Original Article

Long non-coding RNA HAGLROS facilitates the malignant phenotypes of NSCLC cells via repressing miR-100 and up-regulating SMARCA5

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ABSTRACT

Background: Long non-coding RNA (lncRNA) is implicated in the progression of multiple cancers. This study aimed to explore the expression characteristics, biological function and molecular mechanism of lncRNA HAGLROS expression in NSCLC.
Methods: Quantitative real-time polymerase chain reaction (RT-PCR) was adopted to detect HAGLROS expression in NSCLC tissues and normal lung tissues. Survival curve was plotted by Kaplan—Meier method. Gain-of-function and loss-of-function models were respectively established to investigate the biological functions of HAGLROS, miR-100 and SMARCA5. MTT and Transwell assays were carried out to monitor the changes in proliferation, migration and invasion of NSCLC cells. Bioinformatics analysis and dual-luciferase reporter assay were used to verify the binding sites between HAGLROS and miR-100. Western blot was performed to determine the regulatory effects of HAGLROS and miR-100 on SMARCA5 protein expression.
Results: Up-regulated HAGLROS expression was observed in NSCLC tissues and cell lines. Over-expressed HAGLROS promoted the malignant phenotypes of NSCLC cells; conversely, HAGLROS knockdown repressed the malignant phenotypes of NSCLC cells. HAGLROS repressed miR-100 expression to promote SMARCA5 expression in NSCLC cells, and miR-100 overexpression or SMARCA5 knockdown counteracted the oncogenic functions of HAGLROS.
Conclusions: These results conclude that HAGLROS is a tumor promoter in NSCLC, and it regulates the malignant phenotypes of NSCLC cells via miR-100/SMARCA5 axis.

At a glance of commentary

Scientific background on the subject

Long non-coding RNA (lncRNA) HAGLROs have been reported to be associated with the progression of a variety of human cancers, including non-small cell lung cancer. This study aimed to further explore the function and molecular mechanism of HAGLROS in non-small cell lung cancer.

What this study adds to the field

This study found that HAGLROS was up-regulated in NSCLC tissues and cells. Over-expressed HAGLROS promoted the proliferation, migration and invasion of NSCLC cells. In contrast, HAGLROS knockdown had the opposite effect. In addition, HAGLROS promoted the progression of NSCLC by up regulating SMARCA5 through miR-100.

Non-small cell lung cancer (NSCLC) is the most common pathological subtype of lung cancer [1]. The early symptoms of NSCLC are insidious, and more than 40% of the patients are in advanced stage when they are diagnosed with NSCLC, and the prognosis of patients with metastasis or relapse is extremely adverse [2,3]. It's imperative to find novel targets to diagnose and treat NSCLC.

Non-coding RNAs (ncRNAs) have been regarded as potential markers or targets for multiple cancers [4—6]. Long non-coding RNA (lncRNA) is a type of ncRNA, defined as transcripts with lengths exceeding 200 nucleotides which could not be translated into proteins. LncRNAs play crucial roles in a wide range of biological processes [7—10]. For example, lncRNA cancer susceptibility 2 (CASC2) exerts an inhibitory effect on hepatocellular carcinoma via regulating miR-367/F-box and WD repeat domain containing 7 (FBXW7) axis [5]. Up-regulation of lncRNA small nucleolar RNA host gene 12 (SNHG12) promotes the growth and invasion of cervical cancer cells by acting as a molecular sponge for miR-424-5p [11]. Recently, it is reported that HAGLR opposite strand lncRNA (HAGLROS) is highly expressed in NSCLC and it promotes the migration and invasion of NSCLC cells [12]. However, the role of HAGLROS in NSCLC has not been fully clarified.

MicroRNAs (miRNAs) contain about 22 nucleotides, and they regulate gene expression at post-transcriptional level [13—15]. MiR-100 belongs to miR-99 family, which is abnormally expressed in multiple cancers. Reportedly, miR-100 inhibits the growth of glioblastoma cells by targeting SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 5 (SMARCA5) and erb-b2 receptor tyrosine kinase 3 (ErbB3) [16]. Another study reports that miR-100 regulates epithelial-mesenchymal transition and Wnt/β-catenin signaling by targeting HOXA1 and functions as a tumor suppressor in NSCLC [17]. Moreover, HAGLROS can target miR-100 to regulate apoptosis and autophagy of WI-38 cells [18]. However, the specific role of the HAGLROS/miR-100/SMARCA5 axis in NSCLC progression remains unclear.

This study focused on the expression, function, mechanism and clinical implication of HAGLROS in NSCLC. We demonstrated that HAGLROS promoted the malignant phenotypes of NSCLC cells via repressing miR-100 and up-regulating SMARCA5.

Materials and methods

Sample collection

54 NSCLC patients in Xiangyang Central Hospital from 2014 to 2017 were enrolled in this research. Before the collection of the samples, written consents were obtained from the patients. The NSCLC tissues and corresponding adjacent tissues were collected during surgery, and the tissues were frozen and stored in liquid nitrogen at −196 °C before the extraction of total RNA.

Cell cultrue

Normal lung epithelial cells 16HBE, NSCLC cell lines A549, H5N1, H1299, SK-MES-1, H460 and NCI—H23 were purchased from Yaji Biological Co,. Ltd (Shanghai, China). The cells were incubated in RPMI-1640 medium (Gibco, Carlsbad, CA, USA) containing 10% fetal bovine serum (FBS; Gibco, Carlsbad, CA, USA) and 100 U/mL penicillin, and 100 U/mL streptomycin (Hyclone, Logan, UT, USA) at 37 °C in 5% CO_2.

