Abstract. – OBJECTIVE: To investigate whether lncRNA (long non-coding RNA) SNHG15 could regulate the proliferation and migration of lung cancer via microRNA-211-3p and its underlying mechanism.

PATIENTS AND METHODS: SNHG15 expression in 55 LC (lung cancer) tissues and 30 normal lung tissues was detected by qRT-PCR (quantitative Real Time-Polymerase Chain Reaction). The relationship between SNHG15 expression and pathological characteristics of LC patients was analyzed by Kaplan-Meier method. The target microRNA of SNHG15 was predicted by bioinformatics and verified by dual-luciferase reporter gene assay. Viability, cell cycle and migration of LC cells after altering expressions of SNHG15 or microRNA-211-3p were detected by cell counting kit-8 (CCK-8), flow cytometry and transwell assay, respectively.

RESULTS: SNHG15 was highly expressed in LC tissues than that of normal lung tissues. Besides, LC patients with stage I-II presented lower expression of SNHG15 than those with stage III-IV. SNHG15 expression was correlated to tumor size, TNM stage, and lymph node metastasis, whereas not correlated to age and sex of LC patients. For in vitro studies, SNHG15 knockdown resulted in viability reduction, cell cycle arrest and reduced migration of LC cells, which were reversed by the microRNA-211-3p knockdown.

CONCLUSIONS: SNHG15 is highly expressed in LC tissues, which promotes the occurrence and progression of LC via regulating proliferation and migration of LC cells by targeting microRNA-211-3p.

Key Words:
Lung cancer, SNHG15, MicroRNA-211-3p, Proliferation, Migration.

Introduction

Lung cancer (LC) is currently one of the most common malignancies in the world. In recent years, the morbidity and mortality of LC have been astonishingly increased. The incidence of LC ranks the first in male malignancies, which is also the leading cause for tumor death in men. LC ranks the fourth in female malignancies and is the second leading cause for female tumor death1. Currently, surgical resection, platinum-based dual chemotherapy, and radiotherapy are the standard therapies for LC treatment. The combination treatment of epidermal growth factor receptor and tyrosine kinase inhibitors has become the first-line treatment for advanced LC. However, the effectiveness of LC treatments is limited, with the 5-year survival rate of only 17%2,3. Therefore, it is of great significance to explore the specific mechanism and effective targets for invasion and metastasis of LC. Early diagnosis, treatment, and prognosis of LC contribute to better clinical outcomes.

LncRNAs (long non-coding RNAs) are a type of non-coding RNAs with over 200 nt in length4. Studies have shown that some lncRNAs are differentially expressed in tumors. These lncRNAs may participate in biological behaviors of tumors, including cell proliferation, apoptosis, invasion, and immune escape5,6. LncRNA SNHG15 is located on chromosome 7p13, which is involved in the regulation of proliferation, apoptosis, and tumorigenesis7. Chen et al7 found that SNHG15 knockdown can inhibit the proliferation of gastric cancer cells. In addition, other studies showed that SNHG15 is associated with apoptosis and invasion of lung cancer cells8.

MicroRNAs are single-stranded, non-coding RNA molecules of about 22 nucleotides in length. MicroRNAs negatively regulate gene expressions at the transcriptional or post-transcriptional level, which are involved in cell proliferation, differentiation, metabolism, and death9. It has been reported that some microRNAs may also be involved in tumor invasion and metastasis as proto-oncoge-
nes or tumor suppressor genes, such as miR-10b, miR-335, miR-373, miR-520c, miR-9, and miR-211. Relative studies have reported that SNHG15 can promote the development of lung cancer via targeting miR-486. In this work, we aim to investigate whether SNHG15 affects the proliferation and migration of LC by targeting microRNA-211-3p, which provides new suggestions for the development of the clinical diagnosis of LC.

**Patients and Methods**

**Sample Collection**

LC tissues and paracancerous tissues in LC patients undergoing radical resection of pulmonary carcinoma in Affiliated Hospital of Weifang Medical University from April 2012 to July 2017 year were surgically resected. Tissues were immediately preserved in liquid nitrogen for the further experiments. This study was approved by the Ethics Committee of the Affiliated Hospital of Weifang Medical University. The signed written informed consents were obtained from all participants before the study.

