European Review for Medical and Pharmacological Sciences 2019; 23: 10810-10817

Long noncoding RNA SNHG14 exerts oncogenic functions in lung adenocarcinoma through acting as a sponge to miR-613

Z.-N. XU[1], Z.-X. WANG[1], L. XU[1], H.-X. YU[1], K. CHAO[1], L.-L. YANG[1], X.-L. HAN[2], H.-B. SUN[1]

[1]Department of Thoracic Surgery, China-Japan Union Hospital of Jilin Univ[illegible], Cha[illegible]hun, C[illegible]
[2]Department of Radiotherapy, Changchun Tumor Hospital, Changchun, [illegible]a

Zhenan Xu and *Zhenxing Wang* contributed equally to this work

Abstract. – OBJECTIVE: Lung adenocarcinoma is one of the most ordinary malignant tumors. Recent researches have proved that long noncoding RNAs (lncRNAs) are vital factors in many diseases. In this work, lncRNA SNHG14 was studied to identify its function in the development of lung adenocarcinoma.

PATIENTS AND METHODS: Real Time-[illegible] titative Polymerase Chain Reaction (RT[illegible] was utilized to detect SNHG14 express[illegible]in paired lung adenocarcinoma patients' t[illegible]e samples and cells. Then, the function of HG14 was detected through MTT (3-(4,5-dime[illegible] ylthiazol-2-yl)-2,5-diphenyl tet[illegible]bromide assay, colony formation ass[illegible]d t[illegible]well as say in vitro. Besides, mech[illegible]m ass[illegible]and the interaction between SN[illegible]and m[illegible]were conducted.

RESULTS: SNHG1[illegible]s re[illegible]y higher-expressed in lung ad[illegible]carcinom[illegible]ues than in adjacent sampl[illegible]reover, ce[illegible]liferation and invasion [illegible]ng [illegible]nocarcinom[illegible]ere promoted via ove[illegible]xpres[illegible]f SNHG14, while cell proliferati[illegible]nd invasio[illegible]ng adenocarcinoma wer[illegible]nbited via silen[illegible]SNHG14. Moreover, [illegible]qPCR [illegible]sults reve[illegible]ed that miR-613 was [illegible]wnreg[illegible]d via overexpression of SNHG[illegible]ile [illegible]-613 w[illegible]upregulated via knockdown [illegible]14. F[illegible]r experiments showed miR[illegible]as a[illegible]d direct target of SNHG14 [illegible] ade[illegible]oma.

CONCLUSIONS: Our study suggests that SNHG[illegible]nhances lung adenocarcinoma cell pro[illegible]invasion via targeting miR-613, [illegible]ich indi[illegible]tes that SNHG14 may be a potential therapeutic target in lung adenocarcinoma.

Keywords:
Long noncoding RNA, SNHG14, Lung adenocarcinoma, MiR-613.

Introduction

Lung cancer (LC) is one of the leading causes of cancer-related death in males around the world and has been rapidly increasing in females recently. As the predominant type of LC, non-small cell lung cancer (NSCLC) accounts for about 85% of all newly diagnosed cases. Lung adenocarcinoma is a major subtype of NSCLC, accounting for about 40% of NSCLC[1,2]. A primary feature of lung adenocarcinoma is the proliferation and invasion of neoplasms, which is responsible for the high mortality rate[3]. For the past few decades, the conventional therapeutics for advanced lung adenocarcinoma patients include surgical resection, chemotherapy, and radiotherapy. However, the median survival rate of advanced lung adenocarcinoma patients is approximately 9-12 months[3]. Therefore, it is urgent to realize the underlying molecular mechanism of lung adenocarcinoma and find out new therapeutic methods to improve the poor prognosis.

Technology in human genome sequence suggests that most transcripts do not code proteins which are called as non-coding RNAs (ncRNAs), among which those greater than 200 nt are defined as long non-coding RNAs (lncRNAs). Recently, several studies have indicated that lncRNAs play a crucial role in the development of cancers. For example, lncRNA OR3A4, upregulated in breast cancer samples and cells, may be a potential therapeutic target and prognostic marker[4]. The knockdown of lncRNA MALAT1 represents the effects of inhibiting cell proliferation and cell migration in esophageal squamous cell carci-

Corresponding Authors: Hongbin Sun, MM; e-mail: sunhb2003@163.com
Lei Xu, MM; e-mail: xulei041@outlook.com

noma cells[5]. LncRNA HORAIRM1 inhibits the progression of gastric cancer by suppressing the PI3K/AKT pathway[6]. LncRNA CCAT1 promotes cell proliferation and cell migration in esophageal squamous cell carcinoma by regulating the expression of SPRY4 and HOXB13[7].