Quantitative real-time polymerase chain reaction (RT-PCR)

Total RNA was extracted from cancer cells using RNAiso Plus reagent (Takara, Dalian, China). The total RNA was reversely transcribed with cDNA synthesis kit (Takara, Dalian, China). Afterwards, cDNA obtained was considered as a template, and RT-PCR was conducted with SYBR®Premix-Ex-Taq™ (Takara, Tokyo, Japan). The cycle threshold (Ct) values were used to quantify the relative gene expression with $2^{-\triangle\triangle Ct}$ formula. U6 and GAPDH were used as the endogenous controls. The specific primer sequences were available in Table 1.

Establishment of cell lines

The full sequence of HAGLROS lacking poly-A tail was synthesized and subcloned into pcDNA3.1 (GeneChem, Shanghai, China) to construct HAGLROS overexpression plasmid. SMARCA5 siRNA (si-SMARCA5), miR-100 mimics and inhibitors were purchased from GenePharma Co. Ltd. (Shanghai, China). Lipofecmine 2000 (Invitrogen, Carlsbad, CA, USA) was used as the transfection reagent. 48 h after transfection, NSCLC cells were harvested for the validation of over-expression or knockdown, and functional experiments.

MTT assay

NSCLC cells were inoculated in 96-well plate (10^3 cells in each well), and cultured for 12 h. After that, the medium was replaced by a 100 μl of complete medium containing 10 μl of

Table 1 RT-PCR primer sequences.	
Name	Primer sequences
HAGLROS	Forward: 5′-TGTCACCCTTAAATACCGCTCT-3′
	Reverse: 5′-CTTCCTCCCACACAAATACTCC-3′
SMARCA5	Forward: 5′-TCTGTTGCCAGATGTGTTTAATTCA-3′
	Reverse: 5′-CCCAAGGCAGTTGTTTGTATCA-3′
GAPDH	Forward: 5′-AGCCACATCGCTCAGACAC-3′
	Reverse: 5′-GCCCAATACGACCAAATCC-3′
miR-100	Forward: 5′-GCGGCAACCCGTAGATCCGAA-3′
	Reverse: 5′-GTGCAGGGTCCGAGGT-3′
U6	Forward: 5′-GCTTCGGCAGCACATATACTAAAAT-3′
	Reverse: 5′-CGCTTCACGAATTTGCGTGTCAT-3′

MTT reagent (Beyotime, Shanghai, China) at different time points (12 h, 24 h, 48 h, 72 h and 96 h). Following that, the cells were cultured at 37 °C for 4 h. Following that, dimethyl sulfoxide (DMSO; Sigma, Shanghai, China) was added to resolve the formazan crystal. Finally, a microspectrophotometer was used to measure the absorbance of the cells at 570 nm.

Transswell assay

In the migration assay, NSCLC cells were suspended with serum-free medium, and inoculated in the upper chamber of the Transwell system (8 μm pore size; BD Biosciences, San Jose, CA, USA) (about 10^5 cells/well). The lower chamber was full of 500 μl of medium containing 10% FBS. 24 h later, the cells remaining on the upper surface of the filter were gently wiped off with cotton swabs, and cells passing through the filter were fixed with methanol, and then stained with crystal violet solution. Under an inverted microscope, five fields (including the center and periphery of the membrane) were randomly selected to count the number of cells. Matrigel (BD Biosciences, San Jose, CA, USA) was used to coat the filter of the Transwell system in the invasion assay, and the other procedures were the same as the migration assay.

Dual luciferase reporter gene assay

Briefly, DNA oligonucleotides and pMiR-Reporter vectors (Promega, Madison, WI, USA) were used to construct luciferase reporter vectors (pMiR-HAGLROS-wt/pMiR-HAGLROS-mut and pMiR-SMARCA5-wt/pMiR-SMARCA5-mut). pMiR-HAGLROS-wt or pMiR-HAGLROS-mut and miR-100 mimetics or negative controls (NC) were co-transfected into HEK293 cells. The luciferase activity was determined 48 h after the transfection using Dual Luciferase Reporter Assay Kit (Promega, Madison, WI, USA).

Northern blotting

20 μg of total RNA was analyzed on a 15% denaturing polyacrylamide-urea gel. miR-100 and HAGLROS were analysed on a 1.2% agarose gel in 1 × MOPS solution containing 1% formaldehyde. RNAs were separated by electrophoresis and transferred onto a Hyoid-N+ nylon membrane (GE healthcare, Shanghai, China). The membranes were incubated with hydration buffer containing probes in the labelling reaction system (20 mL) containing 2 mL of 10 × T4 PNK ligase buffer and 1 mL of T4 poly nucleotide kinase (NEB, Ipswich, MA, USA) and

2.5 mL of γ-[32 P]-ATP. Then the membranes were pre-hybridized in the hybridization buffer at 65 °C for 1 h and hybridized overnight at 65 °C. The membranes were then exposed to the phosphor screen for at least 12 h to show the bands.

Western blot

RIPA buffer (Beyotime, Shanghai, China) containing protease inhibitor cocktail (Roche, Nutley, NJ, USA) was employed to extract the total protein from NSCLC cells. Protein samples were subjected to SDS-PAGE and then transferred to nitrocellulose (NC) membrane (Millipore, Bedford, MA, USA). After the NC membranes were blocked with 5% fat-free milk, anti-SMARCA5 (Abcam, 1:1000), anti-proliferating cell nuclear antigen (PCNA) (Abcam, 1:1000), anti-matrix metallopeptidase 9 (MMP9) (Abcam, 1:1000) and anti-GAPDH (Santa Cruz, 1: 2000) antibodies were employed to incubate the membrane at 4 °C for 8 h. After the membranes were washed by TBST, the membranes were then incubated with horseradish peroxidase conjugated secondary antibody (1: 2000, Santa Cruz) at room temperature for 1 h. Next, the NC membranes were washed with TBST again, and ECL kit (Amersham Pharmacia Biotech, Little Chalfont, UK) was used to cover the membranes. Ultimately, ChemiDocXRS imaging system was adopted for detecting the protein bands.

