

ORIGINAL ARTICLE

LncRNA-SNHG7 regulates proliferation, apoptosis and invasion of bladder cancer cells

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Summary

Purpose: The purpose of this study was to investigate the expression of long-chain non-coding RNA (lncRNA) SNHG7 in bladder cancer tissues and cell lines, and to explore its impact on bladder cancer cell proliferation, apoptosis and invasion processes.

Methods: Bladder cancer tissues and near-cancer tissues were collected. The expression of lncRNA-SNHG7 in tissues and cell lines was detected by real-time PCR (RT-PCR). The expression of lncRNA-SNHG7 was downregulated by RNA interference (siRNA) as detected by RT-PCR that was used to detect the interference efficiency. CCK-8, flow cytometry and Transwell assays were used to evaluate the effect of lncRNA-SNHG7 on the proliferation, apoptosis and invasion capability of bladder cancer cells. The downregulation effect of lncRNA-SNHG7 on Epithelial-Mesenchymal Transition (EMT) related markers was tested by western blot.

Results: lncRNA-SNHG7 was upregulated in bladder can-

cer cell lines. After the expression of lncRNA-SNHG7 was downregulated, the proliferation of bladder cancer cells was decreased, the apoptosis was increased, and the invasion ability of cells was decreased. The expression of E-cadherin was increased, but the expression of N-cadherin, vimentin and snail were decreased. Increased expression of lncRNA-SNHG7 in cancer tissues was significantly related to tumor size, metastasis and stage.

Conclusions: This study showed that lncRNA -SNHG7 is overexpressed in bladder cancer tissues and cells. Down-regulation of lncRNA-SNHG7 can inhibit the proliferation of bladder cancer cells and promote apoptosis, as well as inhibit cell invasion, which provides a potential molecular target for future tumor targeted therapy.

Key words: bladder cancer, cell invasion, cell proliferation, EMT, lncRNA-SNHG7

Introduction

Bladder cancer is a malignancy that occurs in the bladder mucosa. It is the most common malignant tumor of the urinary system, but also one of the top ten common tumors in humans. It shows the highest incidence of genitourinary tumors in our country, while its incidence in the West is second only to prostate cancer [1]. In 2012, the incidence of bladder cancer in the Chinese Cancer Registration was 6.61/100,000, ranking 9th in in-

cidence among malignant tumors. Bladder cancer can occur at any age, even in children. Its incidence increases with age. The pathological types of bladder cancer include bladder urothelial carcinoma, bladder squamous cell carcinoma, adenocarcinoma of the urinary bladder, and the rare bladder subtypes like clear cell carcinoma, bladder small cell carcinoma and bladder carcinosarcoma. One of the most common is bladder urothelial carcinoma, ac-

counting for more than 90% of the total number of patients with bladder cancer [2].

Long-chain non-coding RNAs (lncRNAs) are non-coding RNAs >200 nucleotides in length that are involved in the development of various diseases [3,4]. Up to date, many researchers found that lncRNAs are abnormally expressed in many malignant tumors and are closely related to tumor cell proliferation, apoptosis and invasion. For instance, lncRNA-NKILA could suppress melanoma invasion-metastasis cascade through modulating NF- κ B pathway [5] and lncRNA-TP73-AS1 is involved in the regulation of liver cancer cell proliferation [6]. However, the current level of expression of lncRNA-SNHG7 in bladder cancer and its function is still unclear. This article focused on lncRNA-SNHG7 expression in bladder cancer tissues and cell lines to explore its impact on bladder cancer cell proliferation, apoptosis and invasion processes, and to provide a strong theoretical basis for clinical diagnosis and treatment.

Methods

Clinical sample collection

Surgical bladder cancer tissues were collected along with matched near-cancer tissues. All samples were quickly placed in liquid nitrogen and immediately stored in -80°C cryogenic refrigerator. This study was approved by the ethics committee of Shunde Hospital of Southern Medical University. Signed written informed consents were obtained from all participants before study entry.