Previous researches have suggested that lncRNA SNHG14 plays an important role in tumor biology and development. However, the function of SNHG14 in lung adenocarcinoma has not been studied so far. Our study demonstrated that SNHG14 was remarkably upregulated in lung adenocarcinoma tissues and cell lines. Moreover, the overexpression of SNHG14 promoted the proliferation and invasion of lung adenocarcinoma, while the knockdown of SNHG14 inhibited the proliferation and invasion of lung adenocarcinoma *in vitro*. In addition, our further experiments explored that the function of SNHG14 in lung adenocarcinoma was also associated with miR-613.

Patients and Methods

Tissue Specimens

62 lung adenocarcinoma patients were e for human tissues and underwent surgery e China-Japan Union Hospital of Jilin Univer Written informed consent was achieved fr participants before the operati is invest gation was approved by the E s C nittee o China-Japan Union Hospit Jilin U ersity.

Cell Culture

Four lung aden inoma r cell lines (SPCA1, H1299 and H35 normal human bronch ial cell (16 E) were purchased fro the S ai Model Cell Bank (Shanghai ina). The c medium consisted of 1 etal bovine seru BS; Invitrogen, Carl , CA. A), Roswell Park Memorial In -164 MI-1640; Invitrogen, Carlsbad, CA, U IU/mL icillin, and 100 µg/mL tom More cells were cultured in an tor c 5% CO_2 at 37°C.

C sfection

irus targeting SNHG14 was cloned to the pLenti-EF1a-EGFP-F2A-Puro vector (Bi- ia Inc., San Diego, CA, USA). The empty tor was also synthesized. Next, SNHG14 lentivirus or empty vector was transfected into SPCA1 lung adenocarcinoma cells through Lipofectamine 2000 reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. Lentivirus expressing short-hairpin RNA (shRNA) directed against SNHG14 was provided by GenePharma (Shanghai, China). Negati trol shRNA was also synthesized. The shRNA or negative control shRNA ransfect ed into PC-9 lung adenocarcinon ls through Lipofectamine 2000 reagent (Inv Carlsbad, CA, USA) according t e ma rer's protocol.

RNA Extraction a eal T ime-quantitativ P a Chain Reactio T-q

Total RNA extracted red lung adenocarci ls or patien mor tissues by using Z ol r t (TaKaRa Bio, Inc., Otsu, Shiga, Japan) and the rse-transcribed to cDNA n reverse Tr ipt ion Kit (TaKaRa, O Shiga, Japan). The p mer sequences used eal Time-qu titative Polymerase Chain Reaction (RT-qPCR) were as follows: SNHG14 forward: 5′-GGGTC TACGTAGACCAGAACC-3′ and rse -CTTCCAAAAGCCTTCTGCCTTA yceraldehyde 3-phosphate dehydrogenase (GAPDH), forward: 5′-GCACCGTGCTGAGAAC-3′ and reverse: 5′-TGGTCGCCAGTGGA-3′. The thermal cycle was as follows: pre-denaturation at 95°C for 1 min, followed by 40 cycles at 95°C for 15 s, 60°C for 30 s, and 72°C for 30 s. The $2^{-\Delta\Delta Ct}$ method was used to calculate the relative expression.

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 Enzyme Linked Immunosorbent Assay (ELISA) reader system (Multiskan Ascent, LabSystems, Helsinki, Finland).

Colony Formation Assay

Cells were placed in a 6-well plate for 10 days. Then, colonies were treated with 10% formaldehyde for 30 min and stained for 5 min with 0.5% crystal violet. The Image-Pro Plus 6.0 (Silver Spring, MD, USA) was used for data analysis.

Transwell Assay

5×10^4 cells in 200 µL serum-free RPMI-1640 were transformed to top chamber of an 8 µm

pore size insert (Millipore, Billerica, MA, USA) coated with 50 µg Matrigel (BD Biosciences, Franklin Lakes, NJ, USA). The bottom chamber was added RPIM-1640 and FBS. 48 h later, once wiped by cotton swab, the top surface of chambers was immersed for 10 min with precooling methanol. Then, they were stained in crystal violet for 30 min. The number of invaded cells was counted by a light microscope (Olympus, Tokyo, Japan).

Luciferase Reporter Gene Assay

DIANA LncBASE Predicted v.2 was used to predict the potential target gene and fragment sequences containing SNHG14 reaction sites. The 3′-UTR of SNHG14 was cloned into the pGL3 vector (Promega, Madison, WI, USA) as wild-type (WT) 3′-UTR. Site-direction mutagenesis of the miR-613 binding site in SNHG14 3′-UTR as mutant (MUT) 3′-UTR was conducted through Quick-change site-directed mutagenesis kit (Stratagene, La Jolla, CA, USA). Then, they were used for transfection of ovarian cancer cells. The luciferase assay was conducted on the [illegible] luciferase reporter assay system (Promega, Madison, WI, USA).