**Cell Culture and Transfection**

H1799 and A549 cells were cultured in Roswell Park Memorial Institute-1640 (RPMI-1640; HyClone, South Logan, UT, USA) containing 10% fetal bovine serum (FBS; Gibco, Rockville, MD, USA), and maintained in 5% CO_2 at 37°C. Cells were seeded in the 6-well plates with 3×10^5 cells per well.

For cell transfection, cells were first divided into si-SNHG15 group, si-SNHG15 + microRNA-211-3p inhibitor group and control group. Transfection was performed following the manufacturer’s instructions of Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA).

**RNA Extraction and qRT-PCR (Quantitative Real-Time Polymerase Chain Reaction)**

The mRNAs of cells were extracted by TRIzol reagent (Invitrogen, Carlsbad, CA, USA) and RNA concentration was determined by NanoDrop1000 Spectrophotometer (Thermo Scientific, Waltham, MA, USA). The extracted mRNAs were reversely transcribed to complementary DNAs (cDNAs) (TaKaRa, Otsu, Shiga, Japan), followed by PCR reaction based on the instructions of relative commercial kits (Applied Biosystems, Foster City, CA, USA). The expression level of mRNA was calculated with the 2−ΔΔCt method. GAPDH (glyceraldehyde 3-phosphate dehydrogenase) was used as internal control.

**Cell Counting Kit-8 (CCK-8) Assay**

H1799 and A549 cells were seeded into the 96-well plates with 2×10^3 cells per well. 10 μL of CCK-8 reagent (Dojindo, Kumamoto, Japan) was added in each well after cell culture for 24, 48, 72, and 96 h, respectively. The optical density was detected at the wavelength of 490 nm using a microplate reader (Bio-Rad, Hercules, CA, USA). Each experiment was performed in triplicate.

**Cell Cycle Detection**

H1799 and A549 cells were digested and collected in 5 mL EP tube. After phosphate-buffered saline (PBS) wash twice, cells were resuspended in 1 mL of PBS and 2 mL of absolute alcohol. Cell density was adjusted to 5×10^5-1×10^6 /mL, followed by cell cycle detection using flow cytometry (Partec AG, Arlesheim, Switzerland).

**Transwell Assay**

Matrigel was diluted with the serum-free medium at a ratio of 1:1 and pre-coated in the 24-well plates. 5 × 10^3 cells and 650 μL of medium containing 20% FBS were added in the upper and lower chamber, respectively. After cell culture for 24 h, cells were fixed with 500 μL of methanol for 20 min and stained with 500 μL of violet crystal for another 20 min. Images were captured using a light microscope (Nikon, Tokyo, Japan).

**Dual-Luciferase Reporter Gene Assay**

Wild-type SNHG15 and mutant-type SNHG15 were constructed. Cells were seeded in the 24-well plates and cotransfected with 0.4 mg firefly luciferase reporter gene vector and 0.08 mg pRL-TK containing Renilla luciferase. Luciferase activity was detected using the dual-luciferase reporter gene assay kit (Applied Biosystems, Foster City, CA, USA).

**Statistical Analysis**

Statistical Product and Service Solutions (SPSS) 20.0 statistical software (IBM, Armonk, NY, USA) was used for data analysis. Measurement data were expressed as mean ± standard deviation (x̄ ± s). Comparison of measurement data was conducted using the t-test. The overall survival was evaluated using the Kaplan-Meier curve, followed by Log-rank test. p<0.05 was considered statistically significant (p<0.05, **p<0.01, ***p<0.001).
Results

**SNHG15 Was Highly Expressed in LC**

SNHG15 expression was higher in 55 LC tissues than that of 30 normal lung tissues detected by qRT-PCR (Figure 1A). Besides, LC patients with stage I-II presented lower expression of SNHG15 than those with stage III-IV (Figure 1B). By analyzing the relationship between SNHG15 expression and prognosis of LC, we found LC patients with higher expression of SNHG15 presented shorter overall survival compared with those with lower expression \((p=0.0410, \text{HR}=2.234)\). (Figure 1C). The SNHG15 expression was correlated to tumor size, TNM stage, and lymph node metastasis, whereas not correlated to age and sex of LC patients (Table I). Subsequently, we detected SNHG15 expression in LC cell lines (H1703, H1799, and A549) and normal lung cell line (EAS-2B). The data suggested that SNHG15 was overexpressed in LC cells than that of normal lung cells.

