Down-regulation LncRNA-SNHG15 Contributes to Proliferation and Invasion of Bladder Cancer Cells

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Research Article

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Abstract

OBJECTIVES: This study aimed to investigate effect of IncRNA-SNHG15 in bladder carcinoma using cell lines experiments and the relationship between clinical characteristics and IncRNA-SNHG15 expression was analyzed.

Methods. Bladder cancer tissues and near-cancer tissues were collected. The expression of IncRNA-SNHG15 in tissues and cell lines was detected by real-time PCR (RT-PCR). The expression of IncRNA-SNHG15 was downregulated by interference (siRNA) as detected by RT-PCR that was used to detect the interference efficiency. CCK-8, and Transwell assays were used to evaluate the effect of IncRNA-SNHG15 on the proliferation, invasion capability of bladder cancer cells. t-test was used for Statistical analyses, were performed using the Statistical Graph pad 8.0.1.224 software.

Result: The expression of IncRNA-SNHG15 was up regulated in 5637, UMUC3 and T24 cell lines compared with corresponding normal controls (P<0.05). up regulation was positively related to tumor stage (P = 0.015), and tumor size (P =0.0465). The down-regulation of IncRNA-SNHG15 with siRNA significantly inhibited UMUC3 and T24 cell proliferation and invasion.

Conclusion: This study showed that IncRNA-SNHG15 is overexpressed in bladder cancer tissues and (5637, UMUC3 T24) cell lines. up regulation was positively related to tumor stage (P = 0.015), and tumor size (P =0.0465). The down-regulation of IncRNA-SNHG15 with siRNA significantly inhibited UMUC3 and T24 cell proliferation and invasion, which provides a potential molecular target for future tumor targeted therapy.

Background

Bladder cancer has a higher incidence in urinary malignancies. Due to its susceptibility to recurrence, progression, and metastasis, the current ideal treatment for bladder cancer is a comprehensive including surgery treatment, chemotherapy and Radiation therapy, but the overall effect is limited, and the invasion and metastasis are the main reasons of bladder cancer treatment failure[1]. Primary bladder tumors usually originate from the muscular is and mucosal epithelium, the latter accounting for about 95%, which about 90–95% are urothelial carcinoma. Bladder cancer has the characteristics of spatial Polycentric and recurrence in time, and has the biological characteristics of local invasion and metastasis and high postoperative recurrence rate. 70–80% of bladder cancer patients are initially diagnosed as noninvasive urinary epithelial papilloma. After active surgery and bladder perfusion treatment, 50% of patients are still diagnosed with non-invasive urinary papilloma. 70% of the patients relapse within 5 years and 10–30% of the patients develop invasive urothelial cancer [2]. As a result of sequencing the human genome, it was found that the proportion of protein-coding genes in the entire human genome sequence was less than 3%, and more than 80% of the sequences were frequently transcribed into RNA without protein-coding functions. These RNA without protein-coding functions are called non-coding RNA, Non-coding RNA is actually a complex network of gene expression regulation, which plays a key role in
regulating many important biological functions of cancer cells. According to the sequence length, it can be divided into short non-coding RNA and long non-coding RNA, Long non-coding RNA (lncRNA) is a class of transcripts with more than 200 nucleotides, and those without protein-coding functions are mostly transcribed by polymerase II[3]. Although lncRNA cannot be translated into protein, it has effects on life activities, such as gene transcription regulation, post-translational modification of proteins, and epigenetic regulation of gene expression. It is closely related to the pathophysiological changes, diagnosis and treatment of the disease[4]. LncRNA -SNHG15, an intergenic IncRNA found on chromosome 7p13, belongs to a non-coding class of RNAs containing snoRNAs[5]. Increasingly studies show that LncRNA-SNHG15 has abnormally expression in many types of tumors such as renal cancer[6], lung cancer [7], colorectal carcinoma [8], prostate cancer[9]. Long non-coding RNA SNHG 15 can promote cell proliferation in glioma microvascular endothelial cells [10], also contributes to proliferation, invasion and autophagy in osteosarcoma cells [11]. However, the expression and function of IncRNA-SNHG15 in bladder cancer is ambiguous. In our study, we investigated the role of IncRNA-SNHG15 in bladder carcinoma using data and cells line PCR, cck-8, and other experimental techniques to detect the expression of IncRNA-SNHG15 in bladder cancer tissues and cells line. The results showed that over- IncRNA-SNHG15 expression was a characteristic molecular change in bladder arcinoma tissues, and in (5637, UMUC3, T24) cell lines. Therefore, the effects of aberrant IncRNA-SNHG15 expression on the biological behavior of UMUC3 and T24 cells line were additionally investigated. The results provided novel insights into the function and mechanisms of IncRNA-SNHG15 bladder carcinoma pathogenesis, and identified IncRNA-SNHG15 as a potential therapeutic target for cancer intervention.