RNA Immunoprecipitation Assay

To confirm the endogenous relationship between SNHG14 and miR-613, the RIP assay was carried out using the EZMA RIP [illegible] protein immunoprecipitation [illegible] (Millipore, Billerica, MA, USA). Treated lung adenocarcinoma 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 with Ago2 antibodies (Millipore, Billerica, MA, USA). IgG acted as a negative control (input group). After incubation for 2 h at 4°C, copreciptated RNAs were isolated and purified by RT-qPCR analysis.

Statistical Analysis

All statistical analyses were performed by Statistical Product and Service Solutions (SPSS) 19.0 (SPSS, Chicago, USA). Independent-sample *t*-test was selected when appropriate. Moreover, $p<0.05$ was considered to indicate a statistically significant difference.

Results

Expression Level of SNHG14 in Tissues and Cells of Lung Adenocarcinoma

RT-PCR was conducted to detect SNHG14 expression in 62 patients' tissues and 4 lung adenocarcinoma cells. The result indicating SNHG14 was significantly upregulated in tumor tissue samples than in adjacent tissues (Figure 1A). Compared to the expression in 16HBE, SNHG14 level was significantly higher in lung adenocarcinoma cells (Figure 1B).

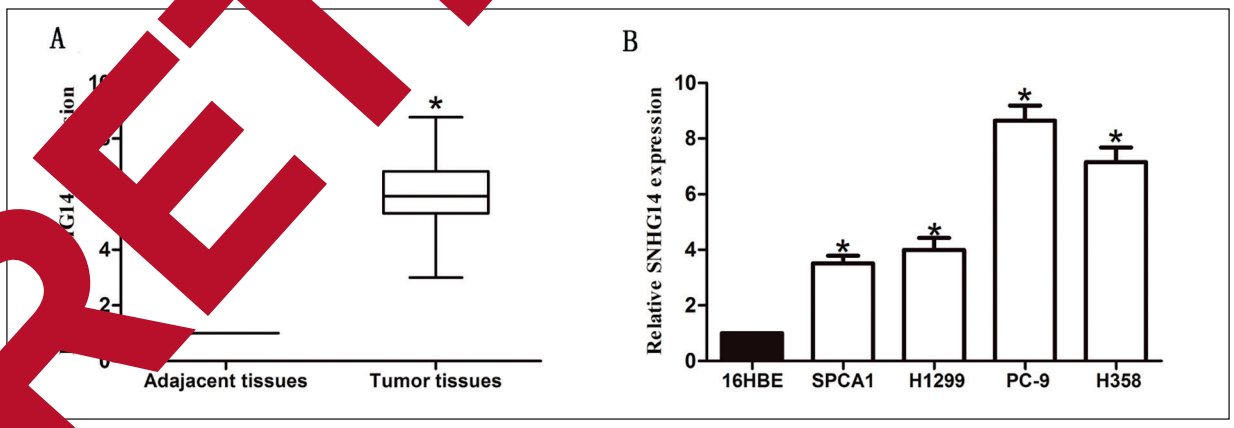

Figure 1. Expression level of SNHG14 was increased in lung adenocarcinoma tissues and cell lines. **A,** SNHG14 expression was significantly increased in the lung adenocarcinoma tissues compared with adjacent tissues. **B,** Expression levels of SNHG14 relative to GAPDH were determined in the human lung adenocarcinoma cell lines and normal human bronchial epithelial cell (16HBE) by RT-qPCR. Data are presented as the mean ± standard error of the mean. *$p<0.05$.

Overexpression of SNHG14 Promoted Cell Proliferation and Invasion in SPCA1 Lung Adenocarcinoma Cells

In our study, we chose the SPCA1 cell line for the overexpression of SNHG14 (Figure 2A). The MTT assay showed that the cell growth ability of SPCA1 cells was markedly increased after SNHG14 was overexpressed (Figure 2B). Colony formation assay showed that the number of colonies was significantly increased after SNHG14 was overexpressed (Figure 2C). Furthermore, the transwell assay showed that the number of invaded cells was markedly increased after SNHG14 was overexpressed (Figure 2D).

