

## ORIGINAL ARTICLE

# LncRNA SNHG16 promotes non-small cell lung cancer development through regulating EphA2 expression by sponging miR-520a-3p

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## Keywords

Development; EphA2; miR-520a-3p; NSCLC; SNHG16.

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## Abstract

**Background:** Recent evidence has found that lncRNA small nucleolar RNA host gene 16 (SNHG16) was associated with cell carcinogenesis in NSCLC. Here, we further investigated the precise functions and mechanisms of SNHG16 in NSCLC progression.

**Methods:** The expression of SNHG16, microRNA (miR)-520a-3p and EPH Receptor A2 (EphA2) was measured using quantitative real-time polymerase chain reaction and western blot, respectively. Cell proliferation was determined using 3-(4, 5)-dimethylthiazoliazolium bromide (MTT) assay. The migrated and invaded cells were measured by Transwell assay. Flow cytometry was used to detect apoptotic cells. The interaction between miR-520a-3p and SNHG16 or EphA2 was confirmed using a dual-luciferase reporter assay.

**Results:** We found that SNHG16 was upregulated in NSCLC tissues and cell lines, knockdown of SNHG16 inhibited cell proliferation, migration, invasion and induced apoptosis in vitro as well as suppressed tumor growth in vivo. MiR-520a-3p directly bound to SNHG16 and miR-520a-3p, and SNHG16 acted as a ceRNA in regulating EphA2 through competitively binding to miR-520a-3p. Additionally, rescue assay exhibited the anticancer activity mediated by SNHG16 knockdown on NSCLC could be reversed by miR-520a-3p inhibition or EphA2 overexpression.

**Conclusion:** SNHG16 promoted NSCLC development by regulating the miR-520a-3p/EphA2 axis, suggesting novel insights for the pathogenesis of NSCLC and new potential therapeutic targets for the treatment of NSCLC.

## Key points

- 1 Knockdown of SNHG16 inhibited NSCLC cell proliferation, migration, invasion and induced apoptosis in vitro as well as suppressed tumor growth in vivo.
- 2 SNHG16 directly interacted with miR-520a-3p.
- 3 EphA2 was a target of miR-520a-3p.
- 4 SNHG16 could regulate the expression of EphA2 by binding to miR-520a-3p.
- 5 SNHG16 promoted NSCLC development by regulating the miR-520a-3p/EphA2 axis.

## Introduction

Non-small cell lung cancer (NSCLC) is one of the main subtypes of lung cancer, accounting for around 80%–85% of all

lung cancers, and has a low five-year survival rate of approximately 15%.<sup>1,2</sup> Although there has been an improvement in the diagnosis and multimodal therapy, the overall survival

rate of NSCLC is still unsatisfactory due to metastasis and recurrence.<sup>3</sup> Thus, there is a great demand for us to better understand the pathological mechanisms of NSCLC in order to develop novel effective therapeutic approaches for the improvement of outcome.

Long non-coding RNAs (lncRNAs) are a class of non-protein coding RNA molecules with the length over 200 nucleotides and have important effects on the regulation of gene expression via chromatin modification, transcription (or post-transcriptional) and translational regulation.<sup>4,5</sup> Increasing evidence has identified that lncRNAs are crucial contributors in regulating malignant physiological or pathological cellular processes, such as tumorigenesis, angiogenesis, drug resistance, and metastasis.<sup>6,7</sup> In addition, numerous studies have gradually revealed that deregulated lncRNAs are associated with the development of many cancers, including NSCLC. For example, Nie *et al.* revealed that lncRNA UCA1 acted as an oncogene to promote cell proliferation by regulating miR-193a-3p/ERBB4 in NSCLC.<sup>8</sup> LncRNA HIT exerted oncogenic functions to promote NSCLC cell growth by interacting with E2F1.<sup>9</sup> Thus, lncRNAs may be promising candidates for developing effective therapeutics of NSCLC. Among these lncRNAs, lncRNA small nucleolar RNA host gene 16 (SNHG16) recently was discovered to be upregulated in NSCLC and was associated with cell carcinogenesis in NSCLC.<sup>10</sup> However, the precise mechanisms underlying the tumorigenesis role of SNHG16 in NSCLC remain largely unclear.

MicroRNAs (miRNAs) are one type of small non-coding RNAs which can modulate gene expression at post-transcriptional level in various cancers.<sup>11</sup> In NSCLC, many miRNAs have been revealed to be involved in the cancer carcinogenesis by functioning as tumor suppressors or oncogenes, and miRNAs are potential diagnostic biomarkers and therapeutic targets in NSCLC.<sup>12–14</sup> Recently, the important roles of the lncRNA-miRNA-mRNA regulatory network and a protein-protein interaction network have also been identified in the development of NSCLC.<sup>15,16</sup>

However, there are few studies to date which concentrate on the interaction network of SNHG16 in NSCLC.

This research aimed to explore the potential biological functions of SNHG16 in NSCLC cell carcinogenesis, investigate the interaction network of SNHG16, as well as how they affect NSCLC development. This study may provide novel therapeutic targets for the treatment of NSCLC.