Methods

Cell lines.

The human bladder cancer cell lines (5637, UMUC3, J82, T24) and the normal bladder epithelial SV-HUC-1 cell line (SV-HUC-1) were obtained from the Chinese Academy of Sciences, Type Culture Collection Cell Bank (Shanghai, China). (SV-HUC-1) cells were cultured in Ham's F12 medium (Sigma, St. Louis, MO, USA). fetal bovine serum (FBS) ,RNA extraction reagent Trizol and reverse transcription kit were purchased from japan(TaKaRa Co. Tokyo Japan), IncRNA-SNHG15 interference RNA (siRNA IncRNA- SNHG15) and si-NC were obtained from Shanghai Jima Co(Shanghai, China), CCK-8 reagent were purchased from the Invitrogen Corporation (Carlsbad, CA, USA), Transwell chamber from the BD Biosciences (Franklin Lakes, NJ, USA).

Tissue samples.

Surgical bladder cancer tissues were collected along with matched near-cancer tissues from 30 patients who were diagnosed with bladder cancer and underwent surgical resection at the First Affiliated Hospital of China Medical University (Shenyang, China) between March and September 2018. The present study was permitted by the Ethics Committee on Human Research of the First Affiliated Hospital of China
Medical University, and written informed agreement was obtained from all patients. The collected tissue samples were stored at 80°C prior to use.

**Real-time PCR**

(1) Design the primer sequence as;

Reverse primer: ACCTGTACTCCGTACTCCGT

Forward primer: GGCCTGGATGACTAGACTG

And we use Takara fluorescence quantitative PCR kit to configure 10 reaction systems according to the TRIZOL method. Add the above 10 μL reaction system to a 96-well plate. Three auxiliary wells were provided for each sample. GAPDH was used as an inner reference when detecting the expression level of LncRNA-snhg15. The green dye method was used to assess the expression of LncRNA- snhg15 in bladder cancer tissues and matched adjacent tissues using GAPDH as an internal reference on a real-time quantitative PCR instrument. Each sample was repeated at least 3 times, and the average CT value was taken for calculation and analysis.

**Cell transfection assay**

Cells were cultured in RPMI1640 medium containing, 10% FBS, placed in 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 μL Lipofectamine 2000 and 200 pmol siRNA LncRNA-SNHG15 mixture were added to each well. LncRNA-SNHG15 siRNA sequence was: GGAUUUAAUAUGUGAAAA.

**Cell proliferation by cck-8 assay.**

Cells were plant seeds in 96-well plates at a thickness 2x10^3 cells/well were transfected with SNHG15 NC siRNAs for 72h in situ. Cell proliferation was evaluated using Cell Counting Kit-8 (CCK-8) assay (Dojindo Molecular Technologies, Inc., Shanghai, China) conforming to the manufacturer's protocol. The decadic was projected at 450 nm each 24 h utilizing a plate reader (Model 680; Bio-Rad Laboratories, Inc., Watford, UK).

