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Long noncoding RNA SNHG14 acts as an oncogene in prostate cancer *via* targeting miR-613

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Abstract. – OBJECTIVE: Prostate cancer is one of the most ordinary malignant tumors. Recently, the role of long non-coding RNAs (lncRNAs) in tumor progression has caught the attention of numerous researchers. In this work, lncRNA SNHG14 was studied to identify how it functioned in the progression of prostate cancer.

PATIENTS AND METHODS: First, Real Time-quantitative Polymerase Chain Reaction (RT-qPCR) was utilized to measure SNHG14 expression in prostate cancer tissues and cell lines. Furthermore, to identify the function of SNHG14 in prostate cancer, functional experiments were conducted *in vitro* and *in vivo*. In addition, by performing Luciferase assays and RNA immunoprecipitation assay (RIP), the underlying mechanism was explored.

RESULTS: In this work, SNHG14 expression was remarkably higher in prostate cancer samples when compared with that in the corresponding ones. Moreover, cell proliferation was inhibited after SNHG14 was silenced in prostate cancer cells and the expression of miR-613 was upregulated after SNHG14 was silenced. Further mechanism analysis revealed that miR-613 was a direct target of SNHG14 in prostate cancer. In addition, tumor formation was inhibited after SNHG14 was knocked-down *in vivo*.

CONCLUSIONS: Our study discovers a potential oncogene in prostate cancer and identifies that SNHG14 enhances cell proliferation *via* targeting miR-613.

Key words:
long non-coding RNA, SNHG14, Prostate cancer,

Introduction

Prostate cancer is one of the most frequent malignancies in males. It is reported to kill more than 29,000 men in America and leads to 13% of cancer-related death in 2018[1]. Most of the patients newly diagnosed with prostate cancer can be treated with surgery or androgen deprivation therapy if they are still at early stage. However, the occurrence and recurrence rates of prostate cancer are significantly increasing in both developed and developing countries which make it the third leading cause of deaths related to cancer in males all over the world[3-6]. Therefore, it is urgent to find out the underlying mechanism and figure out new treatment strategy.

Long non-coding RNAs (lncRNAs) are a subgroup of non-coding RNAs and recent evidence has proved that lncRNAs act as a vital role in the progression of malignant tumors. For example, lncRNA ABHD11-AS1 predicts the prognosis of pancreatic cancer patients and serves as a promoter by activating the PI3K-AKT pathway[7]. LncRNA PCAT-1 modulates TP53-miR-215-PCAT-1-CRKL axis and makes a vital function in tumorigenesis of hepatocellular carcinoma[8]. LncRNA SNHG1 could inhibit the differentiation of Treg cells thereby impeding the immune escape of breast cancer[9].

LncRNA SNHG14 is a novel lncRNA and its function in cancers caught much attention. Our study aimed to identify whether SNHG14 participated in the proliferation of prostate cancer and the potential mechanism.

Patients and Methods

Tissue Specimens

60 paired prostate cancer patients received surgery at The 1st Affiliated Hospital of Kunming Medical University. Their tissue samples got from the surgery were saved immediately at -80°C. All tissues were analyzed by an experienced pathologist. The Research Ethics

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Committee of The 1st Affiliated Hospital of Kunming Medical University ratified this study and written informed consent was offered by the patients.

Cell Culture

Human prostate cancer cell lines LNCaP, DU145 and 22Rv1, and P69 (normal human prostate epithelial cell line) were offered by the Institute of Biochemistry and Cell Biology, Chinese Academy of Science (Shanghai, China). The culture medium consisted of 10% fetal bovine serum (FBS; Gibco, Grand Island, NY, USA), Dulbecco's Modified Eagle's Medium (DMEM; Gibco, Grand Island, NY, USA) as well as penicillin. Besides, cells were cultured in an incubator containing 5% CO_2 at 37°C.

Cell Transfection

ShRNA directed against SNHG14 were synthesized by GenePharma (Shanghai, China). The complementary DNA encoding SNHG14 was amplified and inserted into pcDNA3.1 (GenePharma, Shanghai, China), which were then used for transfection of DU145 prostate ca cells with polybrene (GenePharma, Sha China).

