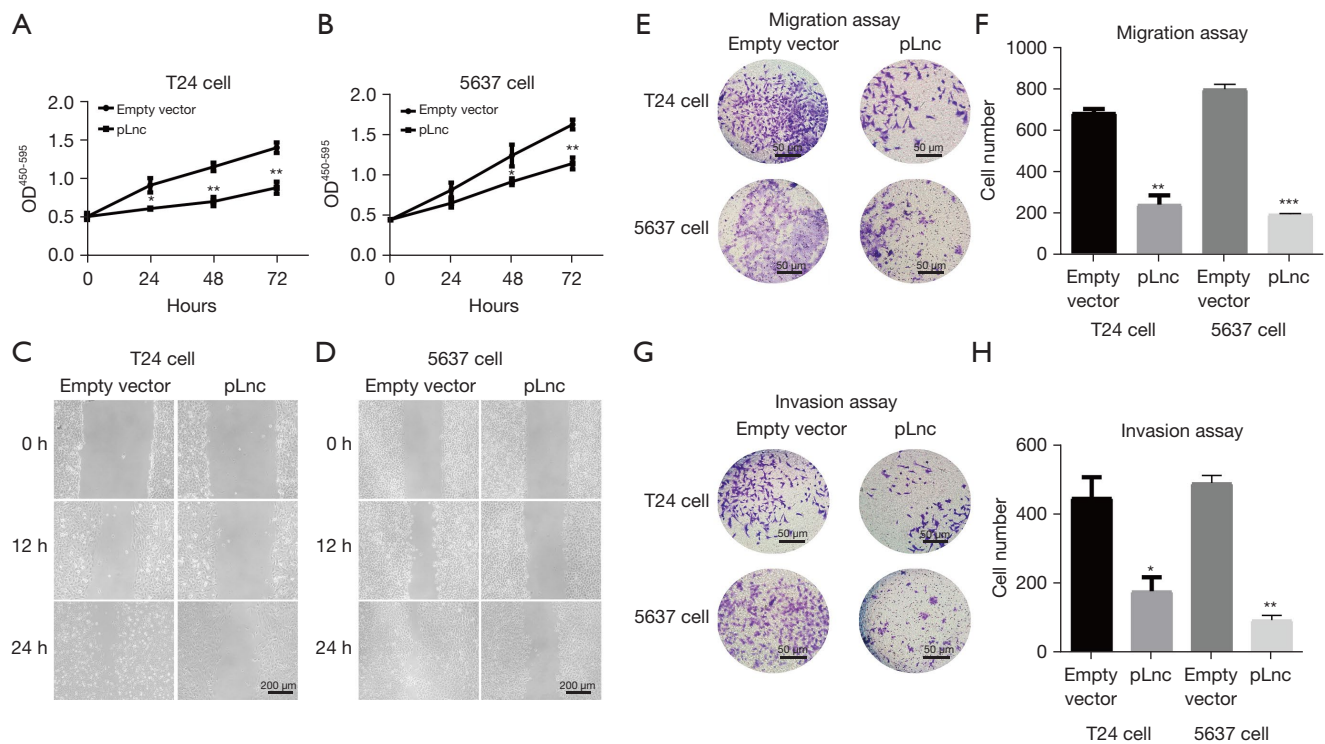


Figure 3 Overexpression of NONHSAT070806 stimulates the proliferation, migration, and invasion of bladder cancer cells *in vitro*. Proliferation of T24 (A) and 5637 (B) cells after transfection with pLnc or empty vector was determined using CCK-8 assays. (C-F) Cell migration was detected using wound healing assays (C,D) and transwell migration (E,F) assays. (G,H) Cell invasion was determined using transwell invasion assays. The empty vector group served as the negative control. Cells were stained with 0.1% crystal violet in (E,G). The P values were analyzed by ANOVA (A,B) or unpaired *t*-test (F,H) (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$). Data are shown as the mean \pm SD. OD, optical density; CCK-8, Cell Counting Kit-8; ANOVA, analysis of variance.