Statistical analysis

All the assays were repeated for three times. SPSS13.0 statistical software (SPSS Inc., Chicago, IL, USA) was adopted to carry out statistical analyses. The Student's t test, one-way ANOVA, Fisher's Exact test or Chi-square test were used to evaluate the differences between different groups. Survival curves were plotted based on the Kaplan–Meier curves and log-rank tests. Correlations among HAGLROS expression, miR-100, and SMARCA5 were analyzed with Pearson's correlation analysis. Differences with $p < 0.05$ were considered to be statistically significant.

Results

HAGLROS expression was up-regulated in NSCLC tissues and cells

The expressions of HAGLROS in 54 pairs of NSCLC samples and adjacent tissues were detected. HAGLROS expression

was observed to be notably up-regulated in NSCLC tissues compared with normal tissues [Fig. 1A]. Furthermore, HAGLROS in NSCLC cell lines including A549 and H1299 cells was be highly expressed [Fig. 1B]. Similarly, the Northern blot analysis demonstrated that the HAGLROS hybridization signal was stronger in NSCLC tissues and cell lines than that in adjacent tissues and 16HBE cells [Fig. 1C and D].

High HGALROS expression was correlated with poor prognosis of NSCLC patients

To explore whether HAGLROS expression was associated with the cliniopathological characteristics of NSCLC patients, the NSCLC samples with HGALROS expression higher than twice of the average HGALROS expression in adjacent tissues, were defined as "high expression", and the other samples were defined as "low expression". Chi-square test indicated that highly-expressed lncRNA HAGLROS in tumor tissues was correlated with T stage ($p = 0.0219$) and local lymph node metastasis ($p = 0.0037$) in NSCLC patients, but had no association with age, sex, smoking history and tumor type ($p > 0.05$) [Table 2]. Additionally, we used Kaplan—Meier plotter database (kmplot.com) to explore the correlation of HAGLROS with the prognosis of NSCLC patients. The results suggested that NSCLC patients with highly expressed HAGLROS had shorter relapse-free survival and overall survival than those with the low expression of HAGLROS [Fig. 2A and B]. The findings above informed us that HAGLROS could probably be used as a biomarker for NSCLC.

HAGLROS facilitated the proliferation, migration and invasion of NSCLC cells

To probe into the impact of HAGLROS on NSCLC, we successfully established HAGLROS over-expression and knockdown cell lines with A549 and H1299, respectively [Fig. 3A]. MTT assay, subsequently, was carried out to monitor the proliferation of NSCLC cell lines A549 and H1299. The results demonstrated that over-expressed HAGLROS could promote the proliferation of NSCLC cells, while knockdown of HAGLROS showed the opposite effect [Fig. 3B]. Transwell assay confirmed the migration and invasion of NSCLC cells were facilitated by over-expressed HAGLROS, but restrained by knockdown of HAGLROS [Fig. 3C and D]. In addition, through Western blot analysis, it was found that overexpression of HAGLROS enhanced the expressions of PCNA and MMP9, and knockdown of HAGLROS inhibited the expressions of PCNA and MMP9 [Fig. 3E and F], which further indicated that HAGLROS regulated the proliferation, migration and invasion of NSCLC cells.

miR-100 interacted with HAGLROS in NSCLC cells

A recent study reported that HAGLROS could regulate the apoptosis and autophagy of WI-38 cells via regulating miR-100/NF-κB axis [18]. What's more, HAGLROS can modulate the apoptosis and autophagy of colorectal cancer cells via regulating miR-100/ATG5 axis [19]. To confirm whether HAGLROS could regulate the expression of miR-100 in
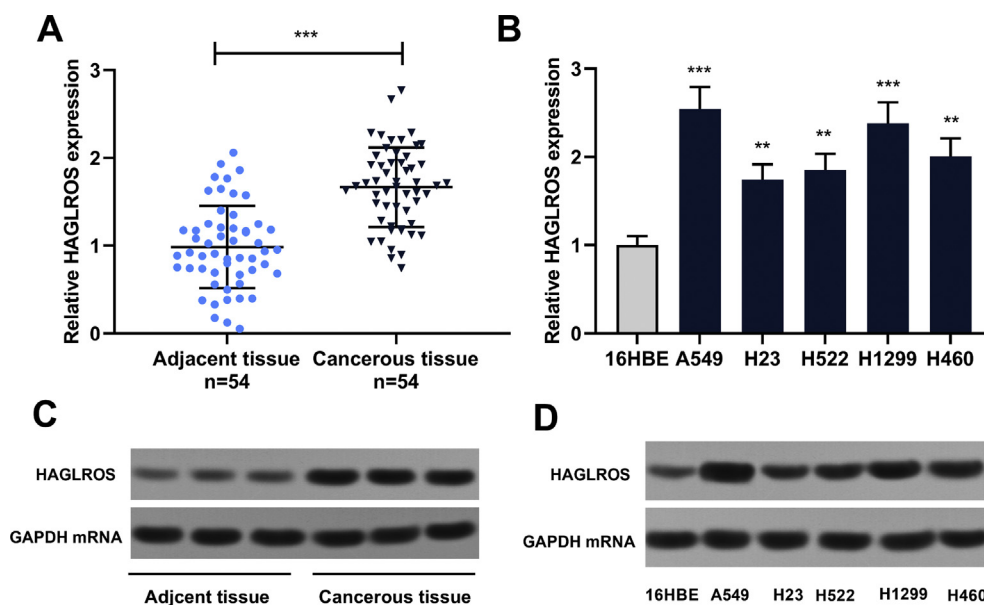

Fig. 1 **HAGLROS was up-regulated in NSCLC tissues and cell lines**. (A) RT-PCR was performed to detect the expression of HAGLROS in 54 pairs of NSCLC tissues and matched adjacent normal tissues. (B) RT-PCR was adopted to detect HAGLROS expression in normal lung epithelial cells and NSCLC cell lines. (C and D) Northern blot was used to detect the expression of HAGLROS in 3 pairs of NSCLC tissues and 6 cell lines. **$p < 0.01$, ***$p < 0.001$.