## Methods

### Patients and specimens

Tumor specimens and adjacent nontumor tissues from 30 NSCLC patients who received surgical resection were

obtained from Dalian University Affiliated Xinhua Hospital and were stored at  $-80^{\circ}\text{C}$  for further analysis. All patients were diagnosed by histopathological examination and did not receive any preoperative treatment. This study was permitted by the Ethics Committee of Dalian University Affiliated Xinhua Hospital and all patients had signed their informed consents for inclusion in the study.

### Cell culture and transfection

Human bronchial epithelial (16HBE) cell line and NSCLC cell lines (A549, NCI-H292, NCI-H460, NCI-H1703) were obtained from Shanghai Academy of Life Science (Shanghai, China) and maintained in the Dulbecco's modified Eagle's medium (DMEM; Gibco, Carlsbad, CA, USA) harboring with 10% fetal bovine serum and 1% penicillin/streptomycin (Gibco) with 5%  $\text{CO}_2$  at  $37^{\circ}\text{C}$ .

The miR-520a-3p mimic (miR-520a-3p), miR-520a-3p inhibitor (anti-miR-520a-3p) and their corresponding negative control (miR-NC or anti-miR-NC) were obtained from RIBOBIO (Guangzhou, China). The short hairpin RNA (shRNA) targeting SNHG16 (sh-SNHG16), shRNA scramble control (sh-NC), the small interfering RNA (siRNA) sequences targeting SNHG16 (si-SNHG16), siRNA negative control (si-NC), pcDNA, pcDNA SNHG16 overexpression vector (SNHG16), pcDNA Eph Receptor A2 (EphA2) overexpression vector (EphA2) were synthesized by Invitrogen (Carlsbad, CA, USA). All oligonucleotides or vectors were transfected into A549 and NCI-H1703 cells using Lipofectamine 2000 (Invitrogen) following the standard protocol.

### Quantitative real-time polymerase chain reaction (qRT-PCR)

TRIzol reagent (Invitrogen) was utilized to extract total RNA according to the standard procedure. For the detection of SNHG16 and EphA2, total RNA was reversely transcribed into complementary DNA (cDNA) using a high capacity cDNA Reverse Transcription Kit (Qiagen, Valencia, CA, USA), and quantitative PCR was then carried out with SYBR Premix Ex Taq (Qiagen). For miR-520a-3p detection, cDNA was synthesized by using miScript Reverse Transcription reagent (Qiagen) and amplified using SYBR Select Master Mix (Qiagen). Fold changes were analyzed by  $2^{-\Delta\Delta\text{Ct}}$  method and normalized by glyceraldehyde 3-phosphate dehydrogenase (GADPH) or U6 small nuclear B noncoding RNA (U6). The specific primer sequences were listed as follows: SNHG16: F, 5'-GCAGAATGCCATGGTTTCCC-3', R, 5'-GGACAGCTGGCAAGAGACTT-3'; EphA2: 5'-CTGGTCTGCAAGGTGTC TGA-3', R 5'-TTGGACAACCTCCAGTAGGG-3'; miR-520a-3p: F, 5'-ACACTCCAGCTGGGAAAGTGCTTCCC-3', R,

5'-CTCAACTGGTGTCGT GGA-3'. GADPH: F 5'-GATATTGTTGCCATCAATGAC-3', R 5'-TTGATTTTGGAGGGATCTCG-3'; U6: F, 5'-CTCGCTTCGGCAGCAC-3' and R, 5'-ACGCTTCACGAATTTGCGT-3'.

### Cell proliferation assay

Transfected cells were grown in the 96-well plates (5000 cells/well) and incubated overnight. Each well was interacted with 10  $\mu$ L 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution (Sigma, St. Louis, MO, USA) for another four hours. After removing the supernatants, 150  $\mu$ L DMSO was added to each well (Sigma). Finally, the absorbance was detected at 490 nm to plot cell proliferation curves.

### Cell migration and invasion

For migrated cells detection, cells suspended in DMEM without serum were planted in the upper chambers and 500  $\mu$ L DMEM mixed with 10% FBS was added to the lower chambers. After incubation for 24 hours at 37°C, migrated cells were then fixed with polyoxymethylene and stained with 0.1% crystal violet. Finally, cells in five randomly selected fields were counted using a microscope. For invaded cells detection, a matrigel invasion chamber (BD Biosciences, San Jose, CA, USA) was utilized and other measurements were similar to the steps of cell migration detection.

### Cell apoptosis assay

Cell apoptosis was detected using Annexin V-FITC/PI apoptosis detection kit (BD Biosciences) according to the standard protocol. Transfected cells were resuspended with binding buffer, followed by staining with 10  $\mu$ L fluorescein isothiocyanate (FITC) annexin V and propidium iodide (PI). Finally, flow cytometry was used to detect apoptotic cells.

### Dual-luciferase reporter assay

The WT or MUT SNHG16/EphA2 3'UTR containing a miR-520a-3p binding sequence was amplified and cloned into the pmirGLO Vector (Promega, Shanghai, China). Then the construct vectors were transfected into A549 and NCI-H1703 cells with miR-520a-3p or miR-NC for 48 hours. Subsequently, a dual-luciferase reporter assay kit (Promega) was used to detect the relative luciferase activity.