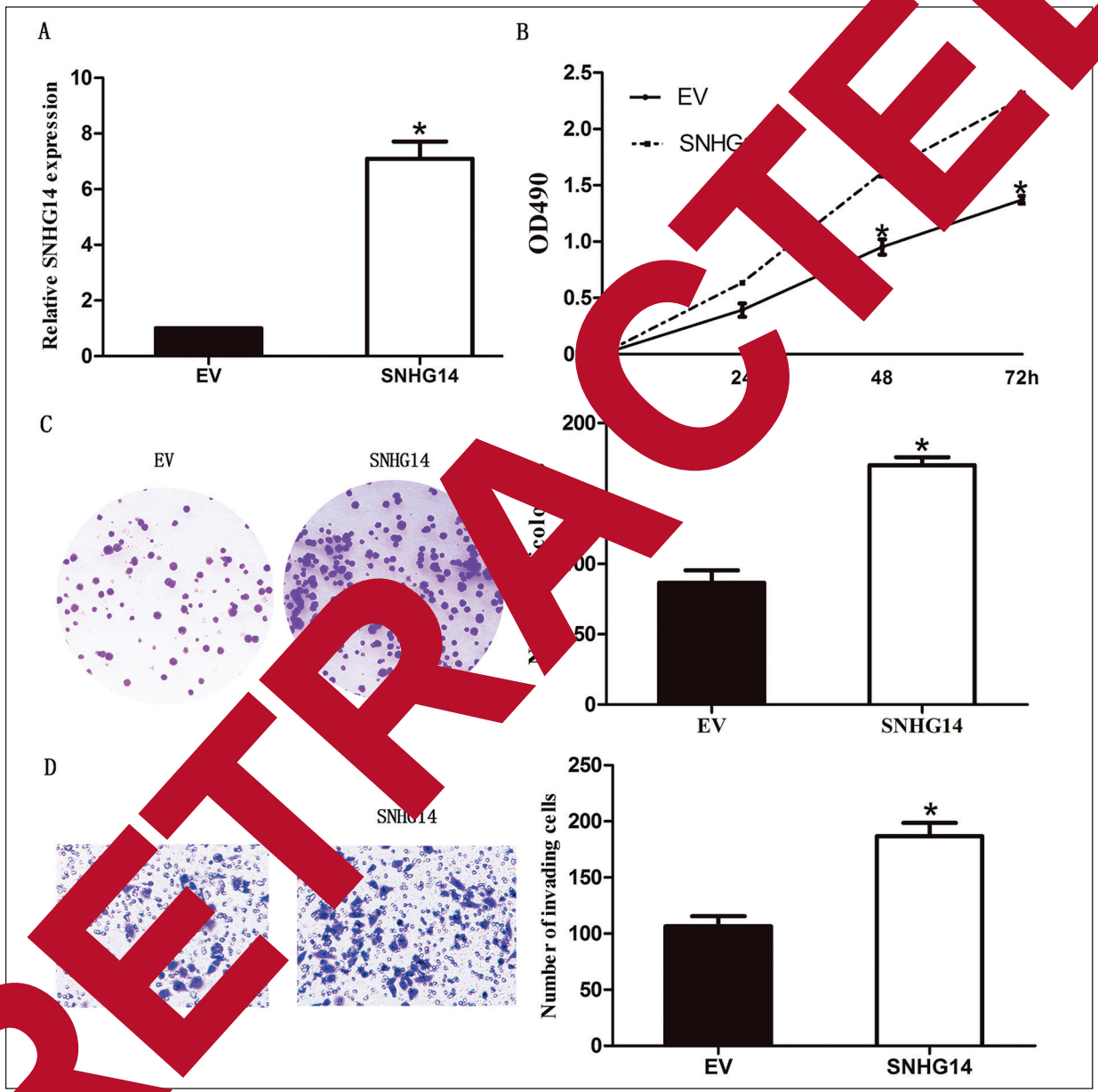

Figure 2. Overexpression of SNHG14 promoted SPCA1 lung adenocarcinoma cell proliferation and invasion. **A,** SNHG14 expression in lung adenocarcinoma cells transfected with empty vector (EV) or SNHG14 lentivirus (SNHG14) was revealed by RT-qPCR. GAPDH was used as an internal control. **B,** MTT assay showed that the cell growth ability of cells in SNHG14 group was significantly increased compared with EV group in lung adenocarcinoma cells. **C,** Colony formation assay indicated that overexpression of SNHG14 significantly enhanced cell proliferation in lung adenocarcinoma cells (magnification: 10×). **D,** Transwell assay discovered that overexpression of SNHG14 significantly promoted cell invasion in lung adenocarcinoma cells (magnification: 40×). The results represent the average of three independent experiments (mean ± standard error of the mean). *$p<0.05$, as compared with the control cells.

Silence of SNHG14 Repressed Cell Proliferation and Invasion in PC-9 Lung Adenocarcinoma Cells

We chose the PC-9 cell line for the knockdown of SNHG14 (Figure 3A). The MTT assay showed that the cell growth ability of PC-9 cells was significantly increased after SNHG14 was silenced (Figure 3B). Colony formation assay showed that the number of colonies was markedly decreased after SNHG14 was silenced (Figure 3C). Furthermore, the transwell assay showed that the number of invaded cells was notably decreased after SNHG14 was silenced (Figure 3D).

