lncRNA-SNHG7 promotes the proliferation, migration and invasion and inhibits apoptosis of lung cancer cells by enhancing the FAIM2 expression

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Abstract. There is growing evidence that long non-coding RNAs (lncRNAs) are related to cancer development. In the present study, we found that the expression levels of lncRNA-SNHG7 mRNA and protein obviously increased in lung cancer tissues compared to adjacent non-cancerous tissues. Simultaneously, the expression levels of Fas apoptotic inhibitory molecule 2 (FAIM2) also increased in lung cancer tissues. In addition, lncRNA-SNHG7 was of positive relevance with FAIM2 in human lung cancer tissues. Silence of lncRNA-SNHG7 by siRNA repressed the level of FAIM2 protein and suppressed cell proliferation, migration and invasion and accelerated apoptosis of A594 cells *in vitro*. Furthermore, silence of FAIM2 by siRNA generated a phenotype similar to silence of lncRNA-SNHG7 by siRNA. Therefore, our research showed that lncRNA-SNHG7 promotes the proliferation, migration and invasion, and inhibits apoptosis of lung cancer cells by enhancing the FAIM2 expression, suggesting that lncRNA-SNHG7 as a key regulator of gene expression, may be a promising therapeutic strategy for the treatment of lung cancer. It may improve the understanding of their biogenesis and function of lung cancer and further provide the theoretical fundamental basis for cancer pathogenesis and treatment.

Introduction

Lung cancer is one of the fastest-growing morbidity and mortality and the most serious threat tohuman health and life due to malignant tumors. Lung cancer is the most important cause of cancer-related deaths worldwide (1-3). It has been reported that more than 160,000 patients died of lung cancer which is more than from colon, prostate and even breast cancer, in the US during 2013 (4). At present, surgery, radiotherapy and chemotherapy have been used in the treatment of lung cancer. Nevertheless, the long-term survival rate is still very low (5). Research has also shown that most patients with lung cancer had been smoking in the US (6), and the genetic damage of lung cancer patients was mainly caused by smoking (7,8). The expression levels of mRNA and protein among thousands of genes can be used to analyze the molecular network among lung carcinogenesis (9,10). At present, epidermal growth factor receptor (EGFR) and echinoderm microtubule associated protein-like 4-anaplastic lymphoma kinase (EML4-ALK) fusion genes have been used to detect lung cancer. Although many known genes and proteins have provided a large amount of information for the treatment of lung cancer, there are also a large number of unknown markers including non-coding RNAs (ncRNAs) which may be crucial regulators of cellular processes such as proliferation, gene regulation and apoptosis and may also serve as novel biomarkers for the treatment of lung cancer.

In recent years, the biomarker research has also focused on non-coding RNAs (ncRNAs), particularly lncRNAs, which are greater than 200 nt and most are transcribed by RNA polymerase (Pol) II/Pol I, small are transcribed by RNA Pol III (11), playing an important role in the regulation of gene expression (12-15). In addition, there is growing evidence that lncRNA is related to cell cycle and cell differentiation (16), apoptosis (17,18) and chromatin remodeling (19-21). It has also been shown that lncRNA expression are concerned with the development of various types of cancer, such as liver (22), lung (23) and breast cancers (24). For example, maternally expressed gene 3 (MEG3) is related to bladder cancer (25), and antisense non-coding RNA in the INK4 locus (ANRIL) is relative to plexiform neurofibromas (26). For lung cancer, recent studies predicted that MALAT1 was a critical regulator of lung cancer cells (27), SCAL1 promoted lung cancer by cigarette smoke (28), AK126698 produced drug resistance

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in lung cancer cells (29). Therefore, lncRNA is considered as an important regulating factor for gene expression, disease as well as cancers. However, the investigation into function and dysregulation of lncRNA in cancer has only just begun; increasing research is urgently needed to lead to a deeper understanding for the lncRNA regulatory network. In our study, we found that lncRNA-SNHG7 was obviously upregulated in lung cancer tissues compared to adjacent non-cancerous tissues. Furthermore, silence of lncRNA-SNHG7 by siRNA suppressed cell proliferation, migration and invasion and accelerated apoptosis of A594 cells *in vitro*.

The present study showed, lncRNA-SNHG7 markedly inhibited apoptosis of A594 cells. Fas apoptotic inhibitory molecule 2 (FAIM2), as a 35 kDa membrane protein, is an anti-apoptotic protein conserved in evolution and known as the Lifeguard (LFG) family which is a distinct gene family of apoptotic-related genes (30). A previous study demonstrated that FAIM2 interacted with Fas upstream of Fas-associated death domain containing protein (FADD) and suppressed apoptosis (31). The high expression of FAIM2 enhanced resistance to Fas regulated apoptosis (32). Therefore, FAIM2 was used as apoptosis detection index. The present study found that the expression levels of FAIM2 also increased in lung cancer tissues. In addition, lncRNA-SNHG7 was of positive relevance with FAIM2 in human lung cancer tissues. Silence of FAIM2 by siRNA suppressed cell proliferation, migration, and invasion and accelerated apoptosis of A594 cells *in vitro*.

In the present study, we found that the expression levels of lncRNA-SNHG7 and FAIM2 obviously increased, and lncRNA-SNHG7 was of positive relevance with FAIM2 in lung cancer tissues. Silence of lncRNA-SNHG7 and silence of FAIM2 by siRNA suppressed cell proliferation, migration and invasion, and also accelerated the apoptosis of A594 cells *in vitro*, respectively. These results indicate that the lncRNA-SNHG7 network may lead to potential therapy for lung cancer in the future.

Materials and methods

Patients and clinical specimens. In the present study, we collected lung cancer and matched adjacent non-cancerous tissues samples (5 cm from the edge of the cancer as assessed by a pathologist) from patients who had undergone treatment for lung cancer between 2013 and 2015 at The First Affiliated Hospital of Southern Medical University, Guangzhou, China. The lung cancer histological diagnosis was confirmed according to the World Health Organization (WHO). Written informed consent was provided from each patient or her/his guardian. All tissue samples were washed with sterile phosphate-buffered saline (PBS) and was immediately saved at -80˚C until use.

