

# Long Non-Coding RNA *SNHG14* Regulates *SPIN1* Expression to Accelerate Tumor Progression in Non-Small Cell Lung Cancer by Sponging *miR-382-5p*

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**Background:** Non-small cell lung cancer (NSCLC) is the most common type of lung carcinoma. Long non-coding RNA (lncRNA) small nucleolar RNA host gene 14 (*SNHG14*) was identified to participate in tumor progression. However, the mechanism and functions of *SNHG14* were rarely reported in NSCLC progression.

**Methods:** The relative gene expression was tested by qRT-PCR. Cell viability, apoptosis, migration and invasion were measured by MTT assay, flow cytometry, and transwell migration and invasion assays, respectively. The interactions between *miR-382-5p* and *SNHG14* or *SPIN1* were predicted by starBase and confirmed by the dual-luciferase reporter assay and RNA pull-down assay. The protein level of *SPIN1* was evaluated by Western blot assay.

**Results:** The levels of *SNHG14* and *SPIN1* were significantly increased, while the level of *miR-382-5p* was apparently reduced in NSCLC tissues and cells. *SNHG14* was verified to sponge *miR-382-5p* and *SPIN1* was identified as a direct target of *miR-382-5p*. *SNHG14* depletion repressed cell viability, migration and invasion, but induced the apoptotic rate by targeting *miR-382-5p*. *miR-382-5p* overexpression blocked cell viability, metastasis and promoted cell apoptosis by regulating *SPIN1*. *SNHG14* silencing down-regulated *SPIN1* expression by sponging *miR-382-5p*.

**Conclusion:** *SNHG14* facilitated NSCLC progression by regulating *SPIN1* expression via targeting *miR-382-5p*.

**Keywords:** lncRNA *SNHG14*, *miR-382-5p*, *SPIN1*, tumor progression, NSCLC

## Introduction

Lung cancer, the most leading causes of cancer-associated death overall the world, is divided into two groups: small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC) which accounted for about 80–85% in all lung cancer patients.<sup>1,2</sup> Despite the improvements in surgeries and therapies in recent decades, the 5-year overall survival of NSCLC is just approximately 15%.<sup>3</sup> One of the most important reasons is metastasis.<sup>4</sup> Hence, it is crucial to explore the mechanism of NSCLC.

Long non-coding RNAs (lncRNAs), a form of long RNA (>200 nucleotides (nt)) with no translation capacity, have been identified to affect target gene expression at posttranscriptional stage and to implicate in the processes of tumor development.<sup>5</sup> In NSCLC, lots of lncRNAs were documented to aberrant expression. For instance, a study indicated that *MALAT1* was elevated in NSCLC, and its

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depletion suppressed metastasis and cell growth in vitro and also restrained xenograft tumor growth in vivo.<sup>6</sup> Liu et al reported that the high level of *HOTAIR* in NSCLC promoted cell metastasis.<sup>7</sup> The similar results of *ANRIL*<sup>8</sup> and *UCA1*<sup>9</sup> were also reported in NSCLC. Small nucleolar RNA host gene 14 (*SNHG14*) is located on human chromosome 15 and reported to accelerate tumor development in many types of cancers, including colorectal cancer,<sup>10</sup> ovarian cancer,<sup>11</sup> bladder cancer,<sup>12</sup> and gastric cancer.<sup>13</sup> However, the mechanism of *SNHG14* was rarely reported in NSCLC.

MicroRNAs (miRNAs) are a family of small RNA (~22 nt) without translation ability and could target message RNA (mRNA) to decrease target gene expression.<sup>14</sup> Also, many miRNAs were reported to be dysregulated in NSCLC. For example, *miR-451* was significantly down-regulated in NSCLC, and its overexpression triggered apoptosis but inhibited cell growth.<sup>15</sup> The similar results of *miR-145*,<sup>15</sup> *miR-193a-3p/5p*<sup>16</sup> were reported in NSCLC. Chen et al documented that miR-382 was strikingly reduced in NSCLC, and miR-382 overexpression blocked cell growth, metastasis by targeting *SETD8* in NSCLC.<sup>17</sup> Nevertheless, the mechanism of *miR-382-5p* was not clear in NSCLC.

Spindlin-1 (*SPIN1*) is located on human chromosome 9, and the ectopic expression of *SPIN1* was documented to be associated with tumor progression. A research in glioma implied that *SPIN1* facilitated cell growth while constrained apoptosis regulated by *miR-489*.<sup>18</sup> Another reporter in breast cancer demonstrated that *SPIN1* enhanced cell growth, metastasis mediated by *miR-1271* in vitro.<sup>19</sup> However, the mechanism of *SPIN1* in NSCLC was scarcely documented. In this research, the mechanism of *SNHG14* in NSLSC was our main study point.

## Materials and Methods

### Tissue Samples

Fifty NSCLC tissue samples were collected from Gansu Gem Flower Hospital, as well as the corresponding adjacent normal tissue samples. The clinicopathologic features of these patients are presented in Table 1. All collected tissue samples were immediately frozen in  $-80^{\circ}\text{C}$  refrigerator until used. The project was authorized by the Ethics Committee of Gansu Gem Flower Hospital and carried out according to the Declaration of Helsinki Principles. All patients were provided informed consents.

**Table 1** Correlation Between Clinicopathological Characteristics and *SNHG14* Expression Level in NSCLC Patients

Characteristics	n	<i>SNHG14</i>		P
		High	Low	
Gender				0.802
Male	26	15	11	
Female	24	13	11	
Age (years)				0.569
$\geq 60$	25	13	12	
$< 60$	25	15	10	
TNM stage				0.023*
I+II;	28	18	10	
IIIa	22	7	15	
Smoking history				0.028*
No	20	13	7	
Yes	30	10	20	
Lymph node metastasis				0.010*
Yes	24	17	7	
No	26	9	17	
Differentiation				0.419
Well/moderate	26	17	9	
poor	24	13	11	

**Note:** \*P < 0.05 was considered statistically significant.

### Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR)

The RNA samples were extracted from NSCLC tissues and cells using TriQuick Reagent (Solarbio, Beijing, China). The random primers (Solarbio) were used for reverse transcription, and the quantitative PCR was performed using SYBR mix (TaKaRa, Dalian, China). The level of *SNHG14* and *SPIN1* was normalized by glyceraldehyde 3-phosphate dehydrogenase (GAPDH) while *miR-382-5p* was normalized via small nuclear RNA U6, and processed with the method of  $2^{-\Delta\Delta C_t}$ . The primers were obtained from Songon (Shanghai, China) and exhibited as follows: *SNHG14*: (GeneBank accession No. NR\_146177; F, 5'-ACCTGCAAGCTTTTTGACCC-3', and R, 5'-AGCAGACAAAGAAAACCCCAAT-3'); *miR-382-5p*: (GeneBank accession No. NR\_029874.1; F, 5'-TAAAA TAGGAGTACTGTCTAA-3', and R, 5'-ATTAGTAAATTGG CTGCTGCAG-3'); *SPIN1*: (GeneBank accession No. NM\_006717.3; F, 5'-GAAGGTGAAGGTCGGAGT-3' and 5'-GATGGCAACAATATCCACTT-3'); GAPDH: (F, 5'-AC CACAGTCCATGCCATCAC-3', and R, 5'-TCCACCA

CCCTGTTGCTGTA-3'), and U6: (F, 5'-ATTGGAACGATA CAGAGAAGATT-3', and R, 5'-GGAACGCTTCACGAAT TTG-3').