**Cell invasion with transwell assay**

A Transwell chambers with a8-μm matrix gel coating hole is inserted into the 24 hole plate. Transwell kit without matrix gel was used for invasive test. After transfection of snhg15 and NC siRNAs for 72 hours, the cells were trypsinized and suspended in RPMI-1640 containing 1% FBS. Subsequently, 0.2 ml of cell
suspension (1 x 10^4 / ml) was added to the upper cavity and 0.6 ml of RPMI-1640 containing FBS was added to the lower cavity. After incubation at 37 °C for 24 hours, the remaining cells in the upper cavity were removed. The cells moving to the lower side were fixed with 4% paraformaldehyde for 10 minutes and stained with 1.0% crystal violet at room temperature for 10 minutes. The image was taken with EVOS™ XL core imaging system (Invitrogen; Thermo Fisher Scientific, Inc.).

Statistics

t-test was used for Statistical analyses, were achieved using the Statistical Graph pad 8.0.1.224 software,

Results

1. The expressions of lncRNA-SNHG15 in bladder cancer tissues and its correlation with clinical features

Interestingly, the results of qRT-PCR showed that the expression of lncRNA-SNHG15 in bladder cancer tissues was higher than that in adjacent tissues (p<0.05, Figure 1a). lncRNA-SNHG15 expression is connected with clinical and pathological features. We examined the relationship between increased lncRNA-SNHG15 expression levels and clinical characteristics in the 30 bladder cancer cases to determine whether lncRNA-SNHG15 expression is related to clinical features. LncRNA-SNHG15 up-regulation was positively correlated with tumor stage (P= 0.015), and tumor size (P= 0.0465). However, lncRNA-SNHG15 expression levels were not correlated with other parameters, such as patient age, gender, tumor number or nodal invasion. (Table 1). This result further indicated that lncRNA-SNHG15 acted as an important molecule modulating the development of bladder cancer.
Table 1
The relationship between the expression of lncrna-snhg15 and clinical statistics

| Factor                | No | LncRNA-SNHG15 | p     |
|-----------------------|----|---------------|-------|
|                       |    | High          | low   |
| Total                 | 30 | 19            | 11    |
| Gender                |    |               |       |
| Male                  | 24 | 15            | 9     | 0.999 |
| Female                | 6  | 4             | 2     |
| Age (year)            |    |               |       |
| ≥50                   | 25 | 17            | 8     | 0.3268 |
| <50                   | 5  | 2             | 3     |
| Tumor size(cm)        |    |               |       |
| ≥3                    | 19 | 15            | 4     | *0.0465 |
| <3                    | 11 | 4             | 7     |
| Tumor stage           |    |               |       |
| Ta & T1               | 9  | 16            | 4     | *0.015 |
| ≥T2                   | 21 | 3             | 7     |

Table 1: The expression level of LncRNA-SNHG15 was related to tumor size (P=*0.0465), degree of immersion (P=* 0.015), metastases, but not related to gender and age.

2. The effect of lncRNA-SNHG15 on bladder cancer cell proliferation, invasion and metastasis

The results of qRT-PCR showed that the expression of lncRNA-SNHG15 in bladder cancer (5637, UMUC3, T24) cell lines was higher than that in normal bladder epithelial (SV-HUC-1) cell line (p<0.05; Figure 1b). To discover the function of lncRNA-SNHG15, an siRNA targeting lncRNA-SNHG15 was transfected into UMUC3 and T24 cells. RT-qPCR indicated that lncRNA-SNHG15 was significantly downregulated at 48 h after transfection of siRNA in the bladder cancer UMUC3 and T24 cell lines compared with the control group (p<0.05; Figure 2). CCK-8 assays showed that siRNA lncRNA-SNHG15 could inhibit the proliferation of lncRNA-SNHG15 in bladder cancer bladder cancer UMUC3 and T24 cell lines. (p<0.05; Figure 3). In addition, the effect of lncRNA-SNHG15 on the migration capacity of UMUC3 and T24 cells was observed via a Transwell invasion assay. The Transwell invasion assay indicates that down-regulation of lncRNA-SNHG15 expression significantly inhibit the invasion of bladder cancer UMUC3 and T24 cell lines (p<0.05, Figures 4).