Table 2 The correlation between lncRNA HAGLROS expression and clinicopathological features (n = 54).

| Clinical features	N	lncRNA HAGLROS expression		Chi-square value	p value
		High expression	Low expression		
Age					
>60	22	14	8	0.1534	0.6953
≤60	32	22	10		
Gender					
Male	31	21	10	0.0379	0.8457
Female	23	15	8		
Smoking history					
Smoker	29	19	10	0.0372	0.8470
Non-smoker	25	17	8		
T stage					
T1-T2	7	2	5		0.034
T3-T4	47	34	13		
Lymph invasion					
N0	24	11	13	8.4375	0.0037
N1—N2	30	25	5		
Histology					
Squamous cancer	16	10	6	1.0000	0.6065
Adenocarcinoma	20	15	5		
Large cell carcinoma	18	11	7		
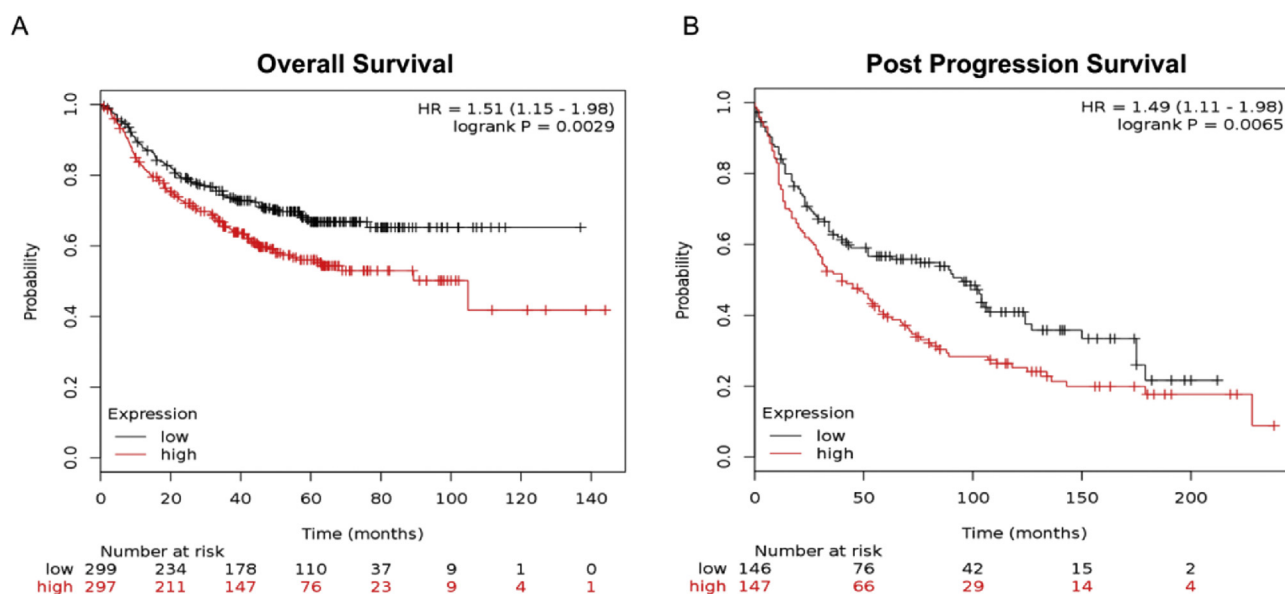
Fig. 2 **Patients with highly expressed HAGLROS in NSCLC have worse prognosis**. (A and B) Kaplan—Meier analysis was carried out to analyze the differences of relapse survival time and overall survival time between NSCLC patients with high expression of HAGLROS and with low expression of HAGLROS.

NSCLC, we examined miR-100 expression in NSCLC cell lines after HAGLROS was knocked down or overexpressed with RT-PCR and Northern blot. It was observed that miR-100 expression was decreased in NSCLC cells with HAGLROS overexpression, while increased in NSCLC cells with HAGLROS knockdown [Fig. 4A]. Luciferase reporter gene assay indicated that miR-100 could directly bind to HAGLROS [Fig. 4B]. The results from RT-PCR further revealed that the expression level of HAGLROS in NSCLC samples was negatively correlated with miR-100 expression [Fig. 4C].