## Cell Culture and Transfection

Two human non-small cell lung cancer cells (H1299, catalog #CBP30016L, and A549, catalog #CBP60084) and human pulmonary bronchial epithelium cell BEAS-2B (catalog #CBP60577) were purchased from Cobioer (Nanjing, China). All cells were cultivated in RPMI-1640 medium (Thermo Fisher Scientific) containing 10% fetal bovine serum (FBS; Thermo Fisher Scientific) and 1% penicillin/streptomycin (Solarbio) in 5% CO<sub>2</sub> incubator at 37°C.

Small interfering RNA (siRNA) against *SNHG14* (si-*SNHG14*, GeneBank accession No. NR\_146177; 5'-GCACAAUAUCUUUGAACUA-3') and its negative control (si-NC), *miR-382-5p* mimics (*miR-382-5p*) and its negative control (miR-NC), *miR-382-5p* antagonist (*miR-382-5p* inhibitor) and its matched control (inhibitor-NC) were synthesized in GenePharma (Shanghai, China). The sequences of *SPINI* were cloned and inserted into pcDNA (GenePharma) to construct *SPINI* overexpression plasmid (pcDNA-*SPINI*). The transfection was performed using Lip2000 Transfection Reagent (Solarbio).

## 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide (MTT) Assay

MTT (Solarbio) was used to detect the cell viability of H1299 and A549 cells. The H1299 and A549 cells ( $2 \times 10^3$  per well) were firstly cultivated in 96-well plate for 24 h. Following the transfection, the transfected H1299 and A549 cells were cultured for another 0 h, 24 h, 48 h, and 72 h. After centrifugation, MTT was injected and incubated for another 4 h. Then, dimethyl sulfoxide (DMSO) was added to dissolve the formazan. The absorbance at 490 nm was measured on a Multiscan Spectrum.

## Flow Cytometry Analysis of Cell Apoptosis

The apoptotic rate of H1299 and A549 cells was assessed using Annexin V-fluorescein isothiocyanate (FITC)/propidium iodide (PI) apoptosis detection kit (Solarbio). The H1299 and A549 cells ( $1 \times 10^5$ ) were resuspended in binding buffer containing Annexin V-FITC and incubated for 10 min at 37°C in the dark. Then, the cell samples were incubated with PI for another 5 min in the dark. The apoptotic rate was evaluated through flow cytometry within 1 h.

## Transwell Assay

Transwell chambers (8.0  $\mu$ m; Solarbio) were used to test the migrated and invaded abilities of H1299 and A549 cells. For cell migration, the lower chamber was injected with RPMI-1640 medium containing 10% FBS, while the upper one was added H1299 and A549 cells ( $5 \times 10^3$ ) into RPMI-1640 medium without FBS and cultivated for 48 h. The cells on the backside of polycarbonate film were fixed and stained with 4% methanol and 0.1% crystal violet, respectively. The random 8 fields were selected, and the cells were counted under a microscope.

For cell invasion, the protocols were similar to that in cell migration. The difference was the upper chamber was covered with a Matrigel matrix (Solarbio).

## Dual-Luciferase Reporter Assay

The wild type and mutant sequences of *SNHG14*, 3'-untranslated regions (3'UTR) of *SPINI* were inserted into pmirGLO vector (Youbio, Changsha, China) to construct luciferase reporters, namely *SNHG14*-WT, *SNHG14*-MUT, *SPINI* 3'UTR-WT, or *SPINI* 3'UTR-MUT. The luciferase report and miR-NC or *miR-382-5p* were co-transfected into H1299 and A549 cells using Lip2000 Transfection Reagent (Solarbio). A luciferase reporter detection kit (Solarbio) was utilized to detect the luciferase activity.

## RNA Pull-Down Assay

Biotin-labeled *miR-382-5p* (Bio-miR-382-5p) and negative control (Bio-miR-NC) were obtained from RiBoBio (Guangzhou, China). The Bio-miR-382-5p or Bio-miR-NC was added into the lysate samples of H1299 and A549 cells. The streptavidin magnetic beads were used to recruit Bio-labelled RNA-RNA complex. After elution, the level of *SNHG14* was evaluated by qRT-PCR.

## Western Blot Assay

The protein in H1299 and A549 cells was extracted using a protein extraction kit (Beyotime, Shanghai, China). Then, the concentration of protein samples was detected using BCA protein assay kit (Beyotime), and the protein samples were separated via sodium dodecyl sulfonate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto a polyvinylidene fluoride (PVDF; GE Healthcare, Piscataway, NJ, USA) membrane. Subsequently, the membrane was sealed in skim milk for 4 h and then incubated with primary antibody overnight at 4°C. The membrane was incubated with secondary antibody for another 2 h at 37°C. The

intensity of bands was measured using eyoECL plus kit (Beyotime). The rabbit primary antibody *SPINI* (1/500; ab196938),  $\beta$ -actin (1/1000; ab8227), and goat anti-rabbit secondary antibody (1/10,000; ab175781) were purchased from Abcam (Cambridge, MA, USA).

## Statistical Analysis

GraphPad Prism 7 (GraphPad Inc., La Jolla, CA, USA) was used to process the experimental data which were repeated for at least three times. The Student's *t*-test was used to compare the data between the two groups, and one-way analysis of variance (ANOVA) was utilized to analyze the comparison among multiple groups. *P*-value less than 0.05 was considered as statistically different.