Materials and reagents

Bladder cancer cell lines (T24, J82 and SW780) and normal human bladder cell line (SV-HUC-1) were purchased from the Institute of Biochemistry and Cell Biology, Shanghai Institutes for Biological Sciences. SV-HUC-1 cells were cultured in Ham's F12 medium (Sigma, St. Louis, MO, USA). RPMI1640 medium, fetal bovine serum (FBS) was purchased from Gibco (Rockville, MD, USA), RNA extraction reagent Trizol and reverse transcription kit were purchased from Japan (TaKaRa Co., Tokyo, Japan), lncRNA-SNHG7 interference RNA (siRNA lncRNA-SNHG7) and si-NC were purchased from Shanghai Jima Co. (Shanghai, China), cell transfection reagent Lipofectamine 2000 and CCK-8 reagent were purchased from the Invitrogen Corporation (Carlsbad, CA, USA), Matrigel and Transwell chamber from the BD Biosciences (Franklin Lakes, NJ, USA), and dimethyl sulfoxide, crystal violet dye and PBS were purchased from the Hyclone (South Logan, UT, USA).

Real-time quantitative PCR

The total RNA was extracted by TRIzol method and the expression of lncRNA-SNHG7 and GAPDH in tissues and cells was detected by Taqman

probe. The upstream primer of lncRNA-SNHG7 was: 5'-GTTGGGGTGTGGCATTCTTGTT-3'; the downstream primer was: 5'-TGGTCAGCCTGGTCACTCTGG-3'; the upstream primer of GAPDH was: 5'-ACCCAGAAGACTGTG GATGG-3'; the downstream primer of GAPDH was: 5'-TTCTAGACGGCAGGTCAGGT-3'.

Cell transfection experiments

Cells were cultured in RPMI1640 medium containing 10% FBS and placed in a 5% CO₂ incubator at 37°C. When the cells were in the logarithmic growth phase, they were seeded in 6-well plates at a density of about 40%. Five μ L Lipofectamine 2000 and 200 pmol siRNA lncRNA-SNHG7 mixture were added to each well. lncRNA-SNHG7 siRNA sequence was: 5'-GCUGGAAUAAAGAGUAACAUU-3'.

Cell proliferation CCK-8 experiments

The transfected cells were seeded in 96-well plates, with 100 μ L RPMI1640 medium without 10% FBS medium per well to ensure that the number of cells per well was about 2000. After 24, 48 and 72 hrs of culturing, each well was supplemented with 10 μ L of CCK-8 reagent and incubated for 2 hrs to measure the optical density (OD) at 450 nm. The experiment was repeated three times in each group.

Flow cytometry

At 48 hrs following transfection, the transfectants were collected and washed twice with PBS. The cells were fixed in ethanol and stained with 50 μ g/ml Annexin V-Fluorescein isothiocyanate (FITC) (BD Biosciences, Franklin Lakes, NJ, USA) and 10 μ g propidium iodide (PI) (Sigma-Aldrich) at 4°C overnight. Stained cells were analyzed by flow cytometry and the experiments were performed in triplicate.

Cell invasion with transwell experiment

Matrigel and 10% FBS plus RPMI-1640 media were diluted 1:5, and 50 μ L of the diluted gel were applied to the transwell chamber for coagulation. Cells in the logarithmic growth phase were digested and resuspended in 10% FBS plus RPMI-1640 medium and added to transwell chambers with approximately 2×10^4 cells in each chamber. 700 μ L medium containing 10% fbs were added to the lower chamber and cells were incubated for 24 hrs. Then the medium was discarded, and the transwell chambers were washed with PBS three times. Anhydrous methanol was used for fixation for 20 min. Following this, crystal violet was used for staining for 30 min. Lastly, transwell chambers were placed under a microscope and 5 fields were randomly selected. The positively stained cells were counted and analyzed.

Statistics

SPSS 19.0 software (IBM, Armonk, NY, USA) was used for statistical analyses. All quantitative data were expressed as mean \pm standard deviation. Comparison between groups was done using the One-Way ANOVA test, followed by *post hoc* test (least significant difference).

Percentage (%) was used to express the enumeration data and χ^2 test was used for data analyses. P value < 0.05 was considered statistically significant.