### Western blot

Western blot assay was carried out as previously described.<sup>17</sup> Several primary antibodies against EphA2 (1:500, ab5386, Abcam, Cambridge, MA, USA),  $\beta$ -actin (1:1000, 4967, Cell Signaling Technology, Danvers, MA, USA) as well as HRP-conjugated secondary antibody (1:1000; ab9482; Abcam) were used for the immunoblot assays. The protein signals were visualized by an ECL method.

### Xenograft experiments in vivo

Athymic BALB/c mice (males, four weeks old) were used to establish xenograft models. A549 cells, stably infected with lentivirus containing sh-SNHG16 or sh-NC were subcutaneously inoculated into the flanks of the nude mice. The tumor size in the nude mice were detected and calculated every five days. After inoculation for 27 days, the tumor masses were excised and weighed. The study was permitted by the Animal Research Committee of Dalian University Affiliated Xinhua Hospital and undertaken following the guidelines of the National Animal Care and Ethics Institution.

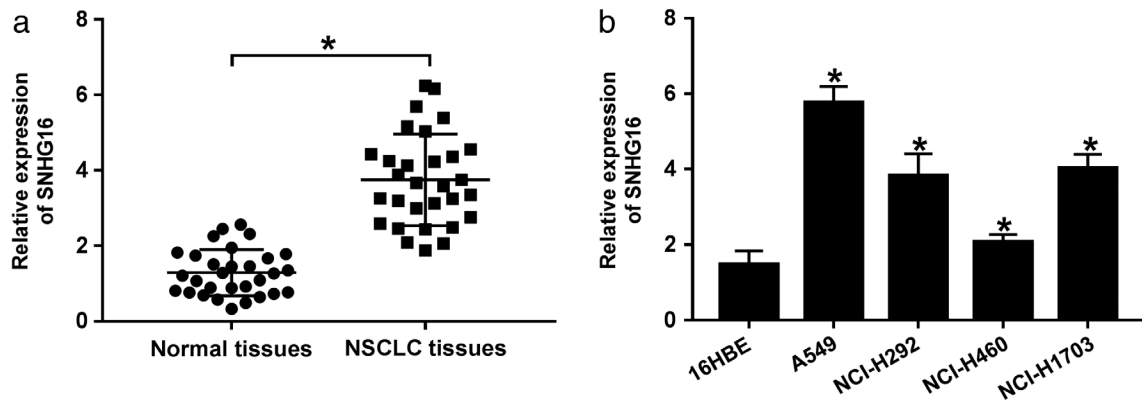
### Statistical analysis

Statistical data from three independent experiments were presented as the mean  $\pm$  standard deviation (SD) and analyzed using GraphPad Prism 7 software (GraphPad Inc., San Diego, CA, USA). Significant differences were analyzed using one-way analysis of variance (ANOVA) or Student's *t*-test. The correlation analysis was analyzed using Spearman's correlation test. A value of  $P < 0.05$  was considered statistically significant.

## Results

### SNHG16 is upregulated in NSCLC tissues and cell lines

SNHG16 expression was investigated to determine whether SNHG16 expression had changed in NSCLC tissues and cell lines. In comparison with nontumor tissues and 16HBE cell line, the results indicated that SNHG16 was significantly elevated in NSCLC tissues and cell lines (Fig 1a,b). Additionally, A549 and NCI-H1703 cells were selected for the following experiments due to the evident upregulation.