## Results

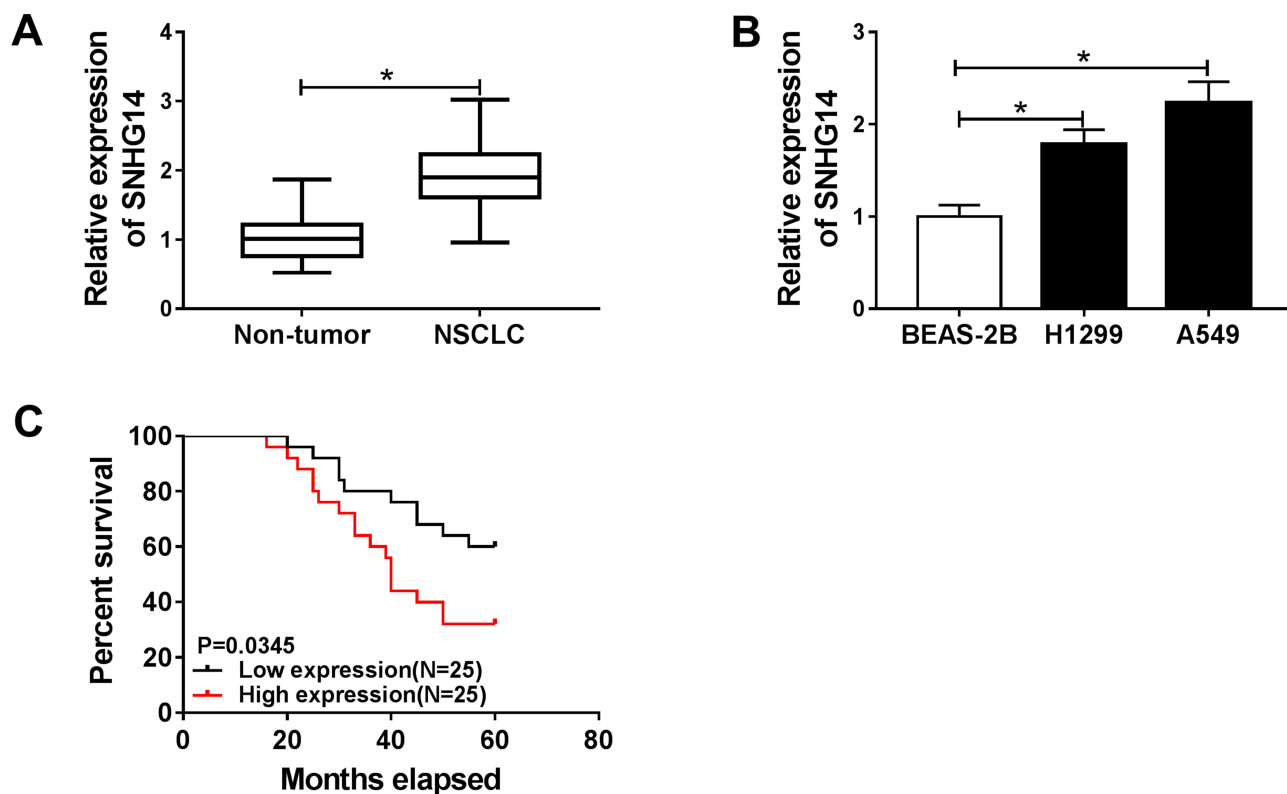
### *SNHG14* Was Highly Expressed in NSCLC Tissues and Cells

In order to search the characters of *SNHG14* in NSCLC, we firstly examined the level of *SNHG14* in NSCLC tissues and cells. The level of *SNHG14* was increased in fifty NSCLC tissues related to that in corresponding adjacent normal tissues

(Figure 1A). Also, *SNHG14* was obviously up-regulated in H1299 and A549 cells compared to that in normal lung cell BEAS-2B (Figure 1B). Kaplan-Meier survival analysis revealed that patients with low *SNHG14* level had longer overall survival time than those in high *SNHG14* level group (Figure 1C). These results indicated that *SNHG14* may play a vital role in NSCLC.

### *SNHG14* Knockdown Constrained Cell Proliferation, Migration, and Invasion While Induced Apoptosis in NSCLC Cells

Next, we investigated the functions of *SNHG14* in NSCLC. Thus, the si-*SNHG14* was transfected into H1299 and A549 cells. The knockdown efficiency was confirmed by qRT-PCR, indicated by the apparent decrease of *SNHG14* in H1299 and A549 cells transfected with si-*SNHG14* (Figure 2A). Following MTT assay exhibited that the transfection of si-*SNHG14* resulted in the striking reduction of cell viability in NSCLC cells in comparison with that in si-NC group (Figure 2B and C). While the apoptotic rate showed the opposite trend. Briefly, the apoptotic rate was dramatically elevated in NSCLC cells transfected with si-*SNHG14* (Figure 2D).



**Figure 1** *SNHG14* was highly expressed in NSCLC tissues and cells. (A and B) The level of *SNHG14* in NSCLC tissues and cells. (C) Overall survival was analyzed using patient post-operative survival by Kaplan-Meier survival assay and Log rank tests. According to the mean of *SNHG14* level, the 50 NB patients were divided into two groups: high *SNHG14* level group (n=25) and low *SNHG14* level group (n=25). \**P*<0.05.