Cell lines. Three human lung cancer cell lines (H125, 95D and A594) and human bronchial epithelial cells (BEAS-2B) were obtained from the Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China). Cells were cultured according to the ATCC protocols, BEAS-2B cells were cultured in bronchial epithelial growth medium (BEGM; PromoCell GmbH, Heidelberg, Germany). H125, 95D and A594 were cultured in RPMI-1640 medium (Invitrogen, Carlsbad, CA, USA), and all the media contained 10% fetal bovine serum (FBS) and 100 U/ml penicillin and 1 μg/ml streptomycin (both from Invitrogen). Then, the cells were cultured in an incubator with 5% CO_2 at 37˚C.

siRNA transfection. According to the protocol, we performed the siRNA transfection using Lipofectamine 3000 (Thermo Fisher Scientific, Rockford, IL, USA). siRNA oligomers were synthesized by Gemma (Shanghai, China). lncRNA-SNHG7 _ siRNA sequence was: 5'-GCUGGAAUAAAGAGUAA CAUU-3'; and FAIM2_siRNA sequence was: 5'-CUGGCU CCAUGCAGUUUAUUU-3'). A594 cells were seeded into 6-well plates (200,000 cells/well), and then transduced with control and lncRNA-SNHG7_siRNA or control and FAIM2_ siRNA (final concentration 50 μM) using Lipofectamine RNAiMax (Life Technologies, Inc., Grand Island, NY, USA). Forty-eight hours after transfection, cells were used forthe tests.

RNA preparation and reverse transcription. According to the manufacturer's instructions, total RNA was extracted from cells or the lung cancer tissues samples by the TRIzol reagent (Invitrogen). RNA (1.0 μg) was used as a template to synthesize corresponding cDNA with random primers using a RevertAid First Strand cDNA synthesis kit (Thermo Fisher Scientific).

Quantitative real-time reverse transcription PCR (qRT-PCR) assay. qRT-PCR was completed using SYBR-Green PCR Master Mix kit (Takara, Shiga, Japan) and an ABI 7500 Real-Time PCR system (Applied Biosystems, Warrington, UK). The primers of endogenous housekeeping gene (GAPDH chosen as internal loading control), lncRNA-SNHG7 and FAIM2 were designed. The sequences of GAPDH primers were: 5'-GTCAGCCGCATCTTCTTTTG-3' (sense) and 5'-GC GCCCAATACGACCAAATC-3' (antisense). The sequences of lncRNA-SNHG7 primers were: 5'-GTTGGGGTGTTGGCA TTCTTGTT-3' (sense) and 5'-TGGTCAGCCTGGTCACTC TGG-3' (antisense). The sequences of FAIM2 primers were: 5'-GGCGTGCTCTTCGTGCTTC-3' (sense) and 5'-TGGCGT CGGTACCCATCA-3' (antisense). The expression level of lncRNA-SNHG7 and FAIM2 mRNA was analyzed relative to the GAPDH mRNA level. All results are shown as the mean ± SD of three independent experiments.

Cell proliferation. Cell proliferation was measured using a Cell Counting Kit-8 (CCK-8 Kit; Beyotime Institute of Biotechnology, Jiangsu, China), transfected were seeded into 96-well plates at a density of $2x10^3$ cells/well with 100 μl RPMI-1640 medium (with 10% FBS) and cultured for 24, 48 and 72 h, respectively. The 10 μl of CCK-8 solution was put into the well at each time point and incubated for 4 h at 37˚C, and then the absorbance at 450 nm was measured using a microplate reader. Each experimental condition was detected in quintuplicates, and the experiments were repeated at least three times.

Flow cytometric analysis of apoptosis. The apoptotic cell death rate was analyzed by flow cytometry and double staining using propidium iodide (PI) and the Annexin V-FITC staining kit (BD Biosciences, San Diego, CA, USA) according to the manufacturer's instructions. A594 cells were digested with

trypsin, dispersed, centrifuged, collected, washed with Dulbecco's modified Eagle's medium (DMEM) medium and cold PBS and resuspended with 1X binding buffer at a concentration of $1x10^6$ cells/ml. PI (5 μl) and Annexin V-FITC were added into A594 cell buffer solution ($1x10^5$ cells) and incubated for 15 min at RT in the dark, and then 400 μl 1X binding buffer was put in each sample tube and analyzed by a BD FACSCalibur cytometer (BD Biosciences). Data were analyzed using FlowJo software version 8.8.6 (Tree Star Inc., Ashland, OR, USA). A594 cells transfected with NC-siRNA were used as the negative control (NC).

Migration and invasion assays. The migration and invasive capacity of the lung cancer cells *in vitro* were detected in 24-well Transwell cell culture chamber with 8-μm pores (both from Corning Costar Corp., Cambridge, MA, USA) according to the manufacturer's instructions. Cells were transfected with 50 μM lncRNA-SNHG7 siRNA, or control and FAIM2 siRNA for 48 h, and then 200 μl A594 cells ($2.5x10^5$/100 μl) were seeded in serum-free media to 8-μm pore cell culture inserts, and complete medium with 10% FBS was added to the lower chambers as a chemoattractant. Cells were incubated for 24 h at 37˚C, and the cells with migration ability spread through the chamber. Paraformaldehyde (4%) was used to fix the cells and 0.1% crystal violet solution was used to stain the lower chamber cells. The cells above the upper surface that did not migrate were cleared with a cotton swab, but did not touch the lower layer of the membrane. The number of migratory cells was confirmed through counting five areas of constant size/well under the microscope using a 20x objective. For the invasion assays, the polycarbonate membrane of Transwell inserts were covered with 10 μl 1:3 diluted Matrigel (BD Biosciences), and then were dried at 37˚C for 30 min. All experiments were completed in triplicate.