RNA Extraction and Real Time-Quantitative Polym Chain Reaction (RT-qPC

Total RNA from tissue cells w separated by using the TRIzol ag bad, CA, USA). Th al RNA reverse-transcribed to cDN rough the erse Transcription Kit Ka iotechnol Co., Ltd., Dalian, Ch a). Therm ling conditions were as follo re-denaturat t 95°C for 5 min, denat ion at 95°C for 10 nnealing at 60°C fo a tota 5 cycles. The $2^{-\Delta\Delta Ct}$ method was utili iculati relative expression. The mer ences as follows: SNHG14 forw 5'-C TACGTAGACCAGAACC-3' an everse TCCAAAAGCCTTCTGCCT-T lyceraldehyde 3-phosphate dehydrogese (G H), forward 5'-CCAAAATCAGAT-GGGGCAATGCTGG-3' and reverse 5'-TGATG-ATGGACTGTGGTCATTCA -3'.

Cell Proliferation Assay

Following the manufacturer's protocol, 2 ×10^3 transfected cells were seeded in 96-well plates and cell proliferation was assessed by the Cell Proliferation Reagent Kit I (MTT; Roche, Basel, Switzerland) at 0 h, 24 h, 48 h and 72 h. Absorbance at 490 nm was assessed using an ELISA reader system (Multiskan Ascent, LabSystems, Helsinki, Finland).

Ethynyl Deoxyuridine (EdU) Incorporation Assay

EdU Kit (Roche, Basel, Switzerland) was used for detecting cell proliferation nsfected cells. The representative photograp taken by Zeiss Axiophot Photomic pe (C eiss, Oberkochen, Germany).

Luciferase Assay

The 3′-UTR of IG cloned the pGL3 vector (P ega, Ma W SA) as wild-type (W TR. Site-d mutagenesis of the iR- inding si in SNHG14 3′-UTR as mutant (M 3′-UTR was conducted thro ck-change directed mutagenesis ki ratagene, La Jolla, A, USA). They were th used for t nsfection of prostate cancer c The Lucife e assay was conducted on the Du ciferas porter assay system (Promega, Madi SA).

Immunoprecipitation (RIP) Assay

IP assay, Magna RIP RNA-Binding Protein Immunoprecipitation Kit (Millipore, Billerica, MA, USA) was performed according to the protocol. To confirm the endogenous relationship between SNHG14 and miR-613, RIP assays were carried out using the EZMagna RIP RNA-binding protein immunoprecipitation kit (Millipore, Billerica, MA, USA). Treated DU145 cells were collected and lysed using RIP lysis buffer containing protease inhibitor and RNase inhibitor. Cells were incubated with the RIP buffer containing magnetic beads coated with Ago2 antibodies (Millipore, Billerica, MA, USA). IgG acted as a negative control (input group). After incubation for 2 h at 4°C, co-precipitated RNAs were isolated and measured by RT-qPCR analysis.

Tumor Formation Assay

After being transfected, DU145 cells (6×10^5/mL) were replaced into NOD/SCID mice (6 weeks old) subcutaneously. Tumor diameters were detected every 5 days. The tumor volume was calculated as the formula (volume = length × width^2 × 1/2). Tumors were extracted after 4 weeks. The research was approved by the Animal Ethics Committee of Kunming Medical University.

Statistical Analysis

All statistical analyses were carried out using GraphPad Prism 5.0 (La Jolla, CA, USA). The difference between the two groups was compared by the Student's *t*-test. The statistical significance was defined as $p<0.05$.

Results

SNHG14 Expression Level in Prostate Cancer Tissues and Cells

First, Real Time-quantitative Polymerase Chain Reaction (RT-qPCR) was conducted for detecting the SNHG14 expression in 64 patients' tissues and 3 prostate cancer cell lines. As a result, SNHG14 was significantly upregulated in tumor tissue samples (Figure 1A). SNHG14 level of prostate cancer cells was higher than that of P69 (Figure 1B).