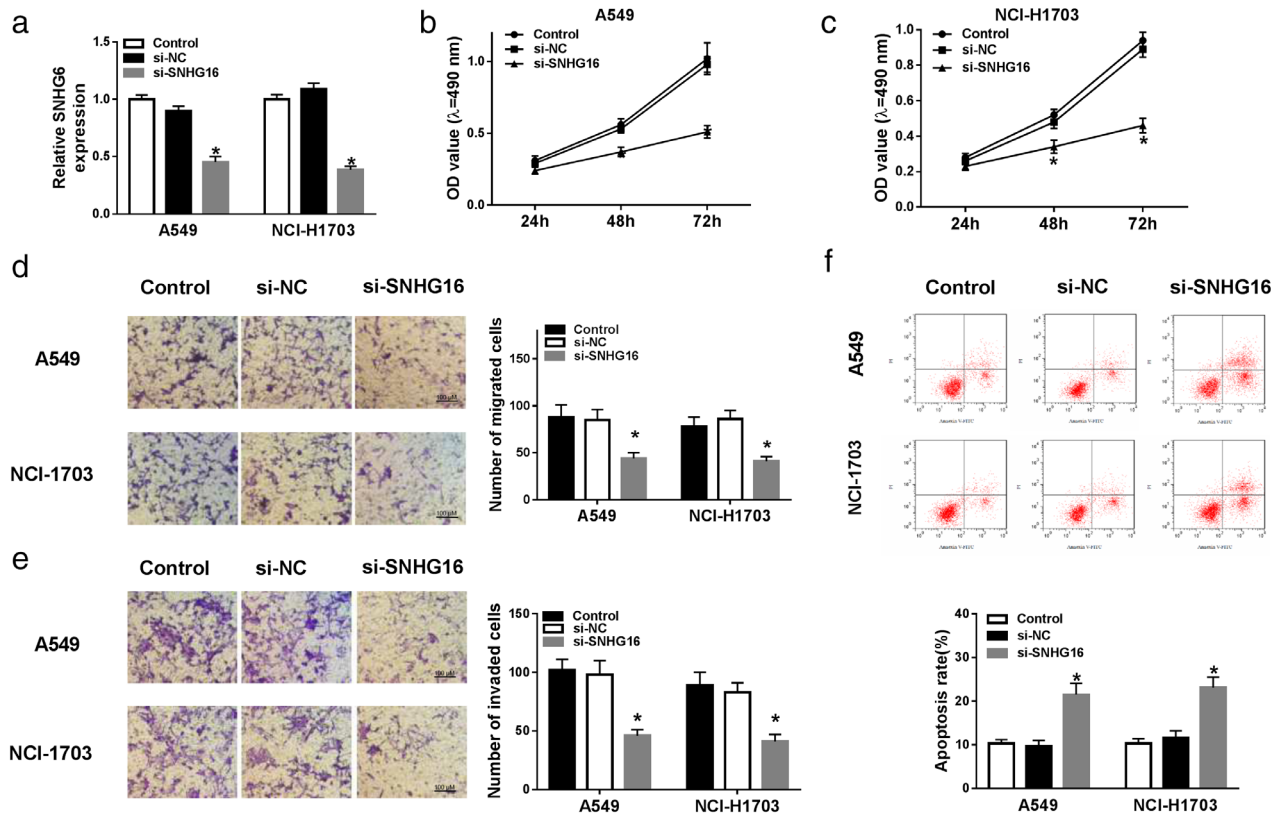


**Figure 1** SNHG16 was upregulated in NSCLC tissues and cell lines. (a, b) The expression of SNHG16 was detected using qRT-PCR in normal tissues, NSCLC tissues, NSCLC cell lines (A549, NCI-H292, NCI-H460, and NCI-H1703), and human bronchial epithelial (16HBE) cell line. \**P* < 0.05.

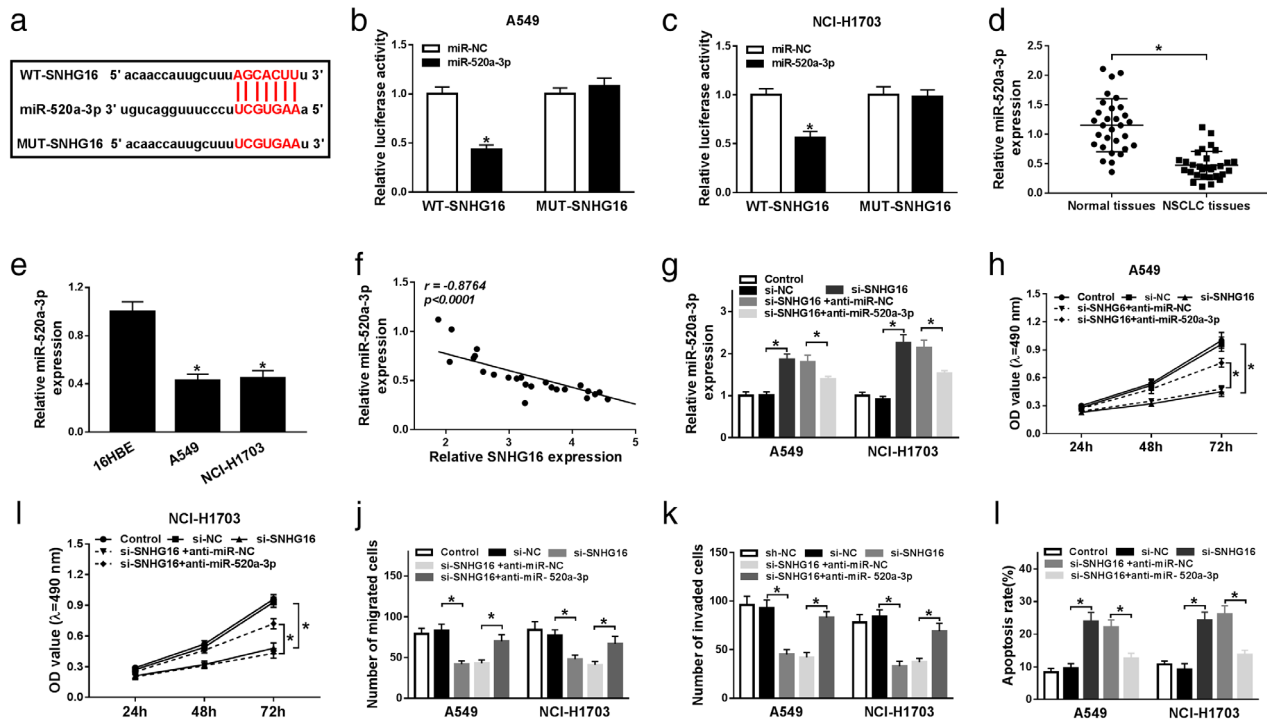
**SNHG16 silence inhibits proliferation, migration and invasion but induces apoptosis of NSCLC cells**

To explore the potential biological functions of SNHG16 in NSCLC cells, SNHG16 was silenced using small interfering

RNA (siRNA) sequences targeting SNHG16 and an obvious reduction of SNHG16 level in A549 and NCI-H1703 cells was observed (Fig 2a). MTT assay showed SNHG16 silence inhibited cell proliferation in NSCLC (Fig 2b,c). Transwell assay subsequently indicated that knockdown of SNHG16 markedly suppressed migration and invasion of



**Figure 2** SNHG16 silence inhibited the proliferation, migration and invasion but induced apoptosis of NSCLC cells. SNHG16 was silenced using small interfering RNA sequences targeting SNHG16 in A549 and NCI-H1703 cells. After transfection, (a) the expression of SNHG16 was measured by qRT-PCR; (b, c) MTT assay was used to detect cell proliferation; (d, e) migrated and invaded cells were examined using transwell assay; (f) apoptotic cells were detected by flow cytometry. \**P* < 0.05.