Western blot analysis. A594 cell homogenates were obtained with lysis buffer containing a protease inhibitor cocktail (P8340; Sigma-Aldrich, St. Louis, MO, USA), and lung cancer and matched adjacent non-cancerous tissue homogenates were obtained with RIPA buffer (Pierce/Thermo Fisher Scientific, Lafayette, CO, USA) containing a protease inhibitor cocktail. A BCA Protein Assay kit (Thermo Fisher Scientific) was used to measure the concentrations of total proteins. Proteins (30 μg) were separated using 8%, 12% Tris/glycine SDS/PAGE gels according to the molecular weight of the objective proteins, and then proteins were transferred to polyvinylidene fluoride (PVDF) membranes (Millipore, Billerica, MA, USA). The PVDF membranes were blocked with 5% skim milk (BD Biosciences) in 1X Tris-buffered saline including Tween-20 (TBST) for 2 h at room temperature, the transferred membranes were incubated with primary antibodies with a proper dilution at 4˚C overnight. Next day, the transferred membranes were washed with 1X Tris-buffered saline including Tween-20 three times, incubated with horseradish peroxidase-conjugated secondary antibodies with a proper dilution for 1 h at room temperature, and then washed with 1X Tris-buffered saline including Tween-20 three times, again. The experimental results were detected with the enhanced chemiluminescence (ECL) substrate kit and the enhanced chemiluminescence detection system (both from Amersham Biosciences, Piscataway, NJ, USA). The anti-human FAIM2 antibody (rabbit, 1:500; Sigma-Aldrich, Taufkirchen, Germany) which was used in the present study is a monoclone antibody. The rabbit anti-GAPDH monoclone antibody (1:4,000; Cell Signaling Technology, Beverly, MA, USA) was used as an internal control.

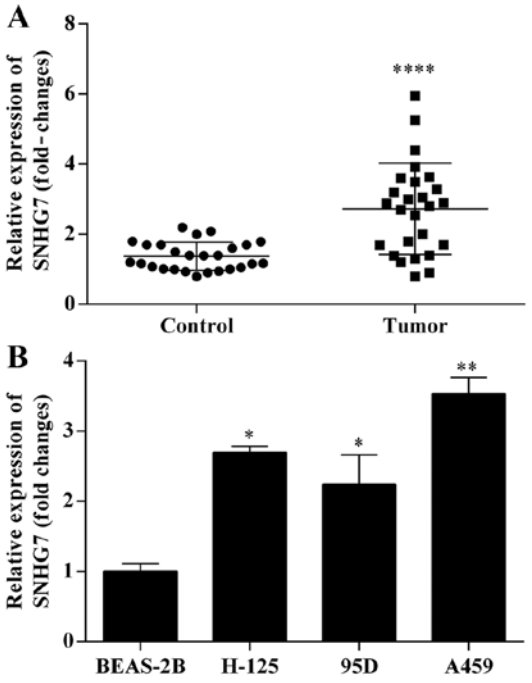

Figure 1. Expression levels of lncRNA-SNHG7 in lung cancer clinical specimens and cell lines. (A) lncRNA-SNHG7 mRNA was upregulated in lung cancer tissues compared to adjacent non-cancerous tissues from 26 lung cancer patients. Total RNA was isolated from lung cancer tissues, and lncRNA-SNHG7 mRNA levels were quantified by qRT-PCR. GAPDH was used as housekeeping-gene controls. (B) Effects of lncRNA-SNHG7 expression in lung cancer cell lines. The expression level of lncRNA-SNHG7 was detected by qRT-PCR and significantly increased in lung cancer cell lines compared with BEAS-2B. The values of expression level are presented as the mean percent of means ± SD (n=3; *P<0.05, **P<0.01 and ***P<0.001).

Statistical analysis. Statistical significance was analyzed using GraphPad (GraphPad Prism Software, La Jolla, CA, USA) and the SPSS 15.0 software [analysis of variance and Student's t-test or one-way analysis of variance (ANOVA)]. The data are presented as the means ± standard deviation (SD). P<0.05 was considered significant. P<0.05, P<0.01 and P<0.001.

Results

High expression of lncRNA-SNHG7 mRNA in lung cancer tissues and lung cancer cell lines. In order to investigate the expression of lncRNA-SNHG7 mRNA in lung cancer tissues, we adopted qRT-PCR assay to analyze the expression level of lncRNA-SNHG7 mRNA in lung cancer tissues relative to adjacent non-cancerous tissues. The results indicated that lncRNA-SNHG7 mRNA was obviously upregulated in lung cancer tissues compared to adjacent non-cancerous tissues (Fig. 1A). Furthermore, we analyzed the effect of lncRNA-SNHG7 mRNA in human bronchial epithelial cells (BEAS-2B) and three human lung cancer cell lines (H125, 95D and A594). qRT-PCR was used to measure the expression