Results

lncRNA-SNHG7 was highly expressed in bladder cancer cell lines

The expressions of lncRNA-SNHG7 in bladder cancer cell lines and the normal cell line were detected via RT-PCR assay. The results of qRT-PCR showed that the expression of lncRNA-SNHG7 in bladder cancer cell lines was higher than that in human normal bladder cells ($p < 0.05$; Figure 1).

lncRNA-SNHG7 modulated bladder cancer cell proliferation and apoptosis

The siRNA lncRNA-SNHG7 was used to interfere with the expression of lncRNA-SNHG7 and to verify its efficiency. The results of qRT-PCR showed that, compared with si-NC (normal control), the expression of lncRNA-SNHG7 was significantly lower ($p < 0.05$) (Figure 2A). The results also showed that siRNA lncRNA-SNHG7 could inhibit the expression of lncRNA-SNHG7 in bladder cancer cells. The siRNA lncRNA-SNHG7 and si-NC were transfected into cells, and the cell proliferation

was detected by CCK-8 proliferation assay. The results showed that there was a significant difference in the proliferation at 48 and 72 hrs ($p < 0.05$). After the expression of lncRNA-SNHG7 was down-regulated, cell proliferation was significantly reduced (Figures 2B and C).

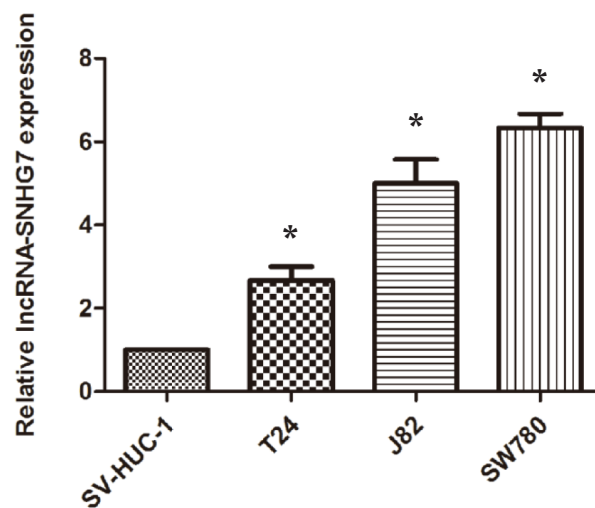


Figure 1. lncRNA-SNHG7 was upregulated in cell lines. The relative expression of lncRNA-SNHG7 was detected in tumor cell lines (T24, J82 and SW780) and compared with the normal cell line (SV-HUC-1) by RT-PCR. GAPDH was regarded as an internal control. * $p < 0.05$.