**Figure 3** SNHG16 silence exerted antitumor effects in NSCLC cells by directly sponging miR-520a-3p. (a) A graphical representation of the miR-520a-3p binding site within SNHG16 is presented. (b, c) The interaction between miR-520a-3p and SNHG16 was confirmed using a dual-luciferase reporter assay in A549 and NCI-H1703 cells. (d, e) The expression of miR-520a-3p was detected in normal tissues and NSCLC tissues (d), as well as NSCLC cell lines (A549 and NCI-H1703) and human bronchial epithelial (16HBE) cell line (e) using qRT-PCR. (f) The correlation between miR-520a-3p and SNHG16 expression was assessed in NSCLC tissues by Spearman's correlation test. A549 and NCI-H1703 cells were transfected with si-NC, si-SNHG16, si-SNHG16 + anti-miR-NC, or si-SNHG16 + anti-miR-520a-3p. After transfection, (g) the level of miR-520a-3p was measured in A549 and NCI-H1703 cells by qRT-PCR; (h, i) MTT assay was used to detect cell proliferation; (j, k) cell migration and invasion were determined by transwell assay; (l) apoptotic cells were examined by flow cytometry. \* $P < 0.05$ .

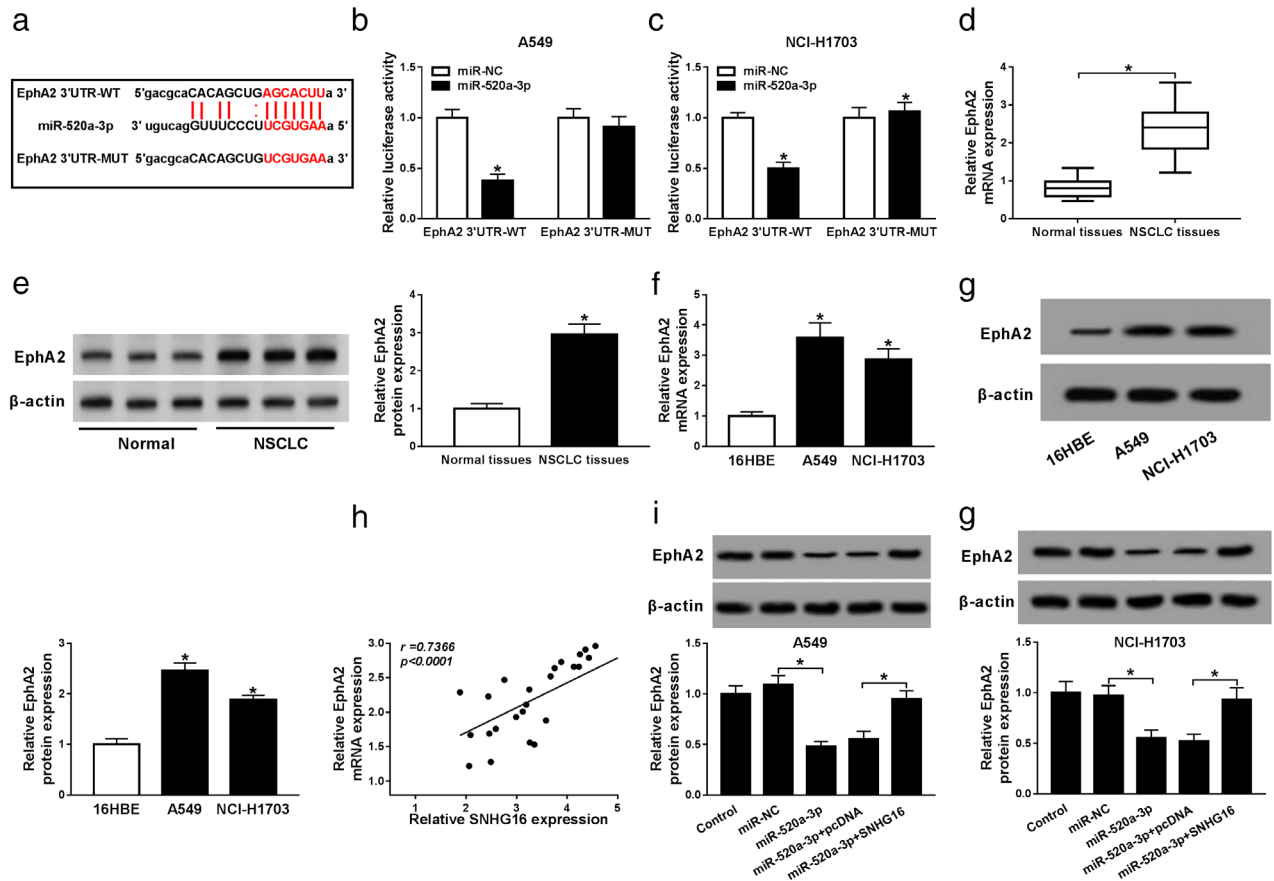
A549 and NCI-H1703 cells (Fig 2d,e). Furthermore, we also found apoptotic cells were significantly increased by SNHG16 deletion in NSCLC (Fig 2f). Therefore, we demonstrated that SNHG16 silence restrained cell carcinogenesis in NSCLC.