Knockdown of SNHG14 Inhibited Cell Proliferation in DU145 Prostate Cancer Cells

In our study, we chose the DU145 cell lin[e] for the knockdown of SNHG14. Then, RT-qPC[R] utilized for detecting the SNHG14 expre[ssion] (Figure 2A). Moreover, MTT assay showed [that] the cell growth ability of DU145 [c]ells was s[ig]nificantly repressed after SNH[G14] knocke[d] down (Figure 2B). Furthe[r]e, Ed[U] incorpo[ration] assay showed that [the] positiv[e] [c]ells were reduced after knockd[own] [of] SH[G14] cells (Figure 2C).

The Interaction Between MiR-613 and SNHG14 in Prostate Cancer

Starbase v2.0 (http://starbase.sysu.edu.cn/mirLncRNA.php) was used to find the miRNAs that contained complementary base with SNHG14. Because miR-613 was a tumor suppressor and was able to suppress cancer cell proli[feration], we focused on miR-613 among thes[e miRNAs] which interacted with SNHG14 (Fi[gure] 3A). Indeed, RT-qPCR assay showed that th[e expression] of miR-613 was higher in sh-SNHG14 [cells] than that in control cells (Fig[ure] 3B). Furthe[r], the Luciferase assay rev[ealed] that co-transfec[tion] of SNHG14-WT and miR-613 l[ed] to decreased the Luciferase act[ivity], w[hile co]-transfe[ction] of SNHG14-MUT and miR-61[3] did n[ot] affect on the Luciferas[e] activity either (Figure 3C). Meanwhile, RIP assay [v]erified that SNHG14 and miR-613 were marke[dly] enriched in Ago2-containi[ng] cells compared [to] the input group (Figure 3D). These data demonstrated that miR-613 w[as] a direct targ[et] of SNHG14.

SNHG14 Knockdown Inhibited Tumorigenesis In Vivo

The ability of SNHG14 in tumor formation was detected *in vivo*. The tumor size in the sh-SNHG14 group was smaller compared with the empty vector group (Figure 4A). The weight of dissected tumors in the sh-SNHG14 group was smaller compared with the empty vector group (Figure 4B). Moreover, the expression level of SNHG14 and miR-613 in extracted tumor tissues was detected by RT-qPCR. The results showed

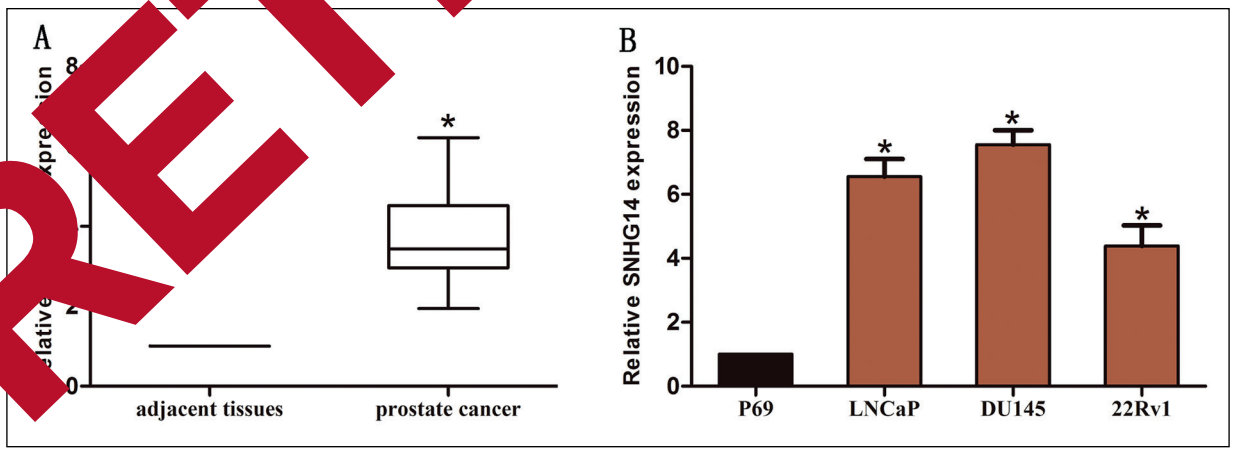

Figure 1. Expression levels of SNHG14 were increased in prostate cancer tissues and cell lines. **A,** SNHG14 expression was significantly increased in the prostate cancer tissues compared with the adjacent tissues. **B,** Expression levels of SNHG14 relative to GAPDH were determined in the human prostate cancer cell lines and normal ovarian cell ISOE80 by RT-qPCR. Data are presented as the mean ± standard error of the mean. *$p<0.05$.