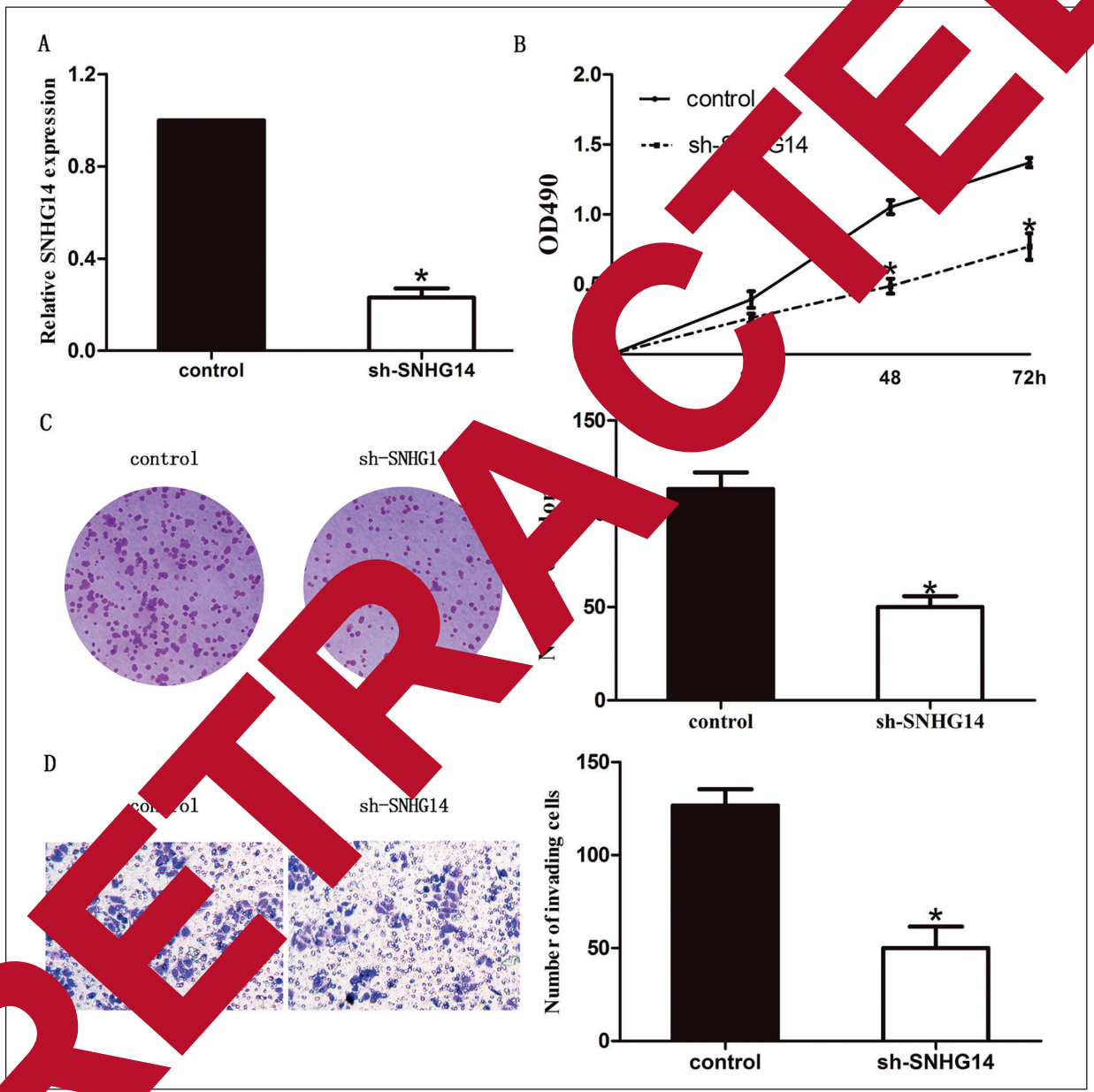

Figure 3. Knockdown of SNHG14 inhibited PC-9 lung adenocarcinoma cell proliferation and invasion. **A,** SNHG14 expression in lung adenocarcinoma cells transfected with control shRNA (control) or SNHG14 shRNA (sh-SNHG14) was detected by RT-qPCR. **B,** MTT assay showed that the cell growth ability of cells in sh-SNHG14 group was markedly decreased compared with control group in lung adenocarcinoma cells. **C,** Colony formation assay indicated that knockdown of SNHG14 significantly inhibited cell proliferation in lung adenocarcinoma cells (magnification: 10×). **D,** Transwell assay discovered that knockdown of SNHG14 significantly repressed cell invasion in lung adenocarcinoma cells (magnification: 40×). The results represent the average of three independent experiments (mean ± standard error of the mean). *$p<0.05$, as compared with the control cells.