These results supported that miR-100 was a target of HAGLROS, and was negatively regulated by HAGLROS in NSCLC tissues and cells. Next, through MTT and Transwell assays, we found that compared with the cells with HAGLROS overexpression, the proliferation, migration and invasion of the cells in HAGLROS overexpression + miR-100 overexpression group were significantly reduced [Fig. 4D,E,F]. These data suggested that HAGLROS could probably regulate the malignant phenotypes of NSCLC cells via repressing miR-100.
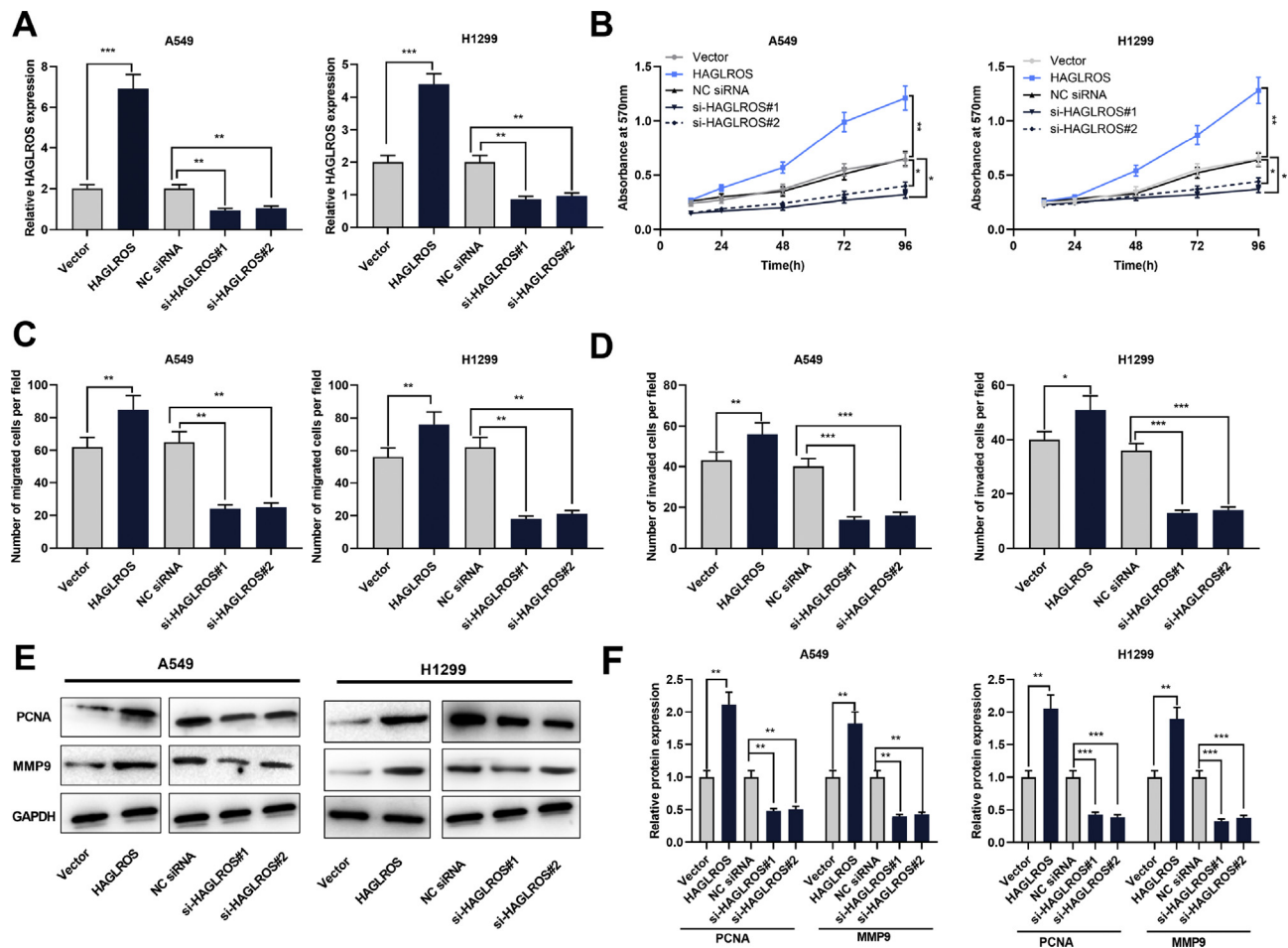
Fig. 3 **HAGLROS could promote the proliferation, migration and invasion of NSCLC cells**. (A) Overexpression and knock-down of HAGLROS in A549 and H1299 cells were detected by RT-PCR. (B) MTT assay was used to monitor the proliferation of A549 and H1299 cells with HAGLROS overexpression or knockdown. (C and D) Transwell assay was conducted to determine the migration and invasion of A549 and H1299 cells with HAGLROS overexpression or knockdown. (E and F) Western blot was used to detect the expressions of PCNA and MMP9 proteins in A549 and H1299 cells with HAGLROS overexpression or knockdown. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

MiR-100 repressed SMARCA5 expression and HAGLROS induced SMARCA5 expression

We demonstrated that the expression of SMARCA5 was significantly down-regulated after miR-100 mimics were transfected into NSCLC cells [Fig. 5A and B]. By searching TargetScan database (http://www.targetscan.org/vert_72/), we found SMARCA5 was a potential direct target of miR-100 [Fig. 5C]. Luciferase reporter gene assay validated that miR-100 directly targeted SMARCA5 mRNA 3′-UTR [Fig. 5D]. We also demonstrated that the expression level of SMARCA5 in NSCLC samples was negatively correlated with miR-100 expression [Fig. 5E]. RT-PCR and Western blot revealed that HAGLROS could positively regulate SMARCA5 on mRNA and protein levels [Fig. 5F and G]. Furthermore, SMARCA5 in NSCLC samples was positively correlated with HAGLROS expression [Fig. 5H]. In addition, the results of Western blot and RT-PCR analysis showed that SMARCA5 protein and

mRNA were significantly highly expressed in NSCLC tissues and cells [Fig. 5I–L]. Collectively, we concluded that HAGLROS/miR-100 axis regulated SMARCA5 expression in NSCLC.