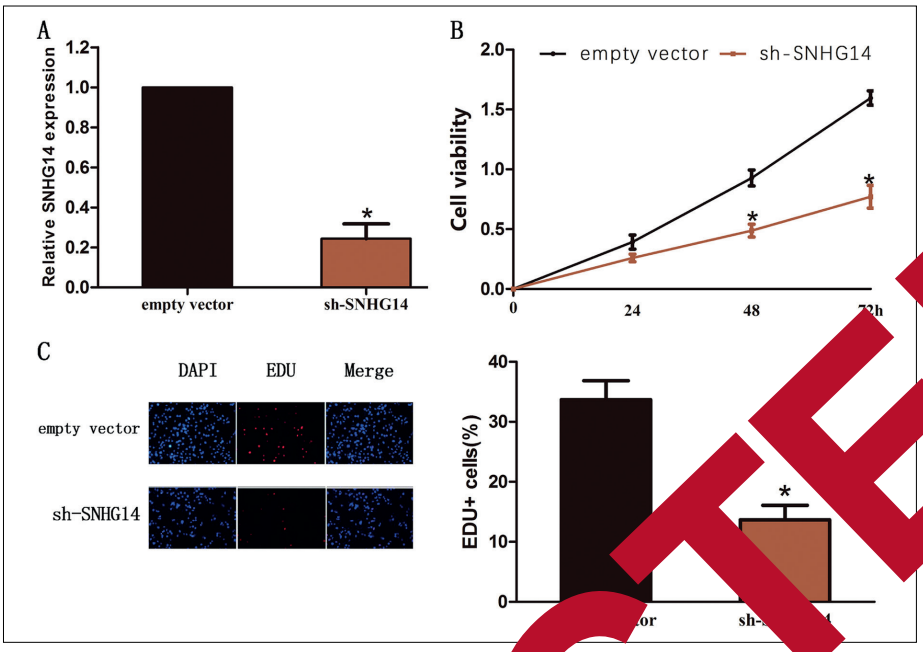

Figure 2. Knockdown of SNHG14 inhibited DU145 prostate cancer proliferation. A, SNHG14 expression in DU145 prostate cancer cells transduced with SNHG14 shRNA (shRNA) and the empty vector was detected by RT-qPCR. GAPDH was used as an internal control. **B,** MTT assay showed that knockdown of SNHG14 markedly inhibited cell growth in DU145 prostate cancer cells. **C,** EdU incorporation assay showed that EdU positive cells were reduced by knockdown of SNHG14 in DU145 cells. The results represent the average of three independent experiments (mean ± standard error). *p<0.05, compared with the control cells.