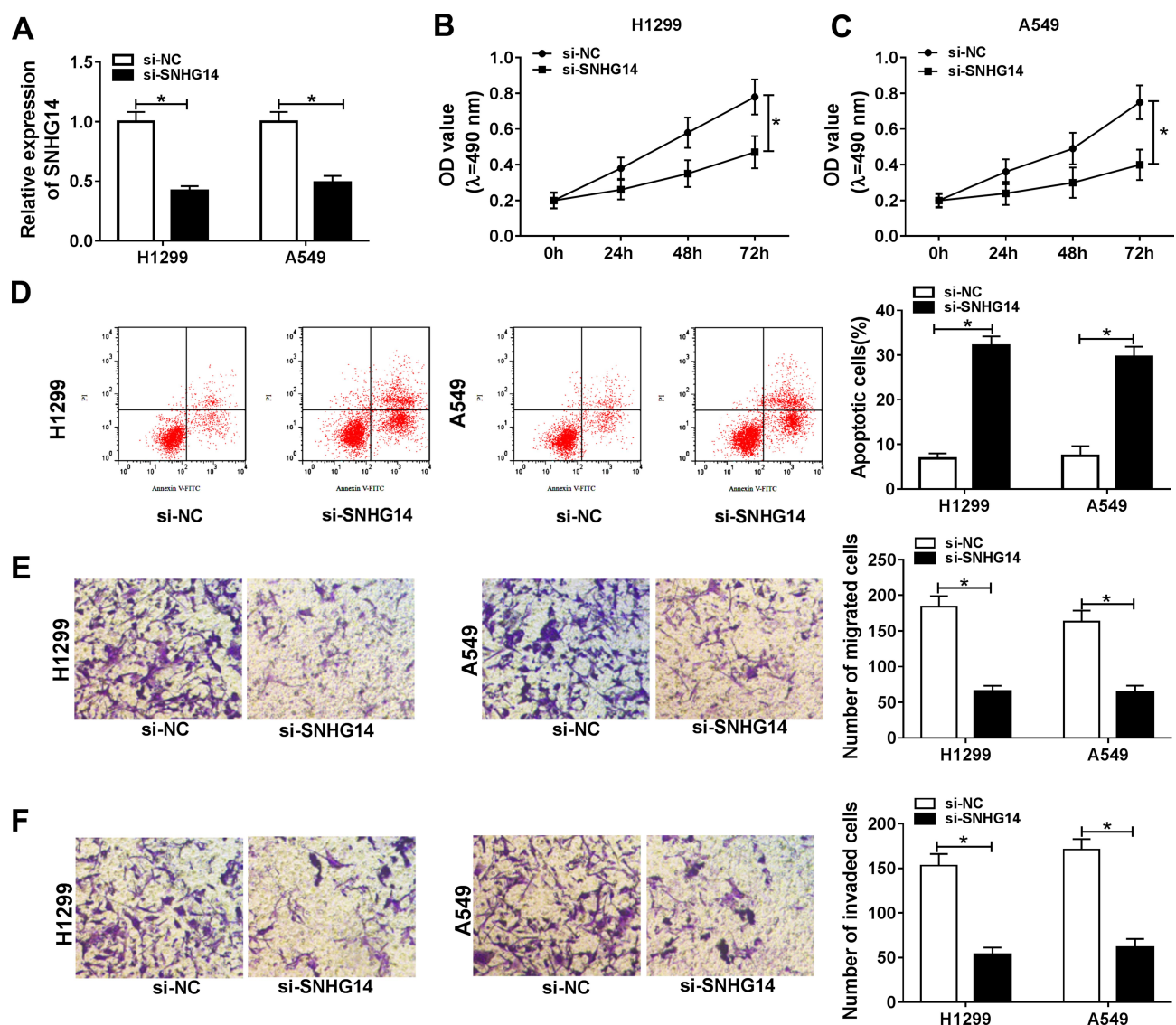


Besides, the migrated and invaded abilities were distinctly declined in si-*SNHG14*-transfected NSCLC cells (Figure 2E and F). Taken together, *SNHG14* silencing repressed cell proliferation, metastasis, but facilitated apoptosis in NSCLC cells.

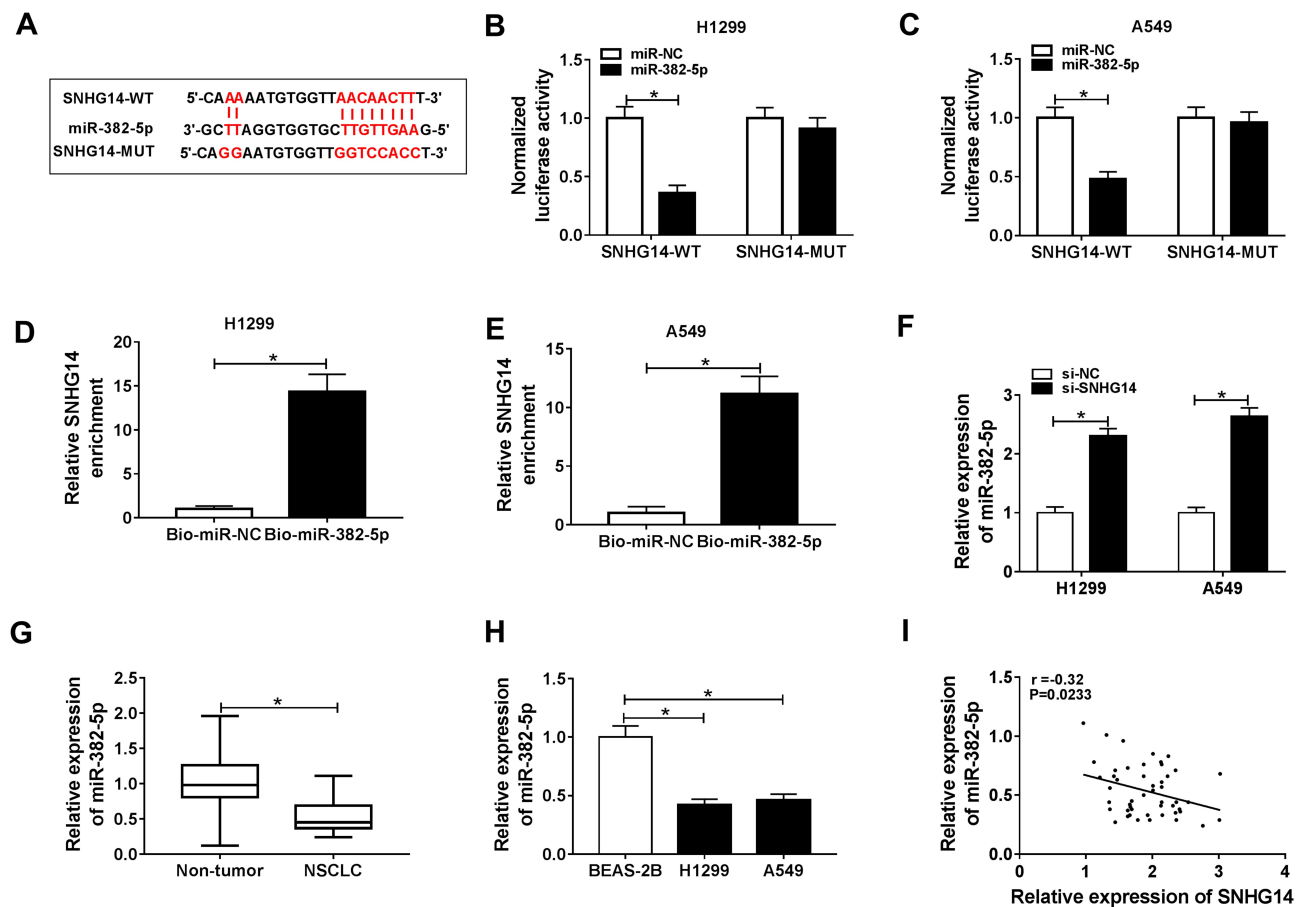
### miR-382-5p Was Negatively Interacted with *SNHG14* in NSCLC Cells