### SNHG16 silence exerts antitumor effects in NSCLC cells by directly sponging miR-520a-3p

To investigate the underlying mechanism of SNHG16 in the development of NSCLC, we used starBase v2.0 program to predict the potential miRNA targets of SNHG16 and miR-520a-3p was identified to have a binding site with SNHG16 (Fig 3a). After that, the dual-luciferase reporter assay was conducted and miR-520a-3p overexpression reduced the luciferase activities of the WT-SNHG16 reporter vector compared to the control group, while there was no obvious change in MUT-SNHG16 reporter after overexpression of miR-520a-3p in A549 and NCI-H1703 cells (Fig 3b,c), indicating SNHG16 directly bound to miR-

520a-3p. Subsequently, the expression of miR-520a-3p was detected and we found miR-520a-3p was decreased in NSCLC tissues and cell lines (Fig 3d,e); in addition, a negative correlation between miR-520a-3p and SNHG16 was demonstrated in NSCLC tissues (Fig 3f), further proving SNHG16 was a sponge of miR-520a-3p. Moreover, qRT-PCR analysis showed the expression of miR-520a-3p was promoted by SNHG16 knockdown, while this promotion could be attenuated by miR-520a-3p inhibition (Fig 3g). Taken together, SNHG16 directly interacted with miR-520a-3p and negatively regulated miR-520a-3p expression.

Based on the relationship between SNHG16 and miR-520a-3p, we further analyzed whether miR-520a-3p participated in SNHG16 deletion mediated regulation on cell tumorigenesis in NSCLC. Thus, rescue assay was conducted and the results suggested that miR-520a-3p inhibition could reverse SNHG16 silence induced inhibition on the proliferation (Fig 3h,i), migration and invasion (Fig 3j,k), promotion on the apoptosis (Fig 3l) of A549 and NCI-H1703 cells. These data demonstrated that SNHG16 silence inhibited NSCLC cells carcinogenesis by directly interacting with miR-520a-3p.



**Figure 4** EphA2 is a target of miR-520a-3p. (a) The binding site between miR-520a-3p and EphA2 is shown. (b, c) The interaction between EphA2 and miR-520a-3p was analyzed using a dual-luciferase reporter assay in A549 and NCI-H1703 cells. (d–g) The expression of EphA2 was measured in normal tissues and NSCLC tissues (d, e), as well as NSCLC cell lines (A549 and NCI-H1703) and human bronchial epithelial (16HBE) cell line (f, g) using qRT-PCR and western blot, respectively. (h) The correlation between SNHG16 and EphA2 expression in NSCLC tissues was assessed by Spearman’s correlation test. (i, j) The level of EphA2 was detected in A549 and NCI-H1703 cells transfected with miR-NC, miR-520a-3p, miR-520a-3p + pcDNA, or miR-520a-3p + SNHG16 using western blot. \*P < 0.05.