Discussion
Antoni S, et-all reported that estimated 430.000 new cases of bladder cancer occurred in 2012, making bladder cancer the ninth most common cancer worldwide [12]. The 5-year recurrence-free survival rate for highly localized invasive bladder cancer after a radical cystectomy ranges from 62–89 % [13]. Emerging evidence have shown that IncRNAs function as oncogenic or suppressor genes in multiple cancers, involving in transcription and post-transcription regulation[14]. The underlying mechanism that regulates bladder cancer development remains largely unknown. There is an urgent essential to develop novel molecular biomarkers for the diagnosis and prognosis of bladder cancer. LncRNA is a transcript with a length of more than 200bp. Because it does not participate in the coding of proteins and has no biological functions, it is considered a byproduct of RNA transcription, and even a "noise" in the transcription process of human genes[15]. However, lately research found that LncRNA participates in the regulation of multiple signal pathways in cells through chromosome modification, transcription, and interference. Our our team Yang Du, Chuize Kong et al 2018, has found that LncRNA-SNHG15 is highly expressed in renal cancer tissues, which is closely related to the proliferation, apoptosis, invasion and migration of renal cancer[16]. In this study, we investigated the expression of LncRNA-SNHG15 in bladder cancer tissues and adjacent normal tissues by RT-PCR and bladder cancer cells. The expression was apparently localized in the adjacent tissues. The results show that the high expression of LncRNA-SNHG15, its effect the tumor malignant, more aggressive, strong invasion ability, and the possibility of recurrence after the surgery. In order to study the potential effect of LncRNA-SNHG15 on bladder cancer cell lines, In vitro cell experiments in this study showed that the expression of LncRNA-SNHG15 in bladder cancer cells was significantly up-regulated in bladder cancer(5637, UMUC3,T24) cell lines. Transfection of specific si-RNA LncRNA-SNHG15 can inhibit the expression of LncRNA-SNHG15 in bladder cancer UMUC3,T24 cell lines. The above experimental results suggest that LncRNA-SNHG15 can inhibit the proliferation and invasion in bladder carcinoma UMUC3 and T24 cell lines, and may provide a potential new method for the diagnosis and treatment of bladder cancer cells.

**Conclusion**

In conclusion, this is the first observation investigating the LncRNA-SNHG15 in bladder carcinoma, which found that the expression of SNHG15 was increased in bladder cancer tissues and cell lines. The current findings showed that SNHG15 may regulate the proliferation and invasion of bladder cancer cells, The results show that LncRNA-SNHG15 as a tumor-promoting gene is closely related to the progress of bladder cancer. LncRNA-SNHG15 was over-expressed in bladder cancer tissue cells. Down-regulating LncRNA-SNHG15 can inhibit the proliferation of bladder cancer cells, and also inhibit the invasion of cells. The results of this study may have new diagnostic and therapeutic insinuations; these data may develop comprehend bladder cancer pathogenesis and progression, and provide information regarding the biological and molecular functions of LncRNA.

**List Of Abbreviations**

FBS Fetal bovine serum
Declarations

Ethics statement

This study was approved by The Research Ethics Committee of the first Affiliated Hospital of China medical university. The informed written consents were collected from all eligible patients and the entire study was performed based on the Declaration of Helsinki.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Conflict of interest

The authors declare no conflict of interest.

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Authors' contributions

Z.Z and C.K. conceived and supervised the study. A.M and Y.D performed the experiments, analyzed the experimental data. A.M wrote the manuscript. All authors read and approved the final manuscript.

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