The Interaction Between MiR-613 and SNHG14 in Lung Adenocarcinoma

DIANA LncBASE Predicted v.2 was used to find the miRNAs that contained complementary base with SNHG14. MiR-613 was selected from these miRNAs which were interacted with SNHG14 (Figure 4A). The RT-qPCR assay showed that the expression of miR-613 was lower in SNHG14 lentivirus group than in empty vector group and the expression of miR-613 was higher in sh-SNHG14 group than in control group (Figures 4B and 4C). Furthermore, the luciferase assay revealed that co-transfection of SNHG14-WT and miR-613 largely decreased the luciferase activity, while the co-transfection of SNHG14-MUT and miR-613 had no effect on the luciferase activity either (Figure 4D). Meanwhile, the RIP assay also identified that SNHG14 and miR-613 were significantly enriched in Ago2-containing beads compared to input group (Figure 4E).

Discussion

Increasing evidence has demonstrated that lncRNAs participate in the regulation of lung adenocarcinoma

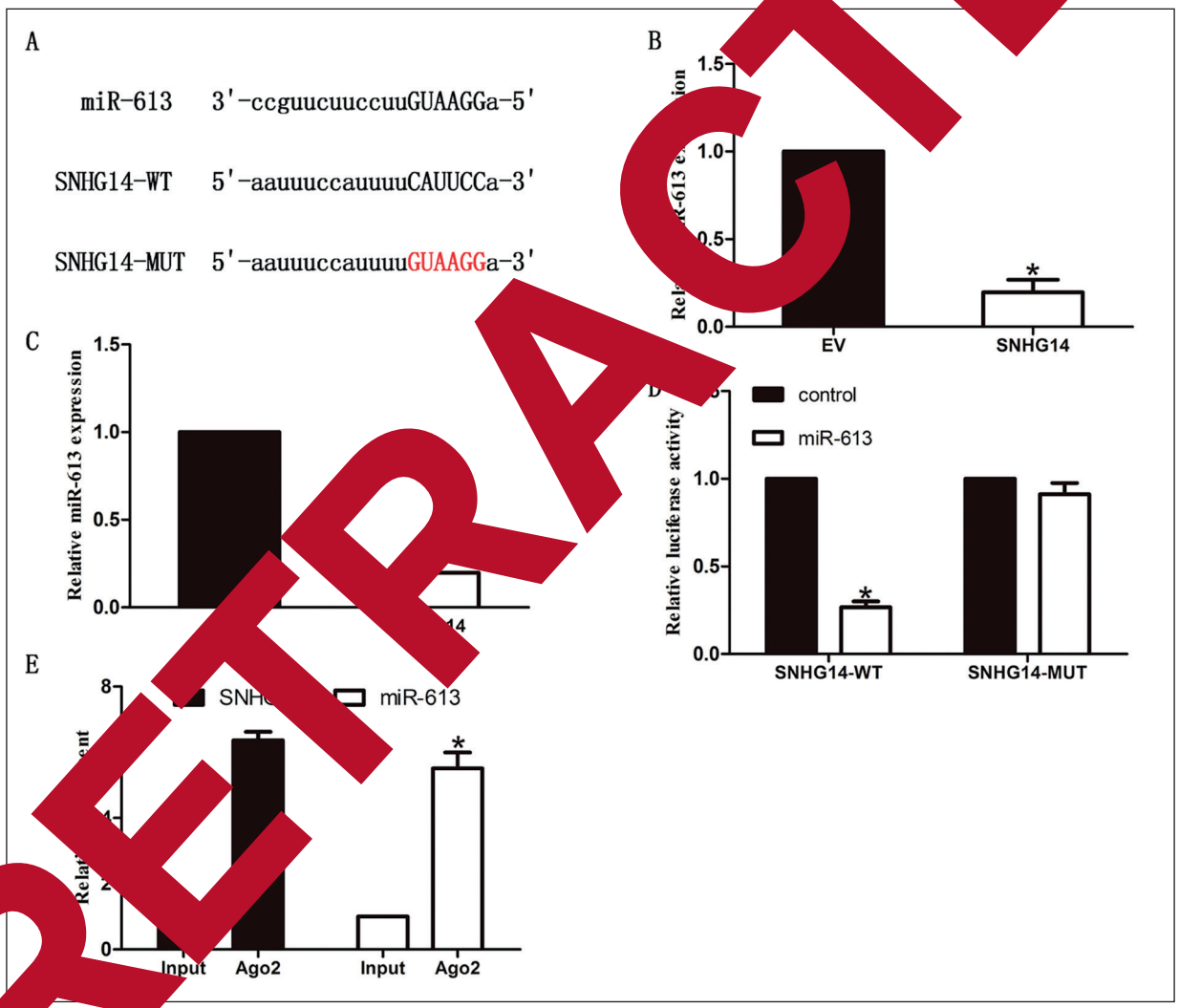

Figure 4. The association between SNHG14 and miR-613 in lung adenocarcinoma. **A,** Binding sites of miR-613 on SNHG14. **B,** MiR-613 expression was decreased in SNHG14 group compared with EV group. **C,** MiR-613 expression was increased in SNHG14 group compared with control group. **D,** Co-transfection of miR-613 and SNHG14-WT strongly decreased the luciferase activity, while co-transfection of miR-613 and SNHG14-MUT did not change the luciferase activity. **E,** RIP assay also identified that SNHG14 and miR-613 were significantly enriched in Ago2-containing beads compared to input group. The results represent the average of three independent experiments. Data are presented as the mean ± standard error of the mean. *$p<0.05$.

enocarcinoma. For instance, the downregulation of lncRNA SFTA1P inhibits cell migration and cell invasion in lung adenocarcinoma[8]. LncRNA CASC2 suppresses cell metastasis and epithelial to mesenchymal transition of lung adenocarcinoma by inhibiting SOX4[9]. LncRNA SNHG3 functions as an oncogene in lung adenocarcinoma and enhances the tumor proliferation which may provide a potential new therapeutic and prognostic target for lung adenocarcinoma[10]. By targeting the expression of p21, lncRNA CRNDE/PRC2 functions as an oncogene in lung adenocarcinoma and contributes to the radiotherapy resistance of lung adenocarcinoma[11].

Small nucleolar RNA host gene 14 (SNHG14), a novel lncRNA located on chromosome 15q11.2, has been reported to be overexpressed and exert its oncogenic activity in various human malignancies. For example, through the H3K27 acetylation, SNHG14 contributes to trastuzumab resistance in breast cancer by regulating expression of PABPC1[12]. SNHG14 facilitates the progression of cervical cancer by regulating miR-206/YWHAZ signaling pathway[13]. By sponging miR-92a-3p, SNHG14 promotes cell apoptosis and suppresses cell proliferation and invasion in glioma[14].