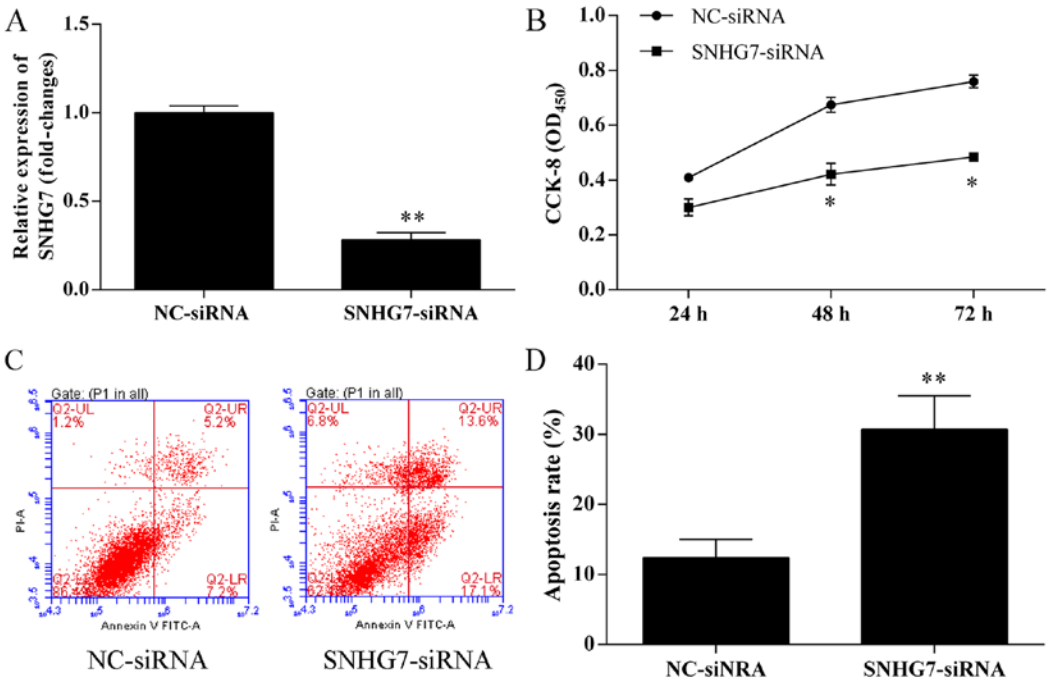

Figure 2. Silence of lncRNA-SNHG7 expression by siRNA inhibits proliferation and promotes apoptosis of A594 cells. (A) The expression effects of silence of lncRNA-SNHG7 mRNA expression by siRNA in A594 cells. Cells were transfected with 50 μM negative control (NC) siRNAs and 50 μM lncRNA-SNHG7 siRNA for 48 h respectively, total RNA was isolated, qRT-PCR showed that expression levels of lncRNA-SNHG7 compared to negative control. (n=3; **P<0.01). (B) lncRNA-SNHG7 promoted the proliferation ability of human lung cancer cells. The cell viability assay was accomplished by CCK-8 at three different durations (24, 48 and 72 h). The quantitative values of cell viability are showed by the mean percent of means ± SD (n=3; *P<0.05). (C and D) lncRNA-SNHG7 inhibits the apoptosis of A594 cells. Apoptotic cell death was detected by flow cytometric analysis with Annexin V-FITC and PI staining in A594 cells transfected with 50 μM lncRNA-SNHG7 siRNA for 48 h. The right lower quadrant indicates the relative proportions of early apoptosis, the right upper quadrant indicates the relative proportions of late apoptosis (**P<0.01).

level of lncRNA-SNHG7 mRNA in the cell lines above. lncRNA-SNHG7 mRNA markedly increased in three human lung cancer cell lines (H125, 95D and A594) compared to human bronchial epithelial cells (BEAS-2B). Among the three human lung cancer cell lines, the expression level of lncRNA-SNHG7 mRNA in A594 cells was the most remarkable (Fig. 1B). Thus, we chose the A594 cells as target cells.

Silence of lncRNA-SNHG7 expression by siRNA inhibits proliferation, migration and invasion, and promotes apoptosis of A594 cells. A594 cells were transfected with negative control (NC) siRNAs and lncRNA-SNHG7 siRNA for 48 h, respectively. The expression effects of silence of lncRNA-SNHG7 mRNA expression by siRNA in A594 cells were measured by qRT-PCR. As shown in Fig. 2A, the expression of lncRNA-SNHG7 was markedly suppressed through lipofectin transfection compared to NC. CCK-8 assay was used to detected cell proliferation ability. As shown in Fig. 2B, the proliferation ability of A594 cells transfected with lncRNA-SNHG7 siRNA was significantly suppressed compared with the control group (P<0.05). Apoptotic cell death was detected by flow cytometric analysis with Annexin V-FITC and PI staining in A594 cells transfected with lncRNA-SNHG7 siRNA for 48 h. Knockdown of lncRNA-SNHG7 in A594 cells by siRNA accelerated apoptosis of A594 cells. As shown in Fig. 2C and D, the apoptosis cells percentage of A594 cells transfected with lncRNA-SNHG7 siRNA was 30.7%, while the control group was 12.4%. Thus, the apoptotic cell percentage of in A594 cells with knockdown of lncRNA-SNHG7 by

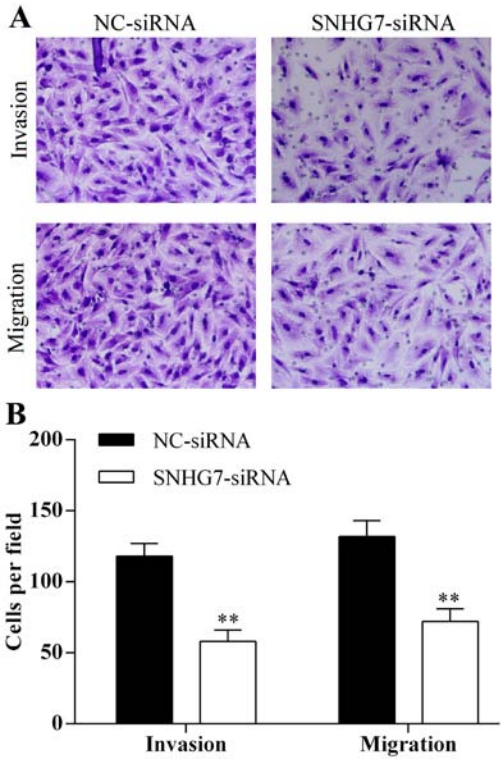

Figure 3. Silence of lncRNA-SNHG7 expression by siRNA inhibits migration and invasion ability of A594 cells. A594 cells transfected with negative control (NC) and lncRNA-SNHG7 siRNA for 48 h and seeded in Transwell. (A) Migrated and invasive cells were stained with crystal violet solution and (B) quantification of migrated and invasive cells is shown as percentage of control. Magnification, x200; scale bars, 10 μm (**P<0.01).