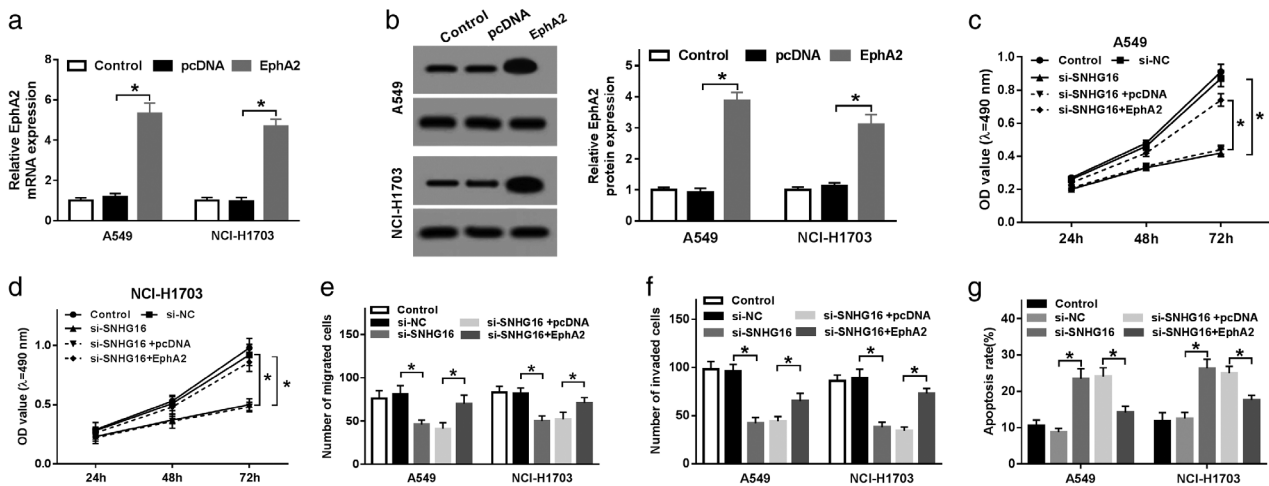
**EphA2 is a target of miR-520a-3p**

We further investigated the molecular mechanisms by which the SNHG16/miR-520a-3p axis regulated NSCLC cell carcinogenesis, and the potential target genes of miR-520a-3p were identified using starBase2.0 software and EphA2 was found to contain the putative binding site of miR-520a-3p (Fig 4a). Subsequently, the dual-luciferase reporter assay results suggested that miR-520a-3p mimic reduced the luciferase activities of the EphA2-WT reporter vector, but not mutant reporter vector in A549 and NCI-H1703 cells (Fig 4b,c), indicating the direct interaction between miR-520a-3p and EphA2. Synchronously, we detected the level of EphA2 and found the mRNA and protein levels of EphA2 were significantly elevated in NSCLC tissues and cell lines (Fig 4d–g). In addition, co-expression analysis exhibited that SNHG16 and EphA2 expression was positively correlated (Fig 4h), moreover, western blot results indicated the protein expression of EphA2 was inhibited by miR-520a-3p restoration, but this inhibition could

be rescued by following SNHG16 overexpression (Fig 4i,j). Thus, these data suggested EphA2 was a target of miR-520a-3p and regulated by SNHG16 through miR-520a-3p.

**SNHG16 silence inhibits cell carcinogenesis in NSCLC by regulating EphA2 expression**

Based on the above results, we determined that SNHG16 functioned as a ceRNA in regulating EphA2 through competitively binding to miR-520a-3p. Therefore, we wanted to find out whether EphA2 was implicated in SNHG16 deletion mediated antitumor effects. First, A549 and NCI-H1703 cells were transfected with pcDNA or EphA2, and we found the level of EphA2 was obviously elevated in A549 and NCI-H1703 cells after transfection (Fig 5a,b). Next, rescue experiments were performed and results confirmed that overexpressed EphA2 could partially overturn SNHG16 deletion-induced repression on proliferation (Fig 5c,d), migration and invasion (Fig 5e,f),



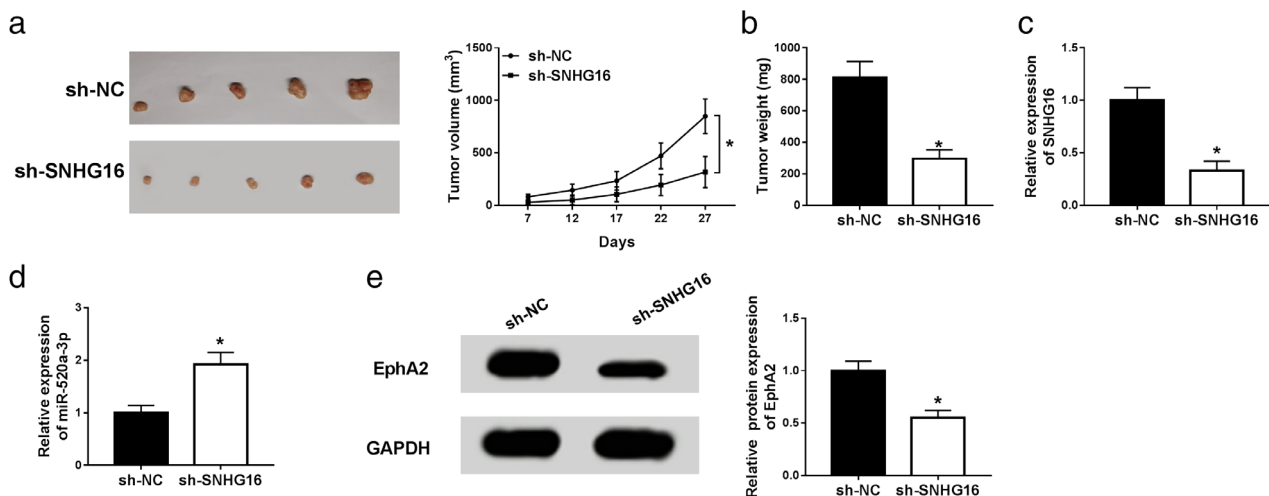
**Figure 5** SNHG16 silence inhibited cell carcinogenesis in NSCLC by regulating EphA2 expression. (a, b) The mRNA (a) and protein (b) expression levels of EphA2 were detected in A549 and NCI-H1703 cells transfected with pcDNA or EphA2 using qRT-PCR and western blot, respectively. A549 and NCI-H1703 cells were transfected with si-NC, si-SNHG16, si-SNHG16 + pcDNA, or si-SNHG16 + EphA2. After transfection, (c, d) MTT assay was used to detect cell proliferation; (e, f) cell migration and invasion were determined by transwell assay; (g) cell apoptosis was analyzed by flow cytometry. \**P* < 0.05.

enhancement on apoptosis (Fig 5g) of A549 and NCI-H1703 cells. Therefore, SNHG16 silence inhibited cell carcinogenesis in NSCLC by regulating EphA2 expression.

### Knockdown of SNHG16 inhibits NSCLC tumor growth in vivo

To further uncover the carcinogenesis roles of SNHG16 in vivo, xenograft models were established using A549 cells infected with lentivirus containing sh-SNHG16 or sh-NC.