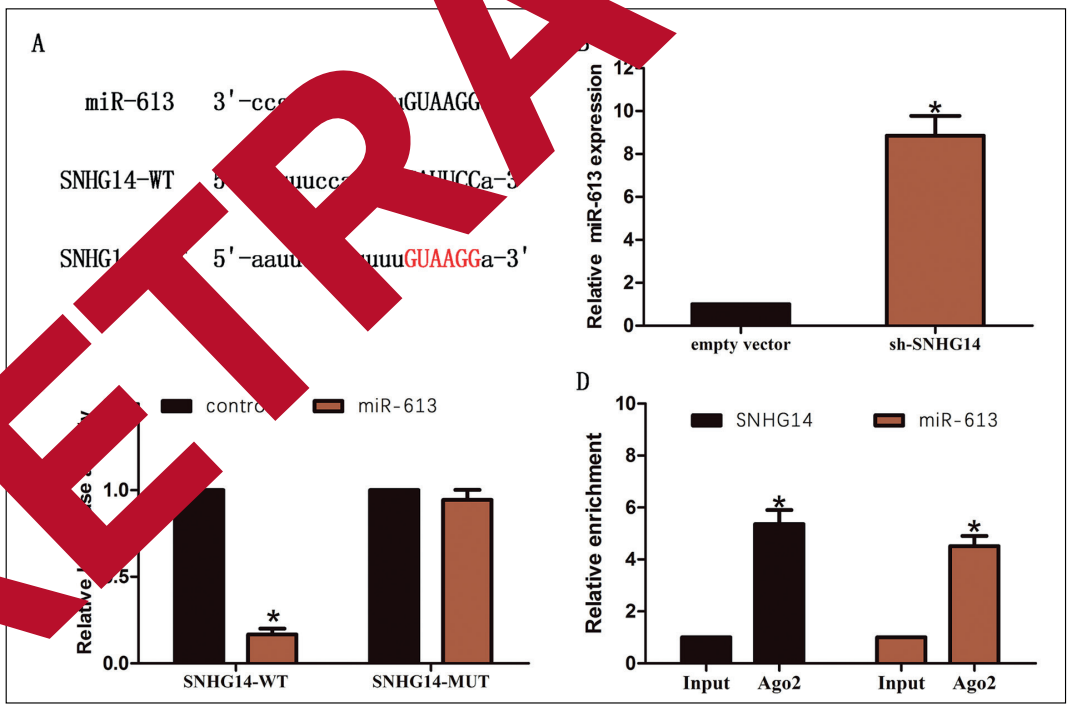

Figure 3. Reciprocal repression between SNHG14 and miR-613. **A,** The binding sites of miR-613 on SNHG14. **B,** The miR-613 expression was increased in the sh-SNHG14 group compared with the empty vector group. **C,** Co-transfection of miR-613 and SNHG14-WT strongly decreased the Luciferase activity, while co-transfection of mir-control and SNHG14-WT did not change the Luciferase activity. **D,** RIP assay results demonstrated that miR-613 could be remarkably enriched in the SNHG14 group compared with the empty vector group. The results represent the average of three independent experiments. Data are presented as the mean ± standard error of the mean. *p<0.05.