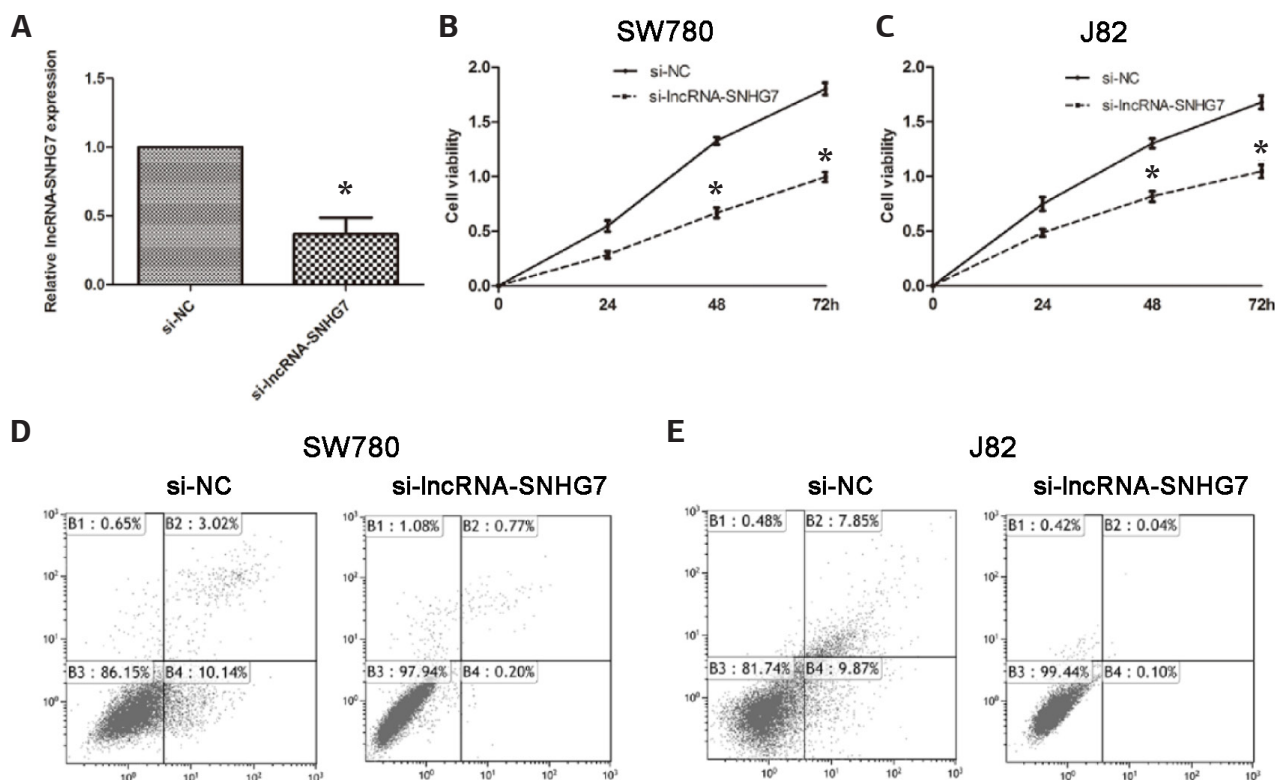


Figure 2. lncRNA-SNHG7 can modulate bladder cancer cell proliferation and cell apoptosis. **A:** Downregulation of lncRNA-SNHG7 by siRNA. * $p < 0.05$, compared with si-NC. **B and C:** CCK-8 assay was performed to detect cell proliferation between si-NC and si-lncRNA-SNHG7. * $p < 0.05$, compared with si-NC. **D and E:** flow cytometry was carried out to evaluate cells apoptosis between si-NC and si-lncRNA-SNHG7.

Flow cytometry was conducted to detect the regulatory effect of lncRNA-SNHG7 on cell apoptosis. The results showed that cell apoptosis of siRNA lncRNA-SNHG7 was markedly higher compared with the control group, suggesting that downregulation of lncRNA-SNHG7 could promote apoptosis of bladder cancer cells (Figures 2D and E).

In summary, silencing lncRNA-SNHG7 can inhibit bladder cancer cell proliferation and promote cell apoptosis.

Downregulation of lncRNA-SNHG7 expression inhibited the invasiveness of bladder cancer cells and regulated the expression of related molecular markers during epithelial-mesenchymal transition (EMT)

The invasiveness of bladder cancer cells transfected with siRNA lncRNA-SNHG7 was significantly lower than that of the transfected si-NC control group by Transwell invasion assay. This result indicates that downregulation of lncRNA-SNHG7

expression significantly suppresses the invasion of bladder cancer cells ($p < 0.05$, Figures 3A and B).

The expression of lncRNA-SNHG7 in EMT was further detected by western blot. The results showed that the expression of E-cadherin was up-regulated and the expression of N-cadherin, vimentin and snail was downregulated after lncRNA-SNHG7 was lowly expressed, which indicated that downregulation of lncRNA-SNHG7 could inhibit the EMT process and bladder cancer cells' invasiveness (Figures 4A and B).

lncRNA-SNHG7 was highly expressed in bladder cancer tissues

Interestingly, the results of qRT-PCR showed that the expression of lncRNA-SNHG7 in bladder cancer tissues was higher than that in adjacent tissues ($p < 0.05$, Figure 5). Based on the clinical information of patients with bladder cancer, we found that the expression of lncRNA-SNHG7 was