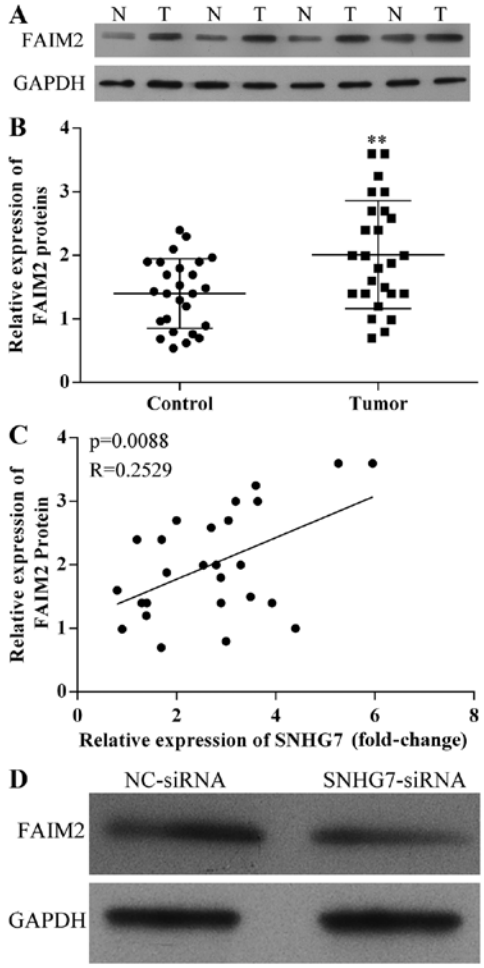

Figure 4. Expression correlation analysis of lncRNA-SNHG7 and FAIM2 in lung cancer clinical specimens and A594 cells. (A) FAIM2 proteins have higher expression in lung cancer tissues. The expression levels of FAIM2 proteins in human lung cancer and paired adjacent non-cancerous tissues from four random clinically-diagnosed lung cancer patients were measured by western blot analysis. GAPDH was used as a loading control. N, paired adjacent normal mucosa tissues; T, tumor tissues. (B) Significant upregulation of FAIM2 mRNA expression in lung cancer tissues compared to adjacent non-cancerous tissues. The expression level of FAIM2 mRNA in human lung cancer tissues was detected by qRT-PCR (n=26; $^{**}P<0.01$). (C) Positive correlation between lncRNA-SNHG7 and FAIM2 gene expression in human lung cancer tissues by qRT-PCR (n=26; $^{**}P<0.01$). (D) Silence of lncRNA-SNHG7 expression by siRNA in A594 cells reduced the expression of FAIM2 proteins. Protein extracts from A594 cells transfected with negative control (NC) and lncRNA-SNHG7 siRNA were assayed to western blot analysis using FAIM2 and GAPDH (loading control) antibody.

siRNA increased 1.48 times. Transwell assay was used to detect migration and invasion abilities of A594 cells transfected with lncRNA-SNHG7 siRNA for 48 h. Knockdown of lncRNA-SNHG7 in A594 cells by siRNA decreased the ability of cell migration and invasion (Fig. 3A and B). Therefore, knockdown of lncRNA-SNHG7 in A594 cells inhibited the capacity of proliferation, migration and invasion and promoted the capacity of apoptosis of A594 cells.

lncRNA-SNHG7 physically interacts with FAIM2 in lung cancer clinical specimens and A594 cells. In our research, we demonstrated that lncRNA-SNHG7 inhibited the capacity of apoptosis of A594 cells. Therefore, FAIM2, an inhibitor of the Fas signaling pathway, was studied in lung cancer. In order to investigate the expression level of FAIM2 in lung cancer tissues, western blotting was used to detect the expression levels of FAIM2 proteins in human lung cancer and paired adjacent non-cancerous tissues from four random lung cancer patients. As shown in Fig. 4A, FAIM2 protein was higher expressed in lung cancer tissues. qRT-PCR was used to measure the expression levels of FAIM2 mRNA in human lung cancer tissues. FAIM2 mRNA was also higher expression in lung cancer tissues (Fig. 4B).

Furthermore, to investigate the correlation between lncRNA-SNHG7 and FAIM2 in human lung cancer tissues qRT-PCR results were analyzed. As shown in Fig. 4C, lncRNA-SNHG7 was of positive relevance with FAIM2 gene expression in human lung cancer tissues when A594 cells were transfected with FAIM2 siRNA for 48 h. The expression level of FAIM2 protein declined relative to NC (Fig. 4C). Therefore, lncRNA-SNHG7 increased the expression level of FAIM2 in lung cancer clinical specimens and A594 cells.

Silence of FAIM2 expression by siRNA inhibits proliferation, migration and invasion, and promotes apoptosis of A594 cells. Based on previous results that positive correlation between lncRNA-SNHG7 and FAIM2 gene expression was shown in human lung cancer tissues, in addition, lncRNA-SNHG7 can promote the expression level of FAIM2 in A594 cells. We further detected the effects of FAIM2 on A594 cell growth, migration, invasion and apoptosis. A594 cells were transfected with NC siRNAs and FAIM2 siRNA for 48 h, respectively. The expression effects of silence of FAIM2 mRNA and protein expression by siRNA in A594 cells were performed by qRT-PCR and western blotting. The expression of FAIM2 was markedly suppressed through transfecting with FAIM2 siRNA compared to NC ($P<0.01$) (Fig. 5A and B). The proliferation ability of A594 cells transfected with FAIM2 siRNA was measured by CCK-8 assay. The proliferation ability of A594 cells transfected with FAIM2 siRNA was significantly decreased compared with the control group ($P<0.05$) (Fig. 5C). Flow cytometric analysis was used to detect the apoptotic cell death of A594 cells transfected with FAIM2 siRNA with Annexin V-FITC and PI staining. We found that the apoptotic cell percentage of A594 cells transfected with FAIM2 siRNA was 32.3%, while the control group was 7.4%. Thus, the apoptotic cell percentage of A594 cells with knockdown of FAIM2 by siRNA increased 3.36 times (Fig. 5D and E). Thus, we indicated that silence of FAIM2 expression by siRNA increased apoptosis of A594 cells. Furthermore, we evaluated the ability of migration and invasion of A594 cells transfected with FAIM2 siRNA using Transwell assay. Silence of FAIM2 expression by siRNA in A594 cells reduced the ability of cell migration and invasion (Fig. 6A and B). Therefore, we indicated that knockdown of FAIM2 in A594 cells reduced the capacity of proliferation, migration and invasion and enhanced the capacity of apoptosis of A594 cells.