To illustrate the potential mechanism of *SNHG14* in NSCLC, starBase online database (<http://starbase.sysu.edu.cn/starbase2/index.php>) was used to predict the putative candidates of *SNHG14*. The results showed that *miR-382-5p* had complementary binding sites with *SNHG14* (Figure 3A). Subsequently, the transfection of *miR-382-5p* in NSCLC

cells contributed to the conspicuous down-regulation of luciferase activity of *SNHG14*-WT reporter in contrast to that in miR-NC group, while the luciferase activity of *SNHG14*-MUT reporter had no apparent fluctuation in any group (Figure 3B and C). Besides, Bio-miR-382-5p enriched much more *SNHG14* in H1299 and A549 cells compared with that in Bio-miR-NC group (Figure 3D and E). Meanwhile, the level of *miR-382-5p* was remarkably enhanced in H1299 and A549 cells transfected with si-*SNHG14* (Figure 3F). In addition, the level of *miR-382-5p* was drastically reduced in NSCLC tissues and cells (Figure 3G and H). The scatter plot exhibited that *miR-382-5p* was negatively linear correlated with *SNHG14* (Figure 3I). These



**Figure 2** *SNHG14* knockdown constrained cell proliferation, migration, and invasion while induced apoptosis in H1299 and A549 cells. (A–F) The H1299 and A549 cells were transfected with si-NC or si-*SNHG14*. (A) The level of *SNHG14* was detected by qRT-PCR. (B and C) The cell viability was monitored via MTT assay. (D) The apoptotic rate was estimated through flow cytometry. (E and F) The migrated and invaded cells were measured by Transwell assay. \* $P < 0.05$ .



**Figure 3** *miR-382-5p* was negatively interacted with *SNHG14* in NSCLC cells. (A) The complementary sequences between *miR-382-5p* and *SNHG14* was presented, as well as the mutant sequences of *SNHG14*. (B and C) The luciferase activity of *SNHG14*-WT or *SNHG14*-MUT reporter in NSCLC cells transfected with miR-NC or *miR-382-5p* was assessed by dual-luciferase reporter assay. (D and E) The enrichment of *SNHG14* in H1299 and A549 cells transfected with Bio-miR-NC or Bio-miR-382-5p was evaluated via RNA pull-down assay. (F) The level of *miR-382-5p* in H1299 and A549 cells transfected with si-NC or si-*SNHG14* was tested by qRT-PCR. (G and H) The level of *miR-382-5p* in NSCLC tissues and cells was examined via qRT-PCR. (I) The correlation between *miR-382-5p* and *SNHG14* were analyzed by Pearson test. \* $P < 0.05$ .

data disclosed that *SNHG14* sponged *miR-382-5p* in NSCLC cells.

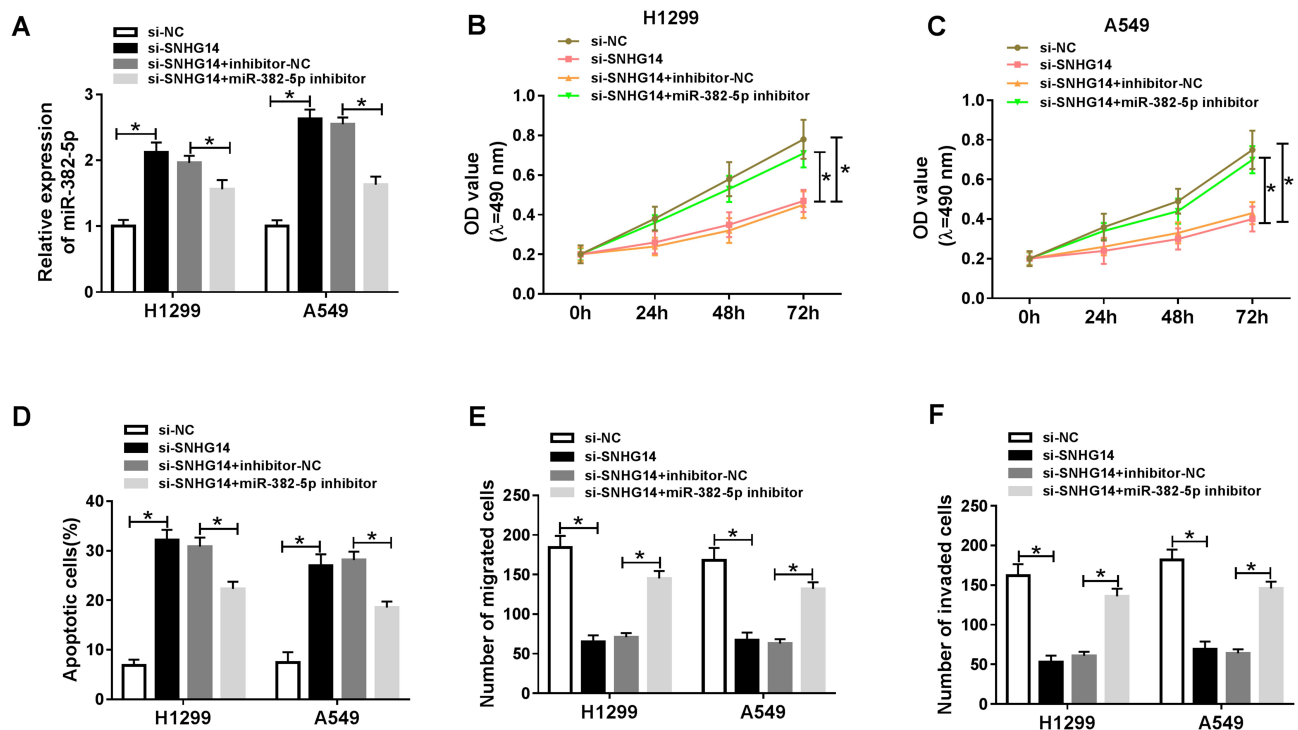
### *SNHG14* Depletion Regulated Cell Behaviors in NSCLC Cells by Regulating *miR-382-5p*

To investigate the functions of *SNHG14* and *miR-382-5p* in NSCLC, si-*SNHG14* and *miR-382-5p* inhibitor were co-transfected into NSCLC cells. As presented in Figure 4A, the level of miR-38-5p was notably elevated in NSCLC cells transfected with si-*SNHG14*, while the introduction of *miR-382-5p* inhibitor mitigated the level of *miR-382-5p* in NSCLC cells. Furthermore, the introduction of *miR-382-5p* inhibitor attenuated the inhibitory impact on cell viability in NSCLC cells repressed by si-*SNHG14* (Figure 4B and C). While the apoptotic rate was evidently facilitated in si-*SNHG14*-transfected H1299 and A549 cells, but

weakened by the introduction of *miR-382-5p* inhibitor (Figure 4D). Besides, the Transwell assay demonstrated that the transfection of *miR-382-5p* inhibitor rescued the restraint effects on the migrated and invaded abilities in H1299 and A549 cells blocked by si-*SNHG14* (Figure 4E and F). These results revealed that *miR-382-5p* inhibitor counteracted the suppressive effects on cell proliferation, migration, and invasion as well as the promoted effect on apoptosis in NSCLC cells caused by si-*SNHG14*.