Knockdown of SMARCA5 attenuated the effect of overexpression of HAGLROS on the progression of NSCLC

To explore whether SMARCA5 was a downstream effector of HAGLROS, si-SMARCA5 was transfected into A549 cells over-expressing HAGLROS. Western blot showed that the expression level of SMARCA5 in HAGLROS + si-SMARCA5 group was significantly lower than that in HAGLROS group [Fig. 6A]. Then the proliferation, migration and invasion of NSCLC cells were detected by CCK-8 and Transwell assays, and the results suggested that knockdown of SMARCA5 attenuated the promoting effects of HAGLROS overexpression on proliferation, migration, invasion [Fig. 6B–D].
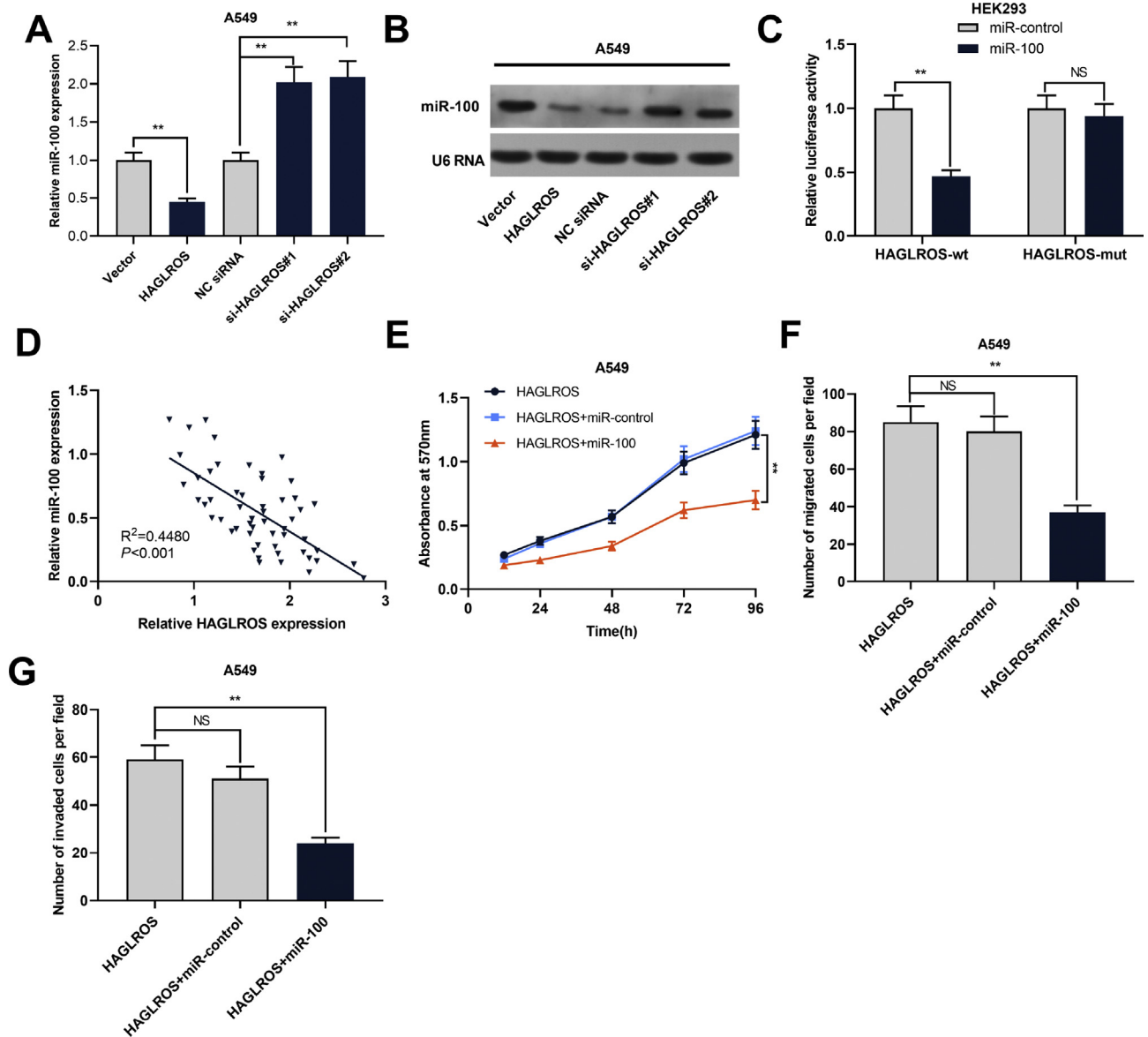
Fig. 4 **MiR-100 interacted with HAGLROS in NSCLC**. (A and B) The changes of miR-100 expression were examined by RT-PCR and Northern blot when HAGLROS was overexpressed or knocked down in NSCLC cells. (C) Dual-luciferase reporter assay was done to check the luciferase activity of cells to verify the binding relationship between HAGLROS and miR-100. (D) RT-PCR was carried out to explore the correlation between HAGLROS expression and miR-100 expression in 54 cases of NSCLC samples. (E) MTT assay was used to monitor the proliferation of A549 cells co-transfected with HAGLROS overexpression plasmids and miR-100 mimics. (F and G) Transwell assay was performed to monitor the migration and invasion of A549 cells co-transfected with HAGLROS overexpression plasmids and miR-100 mimics. **$p < 0.01$, NS: $p > 0.05$.

Discussion

Previously, lncRNA was considered as the "garbage" formed in the transcription, however, recent researches have revealed that lncRNAs features predominantly in regulating multiple biological processes, including tumorigenesis and cancer progression [20—23]. For instance, lncRNA small nucleolar RNA host gene 15 (SNHG15) promotes the proliferation, migration and invasion of breast cancer cells [24]. LncRNA colorectal neoplasia differentially expressed (CRNDE) facilitates the proliferation of gastric cancer cells [25]. LncRNA

urothelial cancer associated 1 (UCA1) meditates the growth and metastasis of pancreatic cancer cells [26]. In the present study, we demonstrated the up-regulation of HAGLROS in NSCLC, which is consistent with the previous report [12]. What's more, it was revealed that over-expressed HAGLROS was associated with unfavorable pathological characteristics and shorter survival time of NSCLC patients. Additionally, HAGLROS overexpression promoted the malignant phenotypes of NSCLC cells. These data suggest that HAGLROS is a promising biomarker and therapy target for NSCLC.