Discussion

BC has the highest incidence among all urinary system tumors in China and accounts for nearly 170,000 deaths worldwide annually (17). The early symptoms are not obvious, which poses a great challenge for early diagnosis and treatment. The most common pathological type of BC is urothelial carcinoma, accounting for >90% of all cases. Other types include squamous cell carcinoma, adenocarcinoma, and other rare subtypes such as clear cell carcinoma, small cell carcinoma and carcinoid. Bladder urothelial carcinoma is one of the most frequently mutated human cancers, following lung and skin cancer, in mutation rates, adverse effecting its clinical treatment (3). Surgery is the traditional treatment for BC, including radical cystectomy and cystoscopy-guided resection of tumor, but it has not improved the high recurrence rate (15–90%) and low 5-year survival rate (50–60%) (17–19). Platinum-based chemotherapy is a first-line option but has disadvantages

such as severe side effects and poor drug tolerance (20). Recently, immunotherapy, including anti-program death-1 (PD-1) and program death-ligand 1 (PD-L1) antibodies, has been approved for clinical application in the treatment of BC, but it still has some challenges such as only a limited number of patients exhibiting clear and durable responses and a subset of patients showing drug resistance (21).

lncRNAs can be divided into five types according to their genomic location and context: sense, antisense, bidirectional, intronic, and intergenic (7). lncRNAs often expressed lower but stronger tissue-specific effects than that of mRNAs (22,23). Moreover, lncRNAs, unlike mRNAs, are found in the cell nuclei, cytoplasm, and mitochondria (24). Studies have shown that lncRNAs affect the pathways of division, growth, and cell differentiation, and are also involved in cellular death processes (7,24). Some lncRNAs, including PCGEM1, H19, MALAT1 and HOTAIR, have been well studied, and have been proved as related to prostate,