### *SPIN1* Was a Target of *miR-382-5p* in NSCLC Cells

To explore the underlying mechanism of *miR-382-5p* in NSCLC, starBase was utilized to search the putative candidate of *miR-382-5p*. As exhibited in Figure 5A, *SPIN1* 3'UTR had complementary binding sites with *miR-382-5p*. Also, the luciferase activity of *SPIN1* 3'UTR-WT reporter was obviously reduced in NSCLC cells transfected with *miR-382-5p*, while



**Figure 4** *SNHG14* depletion regulated cell behaviors in NSCLC cells by regulating *miR-382-5p*. (A–F) The NSCLC cells were introduced with si-NC, si-*SNHG14*, si-*SNHG14* + inhibitor-NC, or si-*SNHG14* + *miR-382-5p* inhibitor. (A) The level of *miR-382-5p* was measured by qRT-PCR. (B and C) The cell viability was detected via MTT assay. (D) The apoptotic rate was monitored through flow cytometry. (E and F) The number of migrated and invaded cells was calculated by Transwell assay. \* $P < 0.05$ .

the luciferase activity of *SPIN1* 3'UTR-MUT reporter had no significant change in any group (Figure 5B and C). Meanwhile, the levels of *SPIN1* mRNA and protein were notably declined in NSCLC cells transfected with *miR-382-5p* compared to that in miR-NC group (Figure 5D and E). Besides, the levels of *SPIN1* mRNA and protein were effectively up-regulated in NSCLC tissues and cells (Figure 5F–I). The scatter diagram displayed that *SPIN1* was negatively linear correlated with *miR-382-5p* (Figure 5J). To sum, these data indicated that *SPIN1* was negatively interacted with *miR-382-5p* in NSCLC cells.

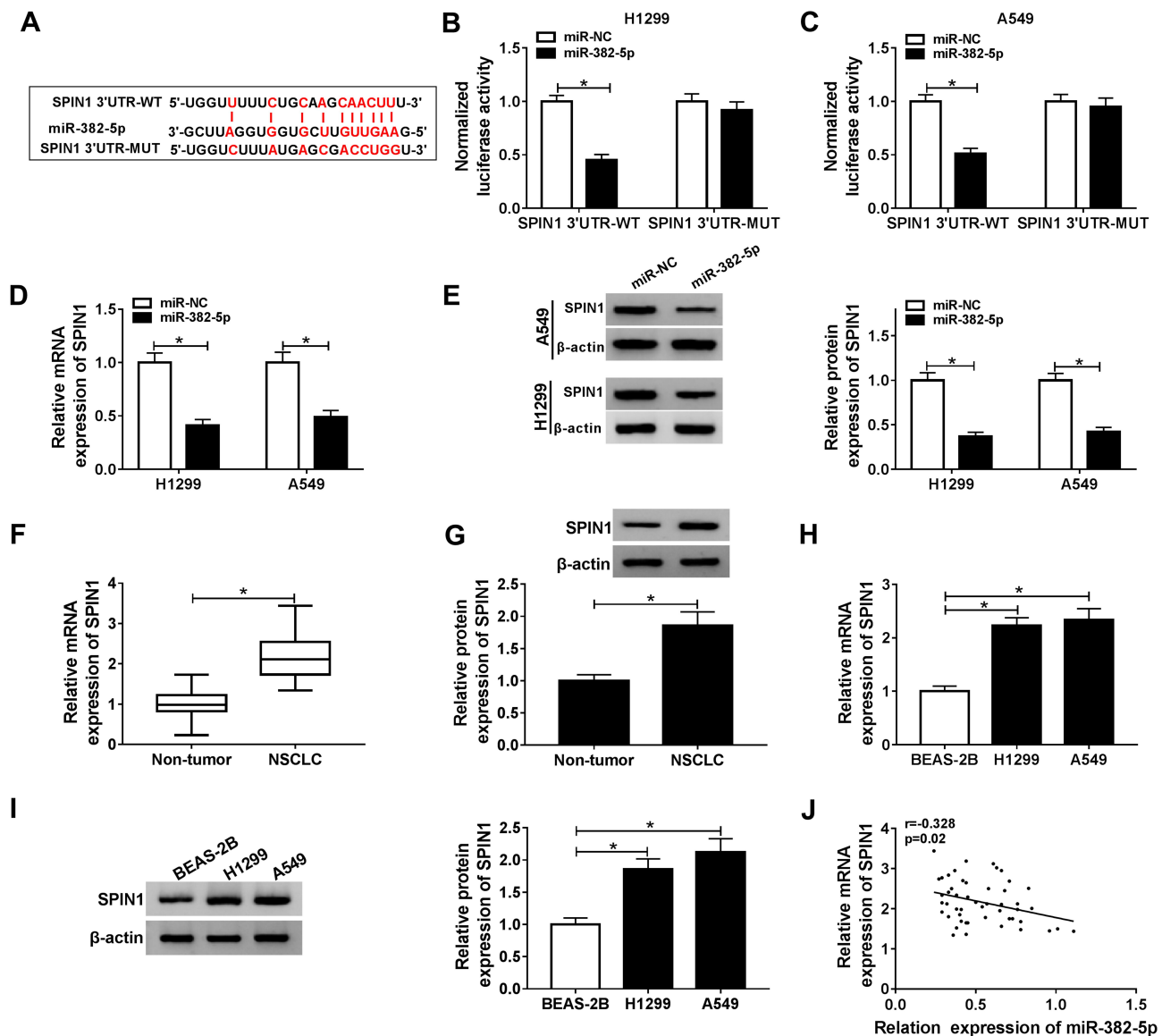
### *miR-382-5p* Overexpression Retarded Cell Proliferation, Metastasis While Facilitated Apoptosis in NSCLC Cells by Targeting *SPIN1*

To illustrate the functions of *miR-382-5p* and *SPIN1* in NSCLC, *miR-382-5p* and pcNDA-*SPIN1* were co-transfected into NSCLC cells. Our data showed the successful overexpression transfection efficiency of pcNDA-*SPIN1* in H1299 and A549 cells (Supplement Figure 1A and B). As presented in Figure 6A–C, the levels of *SPIN1* mRNA and protein were dramatically declined in *miR-382-5p*-transfected H1299 and A549 cells, while partially regained in *miR-382-5p* + pcNDA-

*SPIN1* group. Following MTT assay exhibited that the emergence of pcDNA-*SPIN1* receded the constraint impact on cell viability hindered by *miR-382-5p* mimics (Figure 6D and E). The apoptotic rate was markedly elevated in NSCLC cells transfected with *miR-382-5p*, while partly abated by the introduction of pcDNA-*SPIN1* (Figure 6F). The transfection of *miR-382-5p* resulted in the striking down-regulation of migrated and invaded abilities in NSCLC cells, while restored in *miR-382-5p* + pcDNA-*SPIN1* group (Figure 6G and H). These data demonstrated that the overexpression of *SPIN1* neutralized the repressive effects on cell proliferation, metastasis and the promoted effect on apoptosis in H1299 and A549 cells induced via *miR-382-5p*.