The role of miRNAs in tumors has been extensively studied. For instance, miR-494 inhibits the proliferation of oral
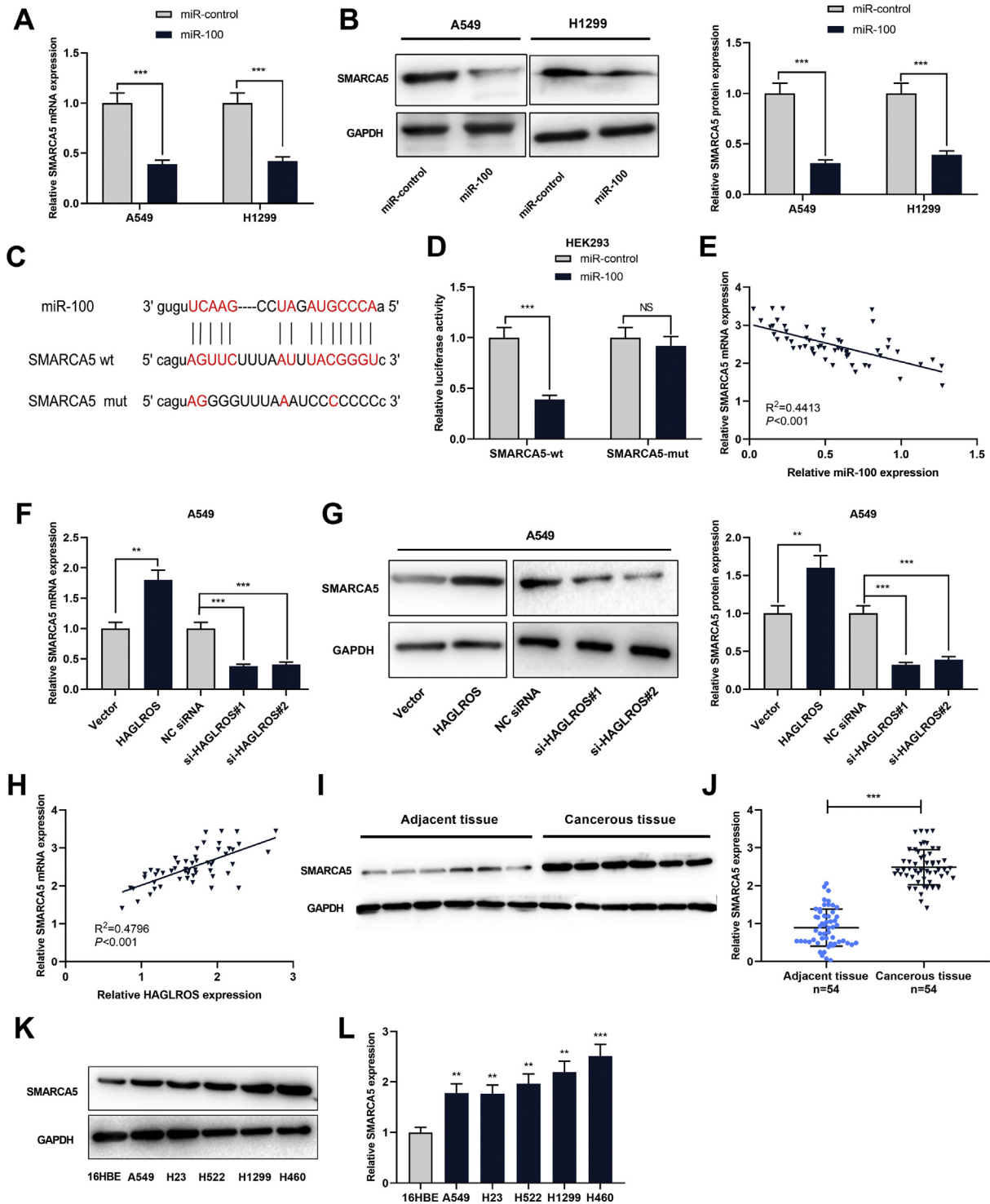
Fig. 5 **In NSCLC, the expression of SMARCA5 can be inhibited by miR-100 and promoted by LncRNA HAGLROS**. (A and B) RT-PCR and Western blot were used to detect SMARCA5 expression in A549 and H1299 cells with overexpressed miR-100. (C) The binding targets of miR-100 and SMARCA5 were predicted according to TargetScan database. (D) Dual-luciferase reporter assay was conducted to measure the luciferase activity to verify the targeting relationship between miR-100 and SMARCA5. (E) RT-PCR was employed to analyze the expression correlation between miR-100 and SMARCA5 in 54 cases of NSCLC samples. (F and G) RT-PCR and Western blot were done to assess the expression level of SMARCA5 mRNA and protein in A549 cells with HAGLROS overexpression or knockdown. (H) The correlation between HAGLROS and SMARCA5 expression in 54 cases of NSCLC samples was determined by RT-PCR. (I and J) Western blot and RT-PCR were used to detect the expression level of SMARCA5 protein and mRNA in NSCLC tissues and adjacent tissues. (K and L) Western blot and RT-PCR were used to detect the expression level of SMARCA5 protein and mRNA in NSCLC cells and 16HBE cells. **$p < 0.01$, ***$p < 0.001$, NS: $p > 0.05$.
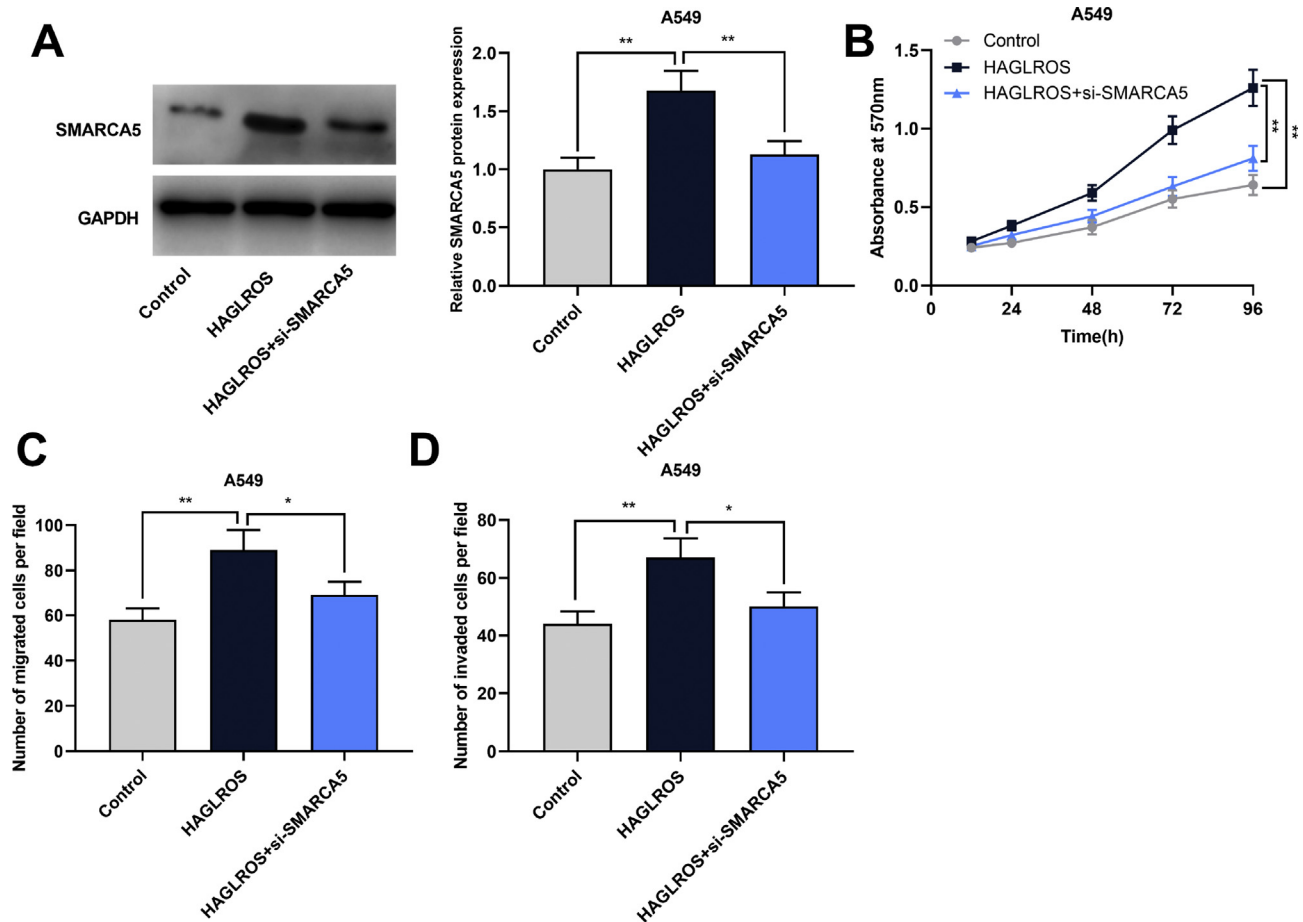
Fig. 6 **The knockdown of SMARCA5 attenuated the effect of overexpression of HAGLROS on the malignant phenotypes of NSCLC cells**. (A) Western blot was used to detect the expression level of SMARCA5 protein in A549 cells after co-transfection. (B—D) CCK-8 and Transwell assay were used to detect the proliferation, migration and invasion of A549 cells, respectively. *$p < 0.05$, **$p < 0.01$.