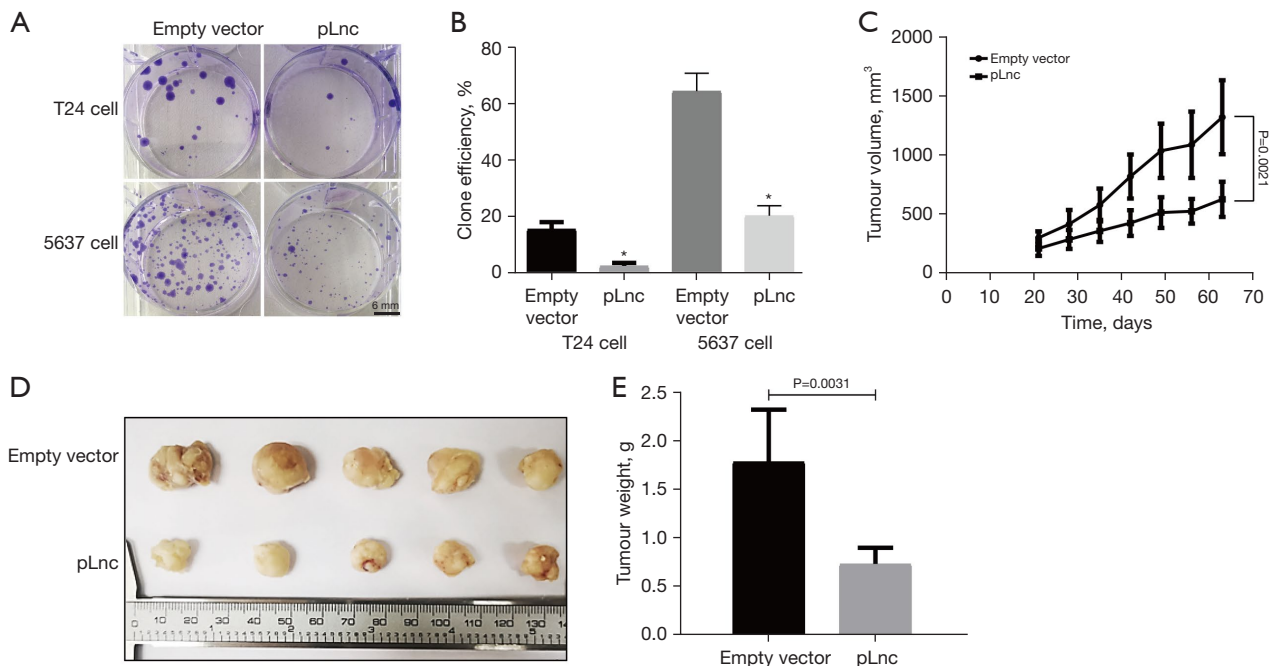


Figure 4 Overexpression of NONHSAT070806 inhibits the tumorigenesis of bladder cancer cells both *in vitro* and *in vivo*. (A,B) *In vitro* tumorigenesis was detected using clonogenic assays. The cells were fixed and stained with 0.1% crystal violet in (A). (C) Tumor volume growth curves over 1 month after injection. (D) Image of tumors removed from nude mice. (E) Histological analysis of tumor weight in the pLnc and empty vector groups. Data are shown as the mean \pm SD. *, $P < 0.05$.

colon, lung, liver, breast, and BC (7,14,25). However, as we know, most of the lncRNAs reported to be involved in the progression of BC have been found to be upregulated in tumor tissues. We identified a novel sense lncRNA located on human chromosome 2, NONHSAT070806, that was downregulated in both BC patients and cells. Additionally, ectopic expression of NONHSAT070806 induced cell apoptosis via the caspase-3 pathway, and inhibited the proliferation, migration and invasion of BC cells. Clonogenic assay and xenograft experiments revealed that overexpression of NONHSAT070806 inhibited the tumorigenicity of BC cells both *in vitro* and *in vivo*, suggesting that NONHSAT070806 may be a tumor suppressor.

Tumor aggressiveness is negatively related to patient prognosis and survival for most malignant tumors, including BC. Indeed, the 5-year survival rate for patients with MIBC is $< 50\%$, which is significantly lower than that for patients with NMIBC (17). Moreover, approximately 50% of MIBC patients ultimately develop disease at distant sites because of disseminated micrometastases (17). In our study, we found that the lncRNA NONHSAT070806

was downregulated in BC tissues and negatively correlated with the tumor invasion level in patients. Moreover, cytological experiments further proved that upregulation of NONHSAT070806 inhibited the invasion of BC cells. These results highlighted the crucial role of NONHSAT070806 in the invasiveness of BC.

At present, the molecular mechanisms of lncRNAs in cancer development remain unclear (26). Emerging evidence indicates that lncRNAs may play their role by either regulating corresponding miRNAs or through posttranslational modifications of key metabolism-related proteins, including ubiquitination, phosphorylation, and acetylation (27-29). In our future studies we plan to clarify the specific miRNAs or genes modulated by NONHSAT070806 and explore the possible signaling pathway which NONHSAT070806 may be involved in, to elucidate its possible molecular mechanism in the development and invasion of BC. Meanwhile, the diagnostic performance of a single lncRNA for cancer remains low at present. Therefore, we hope to add relevant content on the use of multiple lncRNAs in combination with NONHSAT070806 for the diagnosis of bladder cancer in

future studies. Furthermore, we also anticipate increasing the number of BC patients and nude mice.

In summary, NONHSAT070806 may act as a suppressor of BC and may be used as a potential biomarker for local regional metastasis in BC. Meanwhile, our finding highlighted the crucial role of NONHSAT070806 in the invasion of BC and suggests novel ideas for further exploration of the biomarkers of BC.

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Footnote

Reporting Checklist: The authors have completed the MDAR and ARRIVE reporting checklists. Available at <https://tau.amegroups.com/article/view/10.21037/tau-22-644/rc>

Data Sharing Statement: Available at <https://tau.amegroups.com/article/view/10.21037/tau-22-644/dss>

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at <https://tau.amegroups.com/article/view/10.21037/tau-22-644/coif>). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. This research was performed with the approval of the Ethics Committee of PLA General Hospital (No. 20170039). The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). Informed consent was obtained from all subjects included in the study. All animal experimental protocols of the study were in accordance with the national guidelines for the care and use of animals, and were approved by the Animal Care and Use Committee of Beijing Institute of Basic Medical Sciences (No. IACUC-DWZX-2017-057).

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