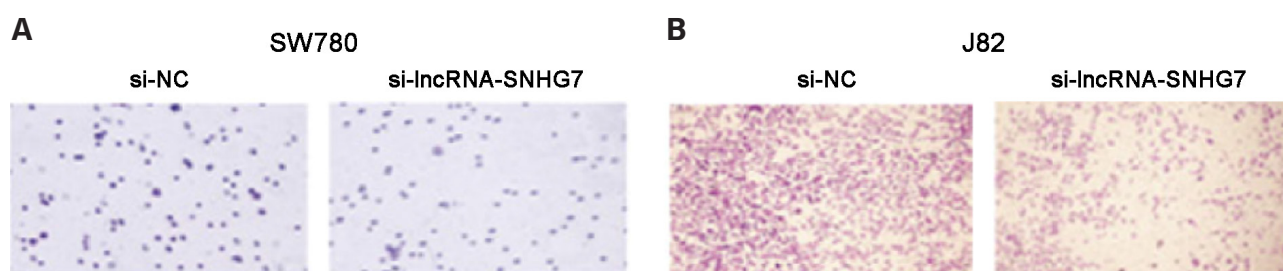


Figure 3. Downregulation of lncRNA-SNHG7 inhibited cell invasion. **A** and **B**: compared with si-NC, si-LNCRNA-SNHG7 significantly inhibited the invasive ability of SW780 and J80 by transwell invasion assay, respectively.

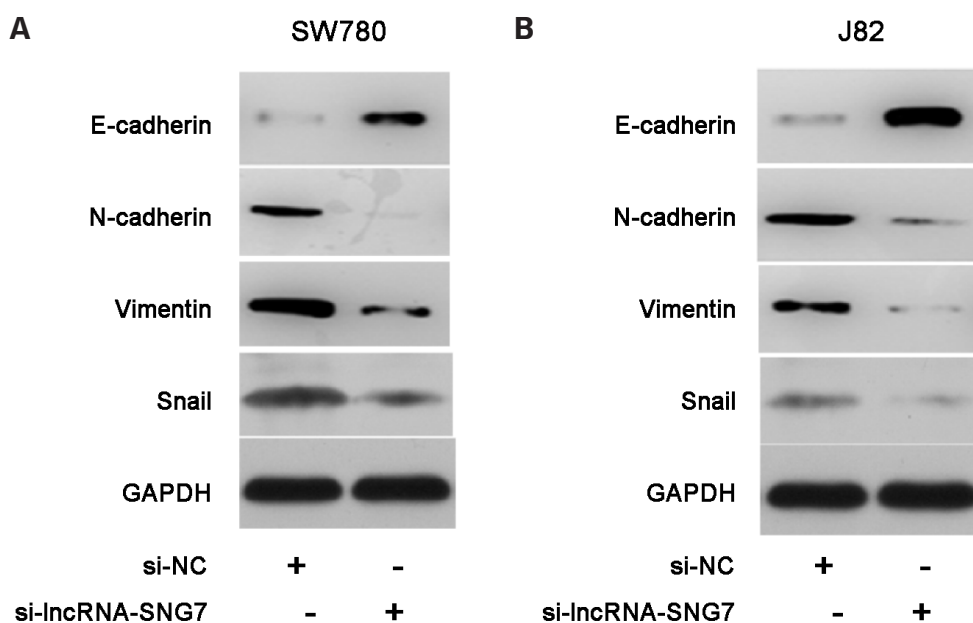


Figure 4. Abnormal expression of lncRNA-SNHG7 regulated EMT-related markers. **A** and **B**: After transfection with si-lncRNA-SNHG7, EMT-related markers such as E-cadherin, N-cadherin, Vimentin and Snail expression levels were detected by western blot assay in cells lines. The results showed that the expression of E-cadherin was upregulated and the expression of N-cadherin, vimentin and Snail were downregulated after lncRNA-SNHG7 was lowly expressed.

not related to the patient's gender and age, but was closely related to tumor size, metastasis and stage (Table 1). This result further indicated that lncRNA-SNHG7 acted as an important molecule modulating the development of bladder cancer.

Discussion

In our country, bladder cancer is one of the common malignant tumors. In recent years, an accumulating number of reports has found that abnormally expressed lncRNAs play an important regulatory role in the development of bladder cancer. lncRNA-n336928 is upregulated, is correlated with tumor progression and can predict poor prog-

nosis for patients with bladder cancer [7]. UCA1 upregulation enhances cisplatin resistance of bladder cancer cells via regulating Wnt signaling depending on Wnt6 [8]. Luo et al. confirmed that upregulated lncRNA H19 enhances bladder cancer metastasis by associating with EZH2 and inhibiting E-cadherin (CAD) expression [9]. Another study reported that H19 induced downregulation of YAP1, which modulates cell proliferation and migration [10]. The role of lncRNA-SNHG7 in cancer development has also been found and reported, e.g. in lung cancer, lncRNA-SNHG7 enhances cell proliferation and invasion and suppresses cell apoptosis by upregulating FAIM2 expression [11]. lncRNA-SNHG7 is increased in non-small cell lung cancer (NSCLC), functions as oncogene, and is correlated with overall survival. She et al. reported that lncRNA-SNHG7 contributes to NSCLC progression by acting as competitive endogenous RNA (ceRNA) antagonizing miR-193b [12].