### *SNHG14* Silencing Impeded *SPIN1* Expression by Sponging *miR-382-5p* in H1299 and A549 Cells

Based on the above results, *SNHG14*, *SPIN1* were elevated but *miR-382-5p* was reduced in NSCLC. To elucidate the relationship among *SNHG14*, *miR-382-5p*, and *SPIN1* in NSCLC, si-*SNHG14* and *miR-382-5p* inhibitor were co-transfected into NSCLC cells. As exhibited in Figure 7A and B, *SPIN1* mRNA and protein were strikingly decreased in NSCLC cells transfected with si-*SNHG14*,



**Figure 5** *SPIN1* was a direct target of *miR-382-5p* in NSCLC cells. (A) The complementary sequences between *SPIN1* 3'UTR and *miR-382-5p* were presented, as well as the mutant sequences of *SPIN1* 3'UTR. (B and C) The luciferase activity of *SPIN1* 3'UTR-WT or *SPIN1* 3'UTR-MUT reporter in NSCLC cells transfected with miR-NC or *miR-382-5p* was evaluated by dual-luciferase reporter assay. (D and E) The H1299 and A549 cells were transfected with *miR-382-5p* or miR-NC. (D) The expression of *SPIN1* was tested via qRT-PCR. (E) The level of *SPIN1* protein was measured by Western blot assay. (F–I) The levels of *SPIN1* mRNA and protein in NSCLC tissues and cells were monitored via qRT-PCR and Western blot assay, respectively. (J) The correlation between *SPIN1* and *miR-382-5p* was processed by Pearson test. \* $P < 0.05$ .

but partly regained in NSCLC cells co-transfected with si-*SNHG14* and *miR-382-5p* inhibitor. These data unraveled that the depletion of *SNHG14* down-regulated *SPIN1* expression by sponging *miR-382-5p* H1299 and A549 cells.

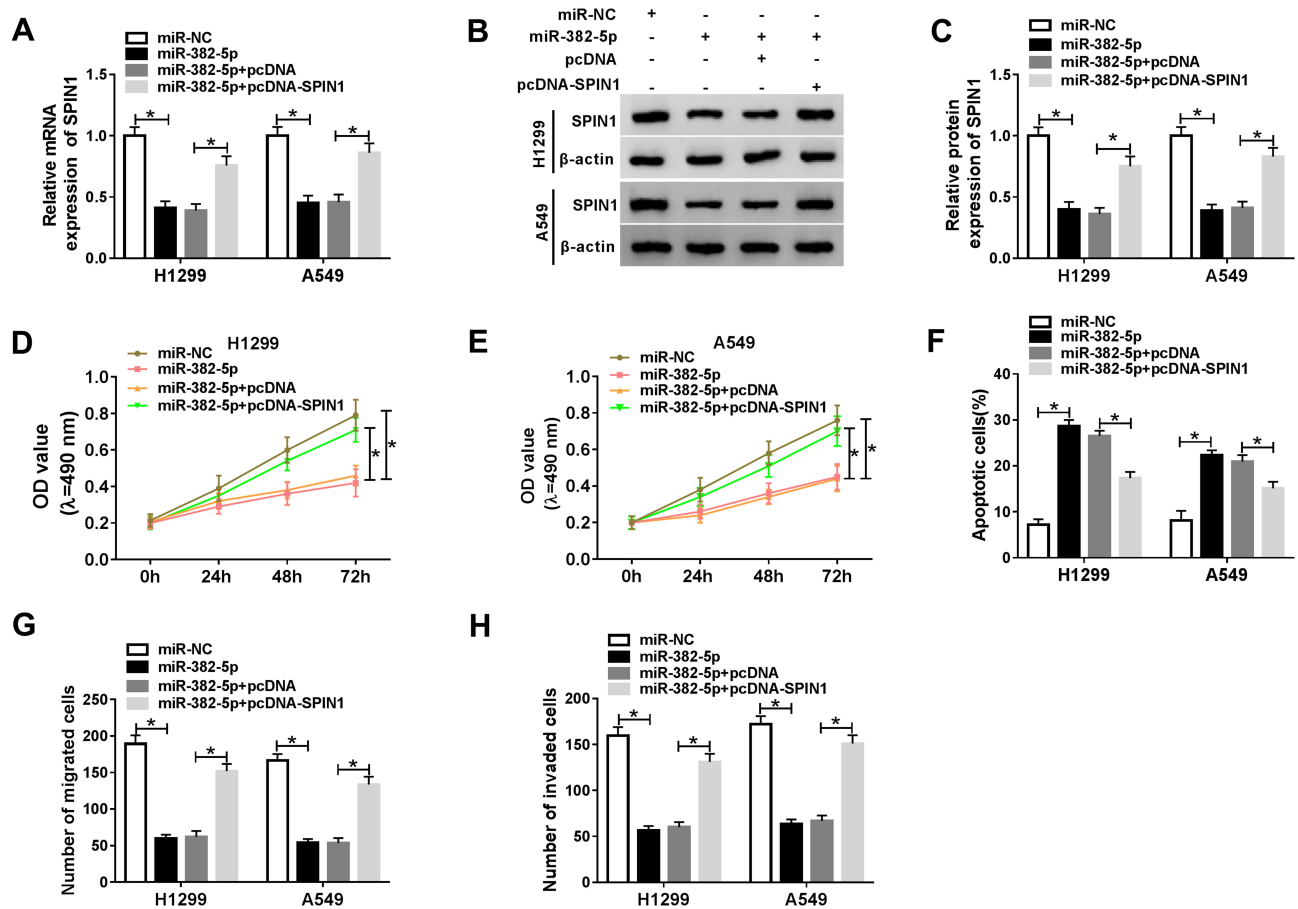
## Discussion

Tumor progression is complex processes in human, and *SNHG14* was identified to be involved in tumor progression in diverse types of cancers. In the current study, we mainly focused on the mechanism of *SNHG14* in NSCLC.