As presented in Fig 6a,b, we found knockdown of SNHG16 inhibited NSCLC tumor growth, reflected by the suppression of tumor volume and weight in sh-SNHG16 group. In addition, molecular analysis showed SNHG16 silence remarkably reduced the expression of SNHG16 and EphA2, but increased miR-520a-3p level in the sh-SNHG16 group compared with sh-NC group (Fig 6c–e). Altogether, these results indicated that knockdown of SNHG16 might inhibit NSCLC tumor growth in vivo by regulating miR-520a-3p and EphA2 expression.



**Figure 6** Knockdown of SNHG16 inhibited NSCLC tumor growth in vivo. Xenograft models were established using A549 cells infected with lentivirus containing sh-SNHG16 or sh-NC. (a) Tumor volume was calculated every five days. (b) Tumor masses were weighed in each group. (c, d) The levels of SNHG16, miR-520a-3p and EphA2 were measured in two groups by qRT-PCR or western blot. \**P* < 0.05.

## Discussion

To date, thousands of lncRNAs have been widely established in mammalian genomes. LncRNAs play critical roles in a wide range of biological processes and whose deregulation contribute to cell carcinogenesis of human cancers.<sup>18,19</sup> For example, lncRNA UICLM functioned as an oncogene to induce colorectal cancer liver metastasis through regulating miR-215/ZEB2 axis.<sup>20</sup> LncRNA NEAT1 contributed to cell proliferation and epithelial-mesenchymal transition (EMT) led to breast cancer progression.<sup>21</sup> In addition, there is also increasing evidence that lncRNA SNHG16 participates in the regulation of tumorigenesis in many types of cancers. For example, in the study by Cao *et al.* SNHG16 contributed to bladder cancer cell proliferation by silencing p21 expression and was associated with poor prognosis.<sup>22</sup> SNHG16 exerted oncogenic function in glioma by regulating miR-4518/PRMT5 regulatory axis to promote cell viability and inhibit cell apoptosis in the report by Lu *et al.*<sup>23</sup> Additionally, Xu *et al.* found SNHG16 performed antitumor effects by inhibiting cell proliferation and chemoresistance in hepatocellular carcinoma.<sup>24</sup> Thus, it is necessary for us to detect the roles of SNHG16 in NSCLC. In this study, SNHG16 was found to be upregulated in NSCLC tissues and cells. Functional experiments showed knockdown of SNHG16 inhibited cell proliferation, migration and invasion but induced cell apoptosis in NSCLC. In addition, *in vivo* experiments also indicated SNHG16 deletion suppressed tumor growth. Therefore, we conclude that SNHG16 is a cell carcinogenesis regulator in NSCLC.

LncRNAs contain multiple miRNA-binding sites and can serve as sponges of miRNA to regulate miRNAs activity as well as the expression of their downstream target genes.<sup>25,26</sup> Here, we further investigated the miRNA targets of SNHG16 and miR-520a-3p was confirmed to be a target of SNHG16. Previous studies have indicated miR-520a-3p was decreased and functioned as a tumor suppressor to suppress cell growth and tumorigenesis in diverse human cancers, such as colorectal cancer, breast cancer, gastric cancer as well as NSCLC.<sup>27–30</sup> In this study, we also found miR-520a-3p was downregulated in NSCLC tissues and cell lines, and in addition a negative correlation between miR-520a-3p and SNHG16 was observed. Subsequently, rescue assay was conducted and results showed SNHG16 silence exerted antitumor effects in NSCLC cells by directly sponging miR-520a-3p. Thus, a SNHG16/miR-520a-3p regulatory axis in the development of NSCLC was identified.

It is well recognized that transmembrane protein receptors are implicated in the prognosis of various tumors and are more and more critical in cancer treatment.<sup>31</sup> EphA2 is one of the protein-tyrosine kinases that is elevated in

almost 90% of NSCLC cell membranes but not in normal lung tissues and cells.<sup>32</sup> Overexpressed EphA2 has been found to be related to the poor clinical outcome and mediate cell proliferation and motility in NSCLC.<sup>33</sup> In addition, EphA2-targeted therapy has also been identified to be an effective therapeutic strategy for NSCLC treatment.<sup>34,35</sup> In this study, EphA2 was predicted and proved to be a target of miR-520a-3p. Subsequently, miR-520a-3p/EphA2 regulatory axis was confirmed using a dual-luciferase reporter assay and co-expression analysis. EphA2 was discovered to be overexpressed in NSCLC tissues and cell lines, and overexpressed EphA2 was positively correlated with SNHG16. Thus, we further investigated the relationship among SNHG16, miR-520a-3p and EphA2, and the results showed SNHG16 could regulate EphA2 expression by binding to miR-520a-3p. Furthermore, rescue assay indicated SNHG16 silence inhibited cell carcinogenesis in NSCLC by regulating EphA2 expression.

In conclusion, this study demonstrated SNHG16 was upregulated in NSCLC tissues and cell lines, and SNHG16 deletion inhibited cell carcinogenesis in NSCLC. Additionally, a SNHG16/miR-520a-3p/EphA2 regulatory axis was identified in NSCLC, and SNHG16 silence exerted antitumor effects on NSCLC cells by regulating miR-520a-3p/EphA2 axis, indicating new potential therapeutic targets for NSCLC treatment.

## Disclosure

The authors declare that they have no conflicts of interest.

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