EMT process refers to the epithelial cells transforming into mesenchymally phenotypic cells [13]. EMT main features are the decrease of expression of cell adhesion molecules (such as E-cadherin), the transformation of cytokeratin, vimentin-based cytoskeleton, and morphologically the cells develop characteristics of mesenchymal cells [14]. By EMT, the epithelial cells lose the epithelial phenotype, such as loss of cell polarity and loss of connectivity with the basement membrane, and obtain higher interstitial phenotypes such as migration and invasion, anti-apoptosis and ability to degrade extracellular matrix. EMT is an important biological process in which epithelial cell-derived

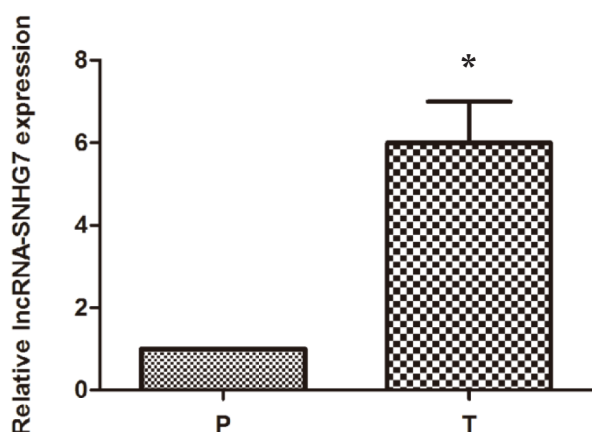


Figure 5. lncRNA-SNHG7 expression was increased in bladder cancer tissues. The relative expression of lncRNA-SNHG7 was detected by RT-PCR assay. P refers to corresponding adjacent samples, T refers to tumor samples. GAPDH served as the control group. * $p < 0.05$.

Table 1. Association between lncRNA-SNHG7 high or low expression and clinical characteristics

Factor	n	lncRNA-SNHG7		p value
		High	Low	
Gender				0.233
Male	87	44	43	
Female	47	21	26	
Age (years)				0.570
<60	40	23	17	
≥60	52	25	27	
Tumor size (cm)				0.000
<3	35	19	16	
≥3	46	14	32	
Lymph nodes metastasis				0.000
No	41	30	11	
Yes	57	19	38	
Tumor stage				0.014
Ta, T1	39	14	25	
≥T2	48	30	18	

malignant cells acquire the ability to migrate and invade [15,16]. Some studies have shown that EMT plays a crucial role in the development, progression and metastasis of malignant tumors, which provides a therapeutic approach in the treatment for those tumors as a target [17,18].

In this study we focused on the role of lncRNA-SNHG7 in the proliferation, apoptosis and invasion of bladder cancer cells. First, the results of *in vitro* cell experiments showed that the expression of lncRNA-SNHG7 in bladder cancer cells was significantly higher than that of normal bladder cells. Transfection of specific siRNA lncRNA-SNHG7 reduced the expression of lncRNA-SNHG7 in bladder cancer cells. Second, this study confirmed by CCK-8, flow cytometry and Transwell experiments that downregulation of lncRNA-SNHG7 could inhibit cell proliferation and promote apoptosis, and in addition, it could inhibit the EMT process, thereby inhibiting cell invasion. Finally, our study showed that lncRNA-SNHG7 expression in bladder cancer

was significantly higher than in the near-cancer tissues, and correlated with tumor size, metastasis and stage. It also showed that lncRNA-SNHG7 as a tumor-promoting molecule is closely related to the development of bladder cancer.

Conclusions

In summary, this study showed that lncRNA-SNHG7 is overexpressed in bladder cancer tissues and cells. Downregulation of lncRNA-SNHG7 can inhibit the proliferation of bladder cancer cells and promote apoptosis, as well as inhibit cell invasion, which provides a potential molecular target for future tumor targeted therapy. Certainly, further potential functions and mechanisms need to be explored.

Conflict of interests

The authors declare no conflict of interests.

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