The results revealed that *SNHG14* facilitated NSCLC progression through *miR-382-5p/SPIN1* axis.

Emerging evidence implied that the ectopic expression of *SNHG14* was associated with tumor progression. For example, Ji et al documented that *SNHG14* was elevated in cervical cancer, and the down-regulation of *SNHG14* curbed cell growth, metastasis but induced apoptosis via miR-206.<sup>20</sup> Another study in ovarian cancer manifested that *SNHG14* was enhanced in ovarian cancer, and the depletion of *SNHG14* inhibited cell growth and metastasis by sponging *miR-219a-5p*.<sup>11</sup> In this research, we validated that *SNHG14*



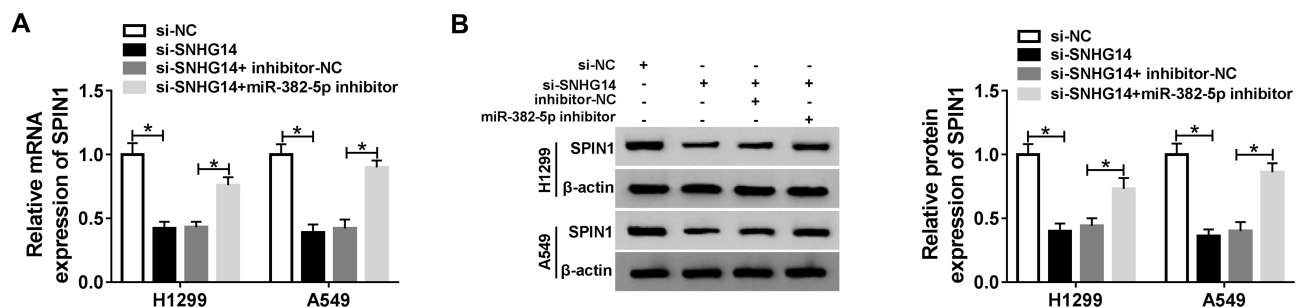


**Figure 6** *miR-382-5p* overexpression retarded cell proliferation, metastasis while facilitated apoptosis in NSCLC cells by targeting *SPIN1*. (A–H) The H1299 and A549 cells were transfected with miR-NC, *miR-382-5p*, *miR-382-5p* + pcDNA, or *miR-382-5p* + pcDNA-*SPIN1*. (A) The expression of *SPIN1* was detected by qRT-PCR. (B and C) The protein level of *SPIN1* was measured via Western blot assay. (D and E) The cell viability was estimated by MTT assay. (F) The apoptotic rate was assessed through flow cytometry. (G and H) The migrated and invaded capacities were evaluated via Transwell assay. \**P*<0.05.

was highly expressed in NSCLC. The depletion of *SNHG14* blocked cell growth, metastasis in NSCLC cells, as well as induced apoptosis in vitro. The above results of *SNHG14* in NSCLC were consistent with the previous report.<sup>21</sup> These data disclosed that *SNHG14* might play important roles in NSCLC.

Recent reports demonstrated that the dysregulation of *miR-382-5p* was implicated in tumor progression. For

instance, a study in glioma unraveled that *miR-382-5p* was obviously declined in glioma, and its overexpression impeded cell growth, metastasis of glioma cells.<sup>22</sup> In this exploration, *miR-382-5p* apparently declined in NSCLC, and *SNHG14* was verified to sponge *miR-382-5p* in NSCLC cells. *SNHG14* silencing confined cell viability, metastasis, but accelerated apoptosis in NSCLC cells by



**Figure 7** *SNHG14* silencing impeded *SPIN1* expression by sponging *miR-382-5p* in NSCLC cells. (A and B) The NSCLC cells were transfected with si-NC, si-*SNHG14*, si-*SNHG14* + inhibitor-NC, or si-*SNHG14* + *miR-382-5p* inhibitor. (A) The level of *SPIN1* mRNA was examined by qRT-PCR. (B) The level of *SPIN1* protein was examined via Western blot assay. \**P*<0.05.

regulating *miR-382-5p*. These results were similar to the report of miR-382 in NSCLC.<sup>17</sup> These data demonstrated that *SNHG14* augmented NSCLC progression by sponging *miR-382-5p*.

Convincing evidence uncovered that the aberrant expression of *SPIN1* was related to tumor progression. A study in breast cancer uncovered that *SPIN1* promoted cell growth and metastasis regulated by *miR-1271*.<sup>19</sup> Li et al reported that *SPIN1* suppressed cell growth but accelerated apoptosis in glioma mediated by *miR-489*.<sup>18</sup> In the present study, *SPIN1* was a target of *miR-382-5p* and was up-regulated in NSCLC. *miR-382-5p* overexpression impeded cell growth and metastasis while impelled apoptosis in NSCLC cells by affecting *SPIN1*. These results of *SPIN1* in NSCLC were in line with the previous study.<sup>23</sup> Furthermore, *SNHG14* positively regulated *SPIN1* expression by sponging *miR-382-5p* in NSCLC cells. These results implicated that *SNHG14* positively modulated *SPIN1* expression to expedite NSCLC progression by sponging *miR-382-5p*.

To better elucidate the effects of *SNHG14* on NSCLC, the mice model experiment will be performed in the future. Accumulating evidence indicated that *PI3K/AKT/mTOR* signaling pathway was implicated in many biological processes in tumor progression.<sup>24</sup> A reporter in gastric cancer disclosed that *SNHG14* boosted gastric cancer development via PI3 K/AKT/mTOR signaling pathway.<sup>13</sup> Next, we will explore whether the *SNHG14/miR-382-5p/SPIN1*-induced tumor progression is mediated by *PI3K/AKT/mTOR* signaling pathway.

In conclusion, *SNHG14* knockdown retarded NSCLC progression by regulating *SPIN1* expression via *miR-382-5p*. This new regulatory network in NSCLC may provide novel therapeutic targets for NSCLC patients.

## Highlights

1. *SNHG14* sponged *miR-382-5p* in NSCLC cells;
2. *SNHG14* knockdown suppressed cell proliferation, migration, and invasion but triggered apoptosis in NSCLC cells by regulating *miR-382-5p*;
3. *SPIN1* was a direct candidate of *miR-382-5p* in NSCLC cells;
4. *miR-382-5p* confined cell proliferation, migration, and invasion while induced apoptosis in NSCLC cells by targeting *SPIN1*;
5. *SNHG14* modulated *SPIN1* by sponging *miR-382-5p* in NSCLC cells.

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## Disclosure

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