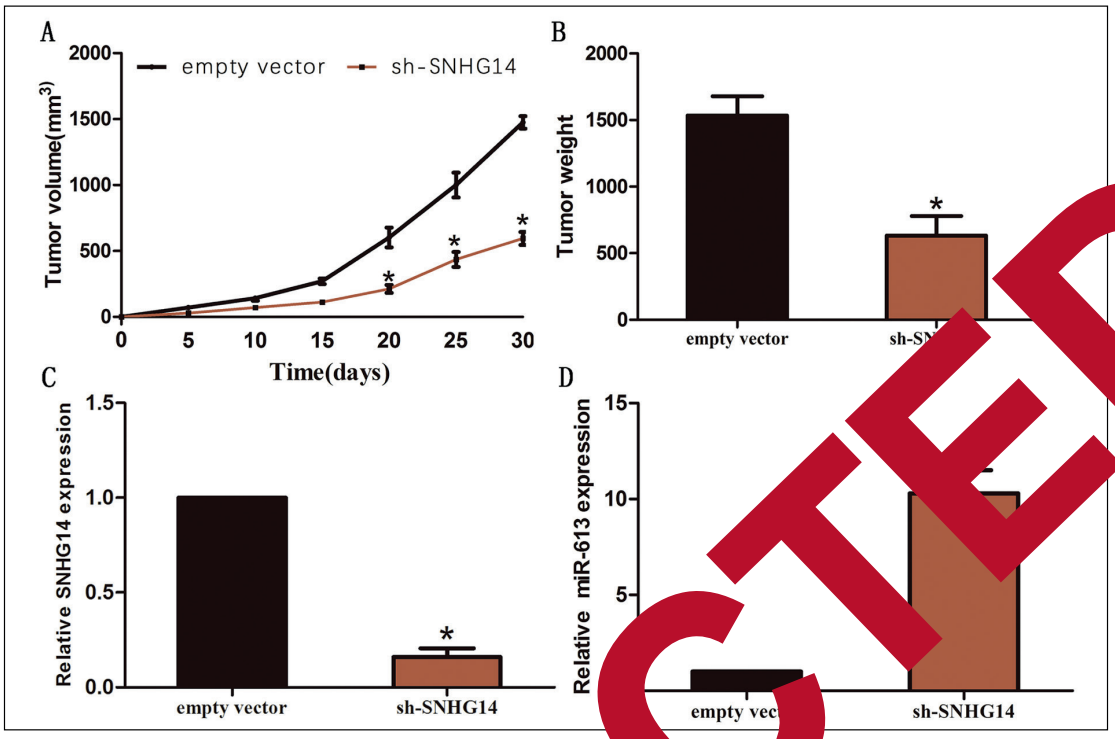

Figure 4. Knockdown of SNHG14 inhibited tumor formation *in vivo*. ... tum... traction, tumor volume was calculated respectively in empty vector or sh-SNHG14 group an... into a graph. ... ...t of dissected tumors in the sh-SNHG14 group was smaller compared with the NC group. **C** ... expression of SNHG14 in tumors were examined by RT-qPCR. **D,** The relative expression of miR-613 in tum... ...re ... by RT-qPCR. Data are presented as the mean ± SD of three independent experiments. *$p<0.05$.

that SNHG14 was lowly-ex...sed in ... sh-SN-HG14 group compared ... the e... vector group (Figure 4C), a... mi... pressed in the sh-S...G14 gr... mpared with the empty vecto... p (Figure ... The above results sugge... th... HG14 co... duce tumor forma...n *in vivo*.

Discussion

Evid... has p... that lncRNA is an im-p...nt fa... development of prostate cancer ce... hich ca... potential indicators of prostate ... In fact, it has been found that lncRNA ...NHG... omotes cell proliferation and cycle progression in prostate cancer through the miR-.../Cyclin D1 pathway. LncRNA MALAT1 and ...TAIR have been reported[11] to play the opposite role in transcriptional regulation in prostate cancer cell which is mediated by estrogen.

LncRNA small nucleolar RNA host gene 14 (SNHG14), as a novel lncRNA, is located in 15q11.2. Our previous work showed that SNHG14 was upregulated in prostate cancer patients. Researches[12-14] have shown that SNHG14 is upregulated in many cancers. Then, we conducted functional experiments and found that after SNHG14 was knocked down, prostate cancer cell proliferation was inhibited, which suggested that SNHG14 could promote tumorigenesis of prostate cancer.

To further identify the underlying mechanism of how SNHG14 affected prostate cancer cell proliferation, we predicted and picked miR-613 as the potential binding microRNA of SNHG14 by using bioinformatic analysis and experimental verification. MiR-613 is widely known as a tumor suppressor in many carcinomas which regulates diverse biological processes. MiR-613 is downregulated in hepatocellular carcinoma and participates in regulating tumor development by targeting YWHAZ[15]. MiR-613 was reported to suppress cell proliferation and metastasis of OC[16]. MiR-613 represses migrated and growth ability through targeting SphK1 in bladder cancer[17].

In the present work, the miR-613 expression could be upregulated after knockdown of SNHG14. Moreover, miR-613 could directly bind

to SNHG14 through a Luciferase assay and miR-613 was significantly enriched by SNHG14 RIP assay. All the results above suggested that SNHG14 might promote tumorigenesis of prostate cancer *via* sponging miR-613. Besides, the tumorigenesis assay revealed that knockdown of SNHG14 could inhibit tumor formation *in vivo*. Through the detection of SNHG14 and miR-613 expression in those extracted tumors, we found that SNHG14 was downregulated and miR-613 was upregulated in nude mice treated with SNHG14 shRNA.

Conclusions

We detected that SNHG14 could enhance prostate cancer cell proliferation by sponging miR-613. These findings implied that lncRNA SNHG14 can act as a prospective therapeutic target for prostate cancer.

Conflict of Interest

The Authors declare that they have no conflict of in

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