Discussion

The crucial results of the present study were that lncRNA-SNHG7 was highly expressed in lung cancer tissues relative to adjacent non-cancerous tissues. FAIM2 was also over-expressed in lung cancer tissues. lncRNA-SNHG7 was

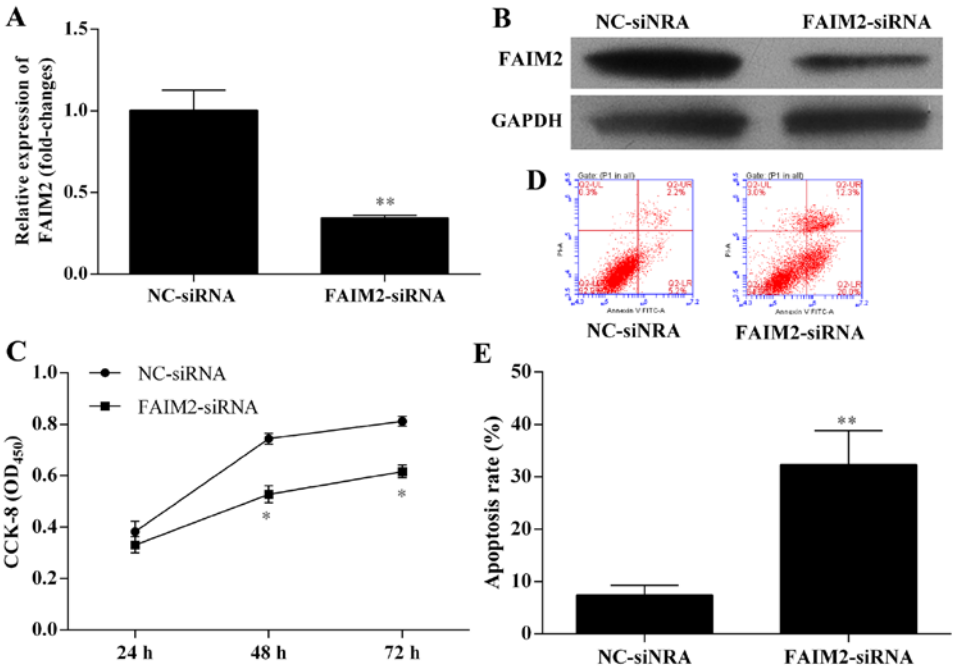

Figure 5. Silence of FAIM2 expression by siRNA inhibits proliferation and promotes apoptosis of A594 cells. (A) The expression effects of silence of FAIM2 mRNA expression by siRNA in A594 cells. Cells were transfected with 50 μM negative control (NC) siRNAs and 50 μM FAIM2 siRNA for 48 h respectively, total RNA was isolated, qRT-PCR showed expression levels of FAIM2 compared to negative control. (n=3; **P<0.01). (B) The expression effects of silence of FAIM2 protein expression by siRNA in A594 cells. Cells were transfected with 50 μM negative control (NC) siRNAs and 50 μM FAIM2 siRNA for 48 h, respectively, total protein was isolated, western blotting analysis of the expression levels of FAIM2 proteins compared to negative control. (C) Silence of FAIM2 expression by siRNA controls the proliferation ability of A594 cells. The cell viability assay was accomplished by CCK-8 at three different durations (24, 48 and 72 h). The quantitative values of cell viability are showed as the mean percent of means ± SD (n=3; *P<0.05). (D and E) FAIM2 inhibits apoptosis of A594 cells. Apoptotic cell death was detected by flow cytometric analysis with Annexin V-FITC and PI staining in A594 cells transfected with 50 μM FAIM2 siRNA for 48 h. The right lower quadrant indicates the relative proportions of early apoptosis, the right upper quadrant indicates the relative proportions of late apoptosis (**P<0.01).

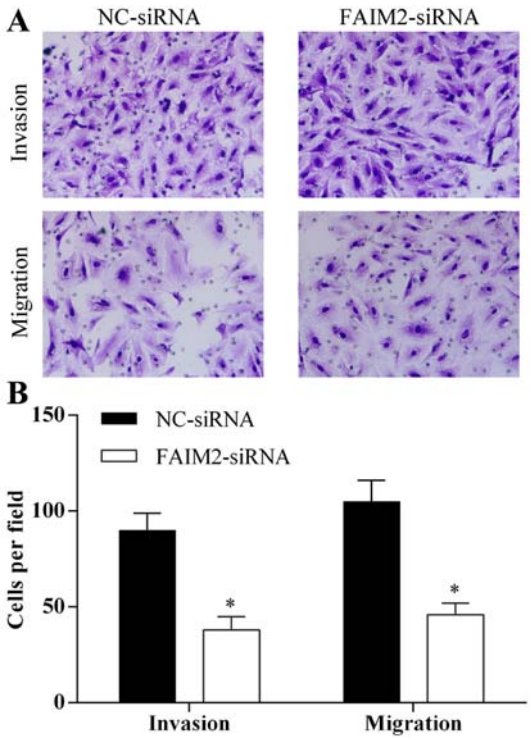

Figure 6. Silence of FAIM2 expression by siRNA inhibits migration and invasion ability of A594 cells. A594 cells transfected with negative control (NC) and FAIM2 siRNA for 48 h and seeded in Transwell. Migrated and invasive cells were stained with crystal violet solution (A) and quantification of migrated and invasive cells is shown as percentage of control (B). Magnification, x200; scale bars, 10 μm (*P<0.01).

associated with FAIM2 and there was a positive correlation between them in lung cancer. Silence of lncRNA-SNHG7 by siRNA repressed the level of FAIM2 protein in A594 cells. Silence of lncRNA-SNHG7 and silence of FAIM2 by siRNA repressed the level of FAIM2 protein and suppressed cell proliferation, migration and invasion and accelerated apoptosis of A594 cells *in vitro*, respectively. Thus, our results showed that lncRNA-SNHG7 promotes the proliferation, migration and invasion and inhibits apoptosis of lung cancer cells by enhancing the FAIM2 expression and may be regarded as a potential therapeutic target for lung cancer.