In the present research, SNHG14 was found to be upregulated in both lung adenocarcinoma tissue and cells. After SNHG14 was overexpressed the ability of cell growth and invasion was promoted. Furthermore, after SNHG14 was knocked down, the ability of cell growth and invasion was suppressed. These results indicate that SNHG14 functions as an oncogene and promotes the tumorigenesis of lung adenocarcinoma.

LncRNAs have been postulated as competing endogenous RNA (ceRNA) or "RNA sponges" and interact with microRNAs so that they can modulate these molecules and reduce their regulatory effect on their target mRNAs. By silencing p27 and sponging miR-101-3p, lncRNA SNHG20 facilitates cell invasion and cell proliferation in cancer through the epithelial-mesenchymal transition. LncRNA GAS5 inhibits tumor growth and metastasis in osteosarcoma through epithelial-mesenchymal transition *via* regulating the miR-221/ARHI pathway[16].

Furthermore, miR-613 was predicted as one of the potential targets of SNHG14 through the bioinformative analysis. MiR-613 is a miRNA conserved in human that which has been previously linked to different types of cancer. For example, miR-613 is downregulated in hepatocellular carcinoma and participates in regulating tumor development by targeting YWHAZ[17]. Besides, miR-613 was reported[18] to suppress cell proliferation and metastasis of OC. MiR-613 represses migration and growth ability by targeting SphK1 in bladder cancer[19].

In the present study, miR-613 was found to be directly targeted by SNHG14 through luciferase assay. Moreover, the miR-613 expression could be downregulated through the overexpression of SNHG14 and miR-613 expression could be regulated through knockdown of SNHG14. Furthermore, miR-613 was significantly enriched by SNHG14 through the RIP assay. All these results showed that SNHG14 could work as a sponge to miR-613 in lung adenocarcinoma.

Conclusions

Our study showed that SNHG14 could enhance lung adenocarcinoma cell proliferation and invasion by sponging miR-613. These findings implied that lncRNA SNHG14 could act as a prospective therapeutic target for lung adenocarcinoma.

Conflict of Interest

The Authors declare that they have no conflict of interests.

Funding Acknowledgements

This work was supported by Jilin Province Direct Health Project (20160101114JC).

References

1) Ramalingam S, Belani C. Systemic chemotherapy for advanced non-small cell lung cancer: recent advances and future directions. Oncologist 2008; 13 Suppl 1: 5-13.
2) Siegel R, Ma J, Zou Z, Jemal A. Cancer statistics, 2014. CA Cancer J Clin 2014; 64: 9-29.
3) Wood SL, Pernemalm M, Crosbie PA, Whetton AD. The role of the tumor-microenvironment in lung cancer-metastasis and its relationship to potential therapeutic targets. Cancer Treat Rev 2014; 40: 558-566.
4) Liu G, Hu X, Zhou G. Long non-coding RNA OR3A4 promotes proliferation and migration in breast cancer. Biomed Pharmacother 2017; 96: 426-433.
5) Wang X, Li M, Wang Z, Han S, Tang X, Ge Y, Zhou L, Zhou C, Yuan Q, Yang M. Silencing of long non-coding RNA MALAT1 by miR-101 and miR-217

inhibits proliferation, migration, and invasion of esophageal squamous cell carcinoma cells. J Biol Chem 2015; 290: 3925-3935.