**SNHG15 Promoted Proliferation and Migration of LC Cells**

We first constructed si-SNHG15 and si-NC, respectively. Transfection efficacies of si-SNHG15 in H1799 and A549 cells were verified by qRT-PCR (Figure 2A). SNHG15 knockdown remarkably inhibited cell viability of H1799 and A549 cells.
The role of SNHG15 in lung cancer

Flow cytometry results demonstrated that LC cells were arrested in the G0/G1 phase after the SNHG15 knockdown, indicating that SNHG15 promotes cell cycle (Figure 2D and 2E). Transwell assay indicated that the amount of penetrating cells was decreased after the SNHG15 knockdown, indicating the promoted migration induced by SNHG15 (Figure 2F and 2G).

**SNHG15 Regulated Proliferation of LC Cells Via Targeting MicroRNA-211-3p**

By online prediction (DIANA, mirRanda, and PicTar), we found that microRNA-211-3p was the target gene of SNHG15 (Figure 3A). Dual-luciferase reporter gene assay further verified that SNHG15 bound to microRNA-211-3p in H1799 and A549 cells (Figure 3B and 3C). To explore the regulatory effect of SNHG15 on microRNA-211-3p, rescue experiments were carried out. We found that the viability reduction and arrested cell cycle induced by SNHG15 knockdown were reversed after the microRNA-211-3p knockdown in H1799 and A549 cells (Figure 3D-3G).

**Discussion**

According to data released in 2016, the morbidity and mortality of LC are one of the highest in all types of cancers. In the past 30 years, the 5-year survival rate of LC is about 16% throughout the world. Therefore, it is of great significance to explore the occurrence and development mechanism of LC, so as to find effective diagnostic indicators and therapeutic targets.

LncRNA has been well studied in tumor researches, which could be serve as the diagnostic indicator and therapeutic target for tumor treatment. Tuo et al. reported that lncRNA UCA1 is upregulated in breast cancer, which regulates cell proliferation and apoptosis through downregulating miR-143. GAS5 is lowly expressed in LC, which is correlated with tumor stage and size. SNHG15 is overexpressed in hepatocellular carcinoma than that of adjacent tissues. The SNHG15 expression is positively related to histological grade, TNM stage, and vascular invasion, which is an independent risk for the prognosis of hepatocellular carcinoma. SNHG15 is highly expressed in gastric cancer tissues that are associated with the depth of tumor infiltration, TNM stage, and lymph node metastasis. SNHG15 knockdown can inhibit proliferation and invasion of gastric cancer cells via regulating MMP2 and MMP9.

In this investigation, we found that SNHG15 is highly expressed in LC tissues, which is in agreement with the above results. In addition, microRNAs also exert a crucial role in tumorigenesis. MicroRNA disorder is closely related to tumorigenesis, drug resistance, and tumor metastasis. Therefore, exploration of microRNA function helps to provide novel tumor treatments. Studies have shown that miR-211 can inhibit proliferation and invasion of gastric cancer cells.
Recent studies have demonstrated that microRNAs participate in proliferation, apoptosis, and tumorigenesis, which are regulated by the upstream lncRNAs. For example, SNHG15 promotes invasion and migration of breast cancer cells via regulating microRNA-211-3p. We showed that SNHG15 promotes proliferation and migration of LC by targeting microRNA-211-3p.

To sum up, we found that SNHG15 promoted the occurrence of LC via regulating microRNA-211-3p, which provides new directions in the prediction and treatment of LC.

Conclusions

We showed that SNHG15 is highly expressed in LC tissues. SNHG15 promotes the oc-
Figure 3. SNHG15 regulated proliferation of LC cells via targeting microRNA-211-3p. 

A, The microRNA-211-3p was the target gene of SNHG15 predicted by online software. 

B-C, The SNHG15 bound to microRNA-211-3p in H1799 and A549 cells. 

D-E, The viability reduction induced by SNHG15 knockdown was reversed after the microRNA-211-3p knockdown. 

F-G, The arrested cell cycle induced by SNHG15 knockdown was reversed after the microRNA-211-3p knockdown.
currence and progression of LC via regulating proliferation and migration of LC cells by targeting microRNA-211-3p. Our results suggest a theoretical basis for developing novel treatment of LC.

Conflict of Interest
The Authors declare that they have no conflict of interest.

References