Increasing number of studies has found that lncRNAs are connected with multiple genetic phenomena, such as transcriptional regulations, DNA methylation and chromatin remodeling (14,33,34). Increasing evidence indicates that lncRNAs are interacted with genes, proteins or chromatin remodeling to influence the expression levels of genes (35,36). LncRNAs which are generated in intronic, intergenic, antisense loci or overlapping regions play a great role in diverse gene regulatory functions (37,38). At present, numerous studies have demonstrated that lncRNAs are involved in tumor carcinogenesis since it can accelerate cell proliferation through controlling correlative proteins (39-41). In the present study, we found that the expression levels of lncRNA-SNHG7 mRNA and protein obviously increased in lung cancer tissues compared to adjacent non-cancerous tissues. lncRNA-SNHG7 promoted the expression of FAIM2 protein and then inhibited A594 cell apoptosis. We also showed that silence

of lncRNA-SNHG7 by siRNA suppressed cell proliferation, migration, and invasion and accelerated apoptosis of A594 cells *in vitro*. These results suggest that lncRNA-SNHG7 is an important molecule for tumor development and may become a potential biomarker for the treatment of lung cancer.

FAIM2, as a gene of the LFG family, will have very important effect in protecting cells against apoptosis by directly bonding the Fas receptor (31,42). It has been discovered that LFG was overexpressed in the mostly neuron and hippocampus cells (31). Recent studies have shown that expression of the FAIM2 was interrelated to high fat (43) and the different methylation levels of the FAIM2 promoter was markedly related to obesity (44). FAIM2 gene is associated with obesity (45), simultaneously, obesity is partly related to tobacco smoking which causes the development of cancer. However, the functional mechanism of FAIM2 is not entirely clear in lung cancer. In the present study, we have demonstrated that the expression level of FAIM2 mRNA increased in lung cancer tissues compared to adjacent non-cancerous tissues. Furthermore, positive correlation between lncRNA-SNHG7 and FAIM2 gene expression in human lung cancer tissues and silence of lncRNA-SNHG7 expression by siRNA reduced the expression of FAIM2 proteins in A594 cells. We also found that silence of FAIM2 expression by siRNA inhibited proliferation, migration and invasion and promoted apoptosis of A594 cells. These results suggest that FAIM2 also is an important molecule for tumor development.

In brief, the present study demonstrated that lncRNA-SNHG7 as an oncogene promoting proliferation, migration and invasion, and inhibiting apoptosis of lung cancer cells by enhancing the FAIM2 expression. This finding shows that lncRNA-SNHG7 is a momentous molecule for tumor progression and provides significant potential to develop new therapies to prevent or treat lung cancer.

References

1. Parkin D, Whelan S, Ferlay J, Teppo L and Thomas D: Cancer Incidence in Five Continents Vol. VIII. IARC Publications 155: 2002.
2. Parkin DM, Bray F, Ferlay J and Pisani P: Global cancer statistics, 2002. CA Cancer J Clin 55: 74-108, 2005.
3. Jemal A, Bray F, Center MM, Ferlay J, Ward E and Forman D: Global cancer statistics. CA Cancer J Clin 61: 69-90, 2011.
4. Siegel R, Naishadham D and Jemal A: Cancer statistics, 2013. CA Cancer J Clin 63: 11-30, 2013.
5. Jemal A, Siegel R, Xu J and Ward E: Cancer statistics, 2010. CA Cancer J Clin 60: 277-300, 2010.
6. Malarcher AM, Schulman J, Epstein LA, Thun MJ, Mowery P, Pierce B, Escobedo L and Giovino GA: Methodological issues in estimating smoking-attributable mortality in the United States. Am J Epidemiol 152: 573-584, 2000.
7. Organization WH: International statistical classification of diseases and related health problems. Vol 1. World Health Organization, 2004.
8. Travis WD, Brambilla E, Muller-Hermelink HK and Harris CC: Pathology and Genetics of Tumours of the Lung, Pleura, Thymus and Heart. IARC Publications, 2004.
9. Granville CA and Dennis PA: An overview of lung cancer genomics and proteomics. Am J Respir Cell Mol Biol 32: 169-176, 2005.
10. Meyerson M and Carbone D: Genomic and proteomic profiling of lung cancers: Lung cancer classification in the age of targeted therapy. J Clin Oncol 23: 3219-3226, 2005.
11. Bierhoff H, Schmitz K, Maass F, Ye J and Grummt I: Noncoding transcripts in sense and antisense orientation regulate the epigenetic state of ribosomal RNA genes. Cold Spring Harb Symp Quant Biol 75: 357-364, 2010.
12. Wang KC and Chang HY: Molecular mechanisms of long noncoding RNAs. Mol Cell 43: 904-914, 2011.
13. Nagano T and Fraser P: No-nonsense functions for long noncoding RNAs. Cell 145: 178-181, 2011.
14. Mercer TR, Dinger ME and Mattick JS: Long non-coding RNAs: Insights into functions. Nat Rev Genet 10: 155-159, 2009.
15. Loewer S, Cabili MN, Guttman M, Loh YH, Thomas K, Park IH, Garber M, Curran M, Onder T, Agarwal S, *et al*: Large intergenic non-coding RNA-RoR modulates reprogramming of human induced pluripotent stem cells. Nat Genet 42: 1113-1117, 2010.
16. Liu X, Li D, Zhang W, Guo M and Zhan Q: Long non-coding RNA *gadd7* interacts with TDP-43 and regulates *Cdk6* mRNA decay. EMBO J 31: 4415-4427, 2012.
17. Lakhotia SC: Long non-coding RNAs coordinate cellular responses to stress. Wiley Interdiscip Rev RNA 3: 779-796, 2012.
18. Paralkar VR and Weiss MJ: A new 'Linc' between noncoding RNAs and blood development. Genes Dev 25: 2555-2558, 2011.
19. Saxena A and Carninci P: Long non-coding RNA modifies chromatin: Epigenetic silencing by long non-coding RNAs. BioEssays 33: 830-839, 2011.
20. Kotake Y, Nakagawa T, Kitagawa K, Suzuki S, Liu N, Kitagawa M and Xiong Y: Long non-coding RNA *ANRIL* is required for the PRC2 recruitment to and silencing of $p15^{INK4B}$ tumor suppressor gene. Oncogene 30: 1956-1962, 2011.
21. Tsai MC, Manor O, Wan Y, Mosammaparast N, Wang JK, Lan F, Shi Y, Segal E and Chang HY: Long noncoding RNA as modular scaffold of histone modification complexes. Science 329: 689-693, 2010.
22. Yang F, Zhang L, Huo XS, Yuan JH, Xu D, Yuan SX, Zhu N, Zhou WP, Yang GS, Wang YZ, *et al*: Long noncoding RNA high expression in hepatocellular carcinoma facilitates tumor growth through enhancer of zeste homolog 2 in humans. Hepatology 54: 1679-1689, 2011.
23. Enfield KS, Pikor LA, Martinez VD and Lam WL: Mechanistic roles of noncoding RNAs in lung cancer biology and their clinical implications. Genet Res Int 2012: 737416, 2012.
24. Piao HL and Ma L: Non-coding RNAs as regulators of mammary development and breast cancer. J Mammary Gland Biol Neoplasia 17: 33-42, 2012.
25. Ying L, Huang Y, Chen H, Wang Y, Xia L, Chen Y, Liu Y and Qiu F: Downregulated MEG3 activates autophagy and increases cell proliferation in bladder cancer. Mol Biosyst 9: 407-411, 2013.
26. Dereure O: Role of non-coding RNA ANRIL in the genesis of plexiform neurofibromas in neurofibromatosis type 1. Ann Dermatol Venereol 139: 421-422, 2012 (In French).
27. Gutschner T, Hämmerle M, Eissmann M, Hsu J, Kim Y, Hung G, Revenko A, Arun G, Stentrup M, Gross M, *et al*: The noncoding RNA *MALAT1* is a critical regulator of the metastasis phenotype of lung cancer cells. Cancer Res 73: 1180-1189, 2013.
28. Thai P, Statt S, Chen CH, Liang E, Campbell C and Wu R: Characterization of a novel long noncoding RNA, SCAL1, induced by cigarette smoke and elevated in lung cancer cell lines. Am J Respir Cell Mol Biol 49: 204-211, 2013.
29. Yang Y, Li H, Hou S, Hu B, Liu J and Wang J: The noncoding RNA expression profile and the effect of lncRNA AK126698 on cisplatin resistance in non-small-cell lung cancer cell. PLoS One 8: e65309, 2013.
30. Hu L, Smith TF and Goldberger G: LFG: A candidate apoptosis regulatory gene family. Apoptosis 14: 1255-1265, 2009.
31. Somia NV, Schmitt MJ, Vetter DE, Van Antwerp D, Heinemann SF and Verma IM: LFG: An anti-apoptotic gene that provides protection from Fas-mediated cell death. Proc Natl Acad Sci USA 96: 12667-12672, 1999.
32. Beier CP, Wischhusen J, Gleichmann M, Gerhardt E, Pekanovic A, Krueger A, Taylor V, Suter U, Krammer PH, Endres M, *et al*: FasL (CD95L/APO-1L) resistance of neurons mediated by phosphatidylinositol 3-kinase-Akt/protein kinase B-dependent expression of lifeguard/neuronal membrane protein 35. J Neurosci 25: 6765-6774, 2005.
33. Mercer TR and Mattick JS: Structure and function of long noncoding RNAs in epigenetic regulation. Nat Struct Mol Biol 20: 300-307, 2013.
34. Geisler S and Coller J: RNA in unexpected places: Long non-coding RNA functions in diverse cellular contexts. Nat Rev Mol Cell Biol 14: 699-712, 2013.
35. Khalil AM, Guttman M, Huarte M, Garber M, Raj A, Rivea Morales D, Thomas K, Presser A, Bernstein BE, van Oudenaarden A, *et al*: Many human large intergenic noncoding RNAs associate with chromatin-modifying complexes and affect gene expression. Proc Natl Acad Sci USA 106: 11667-11672, 2009.