6) Lu R, Zhao G, Yang Y, Jiang Z, Cai J, Zhang Z, Hu H. Long noncoding RNA HOTAIRM1 inhibits cell progression by regulating miR-17-5p/ PTEN axis in gastric cancer. J Cell Biochem 2019; 120: 4952-4965.

7) Zhang E, Han L, Yin D, He X, Hong L, Si X, Qiu M, Xu T, De W, Xu L, Shu Y, Chen J. H3K27 acetylation activated-long non-coding RNA CCAT1 affects cell proliferation and migration by regulating SPRY4 and HOXB13 expression in esophageal squamous cell carcinoma. Nucleic Acids Res 2017; 45: 3086-3101.

8) Zhang H, Xiong Y, Xia R, Wei C, Shi X, Nie F. The pseudogene-derived long noncoding RNA SFTA1P is down-regulated and suppresses cell migration and invasion in lung adenocarcinoma. Tumour Biol 2017; 39: 1010428317691418.

9) Wang D, Gao ZM, Han LG, Xu F, Liu K, Shen Y. Long noncoding RNA CASC2 inhibits metastasis and epithelial to mesenchymal transition of lung adenocarcinoma via suppressing SOX4. Eur Rev Med Pharmacol Sci 2017; 21: 4584-4590.

10) Liu L, Ni J, He X. Upregulation of the long noncoding RNA SNHG3 promotes lung adenocarcinoma proliferation. Dis Markers 2018; 2018: 5736716.

11) Zhang M, Gao C, Yang Y, Li G, Dong J, Ai [illegible] N, Li W. Long noncoding RNA CRNDE/PRO[illegible]r-ticipated in the radiotherapy resistance of hu[illegible] lung adenocarcinoma through targeting p21 [illegible]pression. Oncol Res 2018; 26: [illegible]255.

12) Dong H, Wang W, Mo S, L[illegible] Chen R, Zhang Y, Zou K, Ye M, He [illegible]ang F, [illegible] J, Hu J. Long non-coding RNA SNHG14 induces trastuzumab resistance of breast cancer via regulating PABPC1 expression through H3K27 acetylation. J Cell Mol Med 2018; 22: 4935-4947.

13) Ji N, Wang Y, Bao G, Yan J, Ji S. LncRNA [illegible] promotes the progression of cervic[illegible]cer regulating miR-206/YWHAZ. Pat[illegible]Res Pract 2019; 215: 668-675.

14) Wang Q, Teng Y, Wang R, Deng D, [illegible] Peng Y, Shao N, Zhi F. The long non[illegible]ding R[illegible]HG14 inhibits cell proliferation[illegible] invasion[illegible]ro motes apoptosis by sp[illegible]ng miR-92a-3p[illegible] ma. Oncotarget 201[illegible]12112-[illegible].

15) Yan K, Tian J, Shi [illegible]H, [illegible]. LncRNA SNHG6 is associ[illegible]wi[illegible]ognosis[illegible]gastric cancer and [illegible]otes c[illegible]iferati[illegible]nd EMT through e[illegible]etically sile[illegible]nd sponging miR[illegible]Cell Physiol[illegible]m 2017; 42: 999-1[illegible].

16) Ye K, Wang S, Zha[illegible]Han H, Ma B, Nan W. Long [illegible]ng RNA GA[illegible]uppresses cell growth [illegible]pithelial-mesenc[illegible]al transition in osteosarcoma by regulating the miR-221/ARHI pathway. J Cell Bi[illegible]em 2017; 118: 4772-4781.

17) [illegible]iang X, Wu J, [illegible]ng Y, Wang S, Yu X, Li R, Huang X. [illegible]R-613 fun[illegible]s as tumor suppressor in hepa[illegible]ular [illegible]oma by targeting YWHAZ. Gene 20[illegible]8-174.

18) Fu X, Cui Y, Yang S, Xu Y, Zhang Z. MicroRNA-613 [illegible]hibited ovarian cancer cell proliferation and in[illegible]n by regulating KRAS. Tumour Biol 2016; 37: 6[illegible]7-6483.

19) Yu H, Duan P, Zhu H, Rao D. MiR-613 inhibits bladder cancer proliferation and migration through targeting SphK1. Am J Transl Res 2017; 9: 1213-1221.