36. Mattick JS and Gagen MJ: The evolution of controlled multitasked gene networks: The role of introns and other noncoding RNAs in the development of complex organisms. Mol Biol Evol 18: 1611-1630, 2001.
37. Shamovsky I and Nudler E: Gene control by large noncoding RNAs. Sci STKE 2006: pe40, 2006.
38. Kaikkonen MU, Lam MT and Glass CK: Non-coding RNAs as regulators of gene expression and epigenetics. Cardiovasc Res 90: 430-440, 2011.
39. Cooper C, Guo J, Yan Y, Chooniedass-Kothari S, Hube F, Hamedani MK, Murphy LC, Myal Y and Leygue E: Increasing the relative expression of endogenous non-coding Steroid Receptor RNA Activator (SRA) in human breast cancer cells using modified oligonucleotides. Nucleic Acids Res 37: 4518-4531, 2009.
40. Okamura D, Maeda I, Taniguchi H, Tokitake Y, Ikeda M, Ozato K, Mise N, Abe K, Noce T, Izpisua Belmonte JC, *et al*: Cell cycle gene-specific control of transcription has a critical role in proliferation of primordial germ cells. Genes Dev 26: 2477-2482, 2012.
41. Prensner JR, Iyer MK, Balbin OA, Dhanasekaran SM, Cao Q, Brenner JC, Laxman B, Asangani IA, Grasso CS, Kominsky HD, *et al*: Transcriptome sequencing across a prostate cancer cohort identifies *PCAT-1*, an unannotated lincRNA implicated in disease progression. Nat Biotechnol 29: 742-749, 2011.
42. Fernández M, Segura MF, Solé C, Colino A, Comella JX and Ceña V: Lifeguard/neuronal membrane protein 35 regulates Fas ligand-mediated apoptosis in neurons via microdomain recruitment. J Neurochem 103: 190-203, 2007.
43. Yoganathan P, Karunakaran S, Ho MM and Clee SM: Nutritional regulation of genome-wide association obesity genes in a tissue-dependent manner. Nutr Metab 9: 65, 2012.
44. Wu L, Zhao X, Shen Y, Zhang MX, Yan Y, Hou D, Meng L, Liu J, Cheng H and Mi J: Promoter methylation of fas apoptotic inhibitory molecule 2 gene is associated with obesity and dyslipidaemia in Chinese children. Diab Vasc Dis Res 12: 217-220, 2015.
45. Boender AJ, van Rozen AJ and Adan RA: Nutritional state affects the expression of the obesity-associated genes *Etv5*, *Faim2*, *Fto*, and *Negr1*. Obesity 20: 2420-2425, 2012.