cancer cells via regulating homeobox A10 (HOXA10) expression [27]. MiR-506 impedes the proliferation of esophageal cancer cells via targeting cAMP responsive element binding protein 1 (CREB1) [28]. Besides, miR-124 restrains the proliferation, migration and invasion of lung adenocarcinoma cells by directly targeting SRY-box transcription factor 9 (SOX9) [29]. MiR-100 is a potential molecular marker of NSCLC, which acts as a tumor suppressor by targeting polo-like kinase 1 (PLK1) [30]. Accumulating studies report that lncRNAs, as competitive endogenous RNA, can modulate the expression of miRNA. For instance, lncRNA colon cancer associated transcript 1 (CCAT1) promotes the proliferation, migration and invasion of thyroid cancer cell line FTC-133 by downregulating miR-143 [31]. LncRNA maternally expressed 3 (MEG3) suppresses the proliferation and invasion of melanoma cells by regulating miR-499-5p [32]. In NSCLC, lncRNA growth arrest specific 5 (GAS5) inhibits tumorigenesis by suppressing the expression of miR-23a, and lncRNA HOXA11 antisense RNA (HOXA11-AS) promotes epithelial-mesenchymal transformation of cancer cells via suppressing miR-200 b [33,34]. In this study, we found that HAGLROS could target miR-100 and

repress its expression, and this regulatory relationship is similar with the previous reports [18,19].

SMARCA5 is a component of SWI/SNF chromatin-remodeling complex. It possesses a DNA-stimulated ATPase activity [35]. SMARCA5 is a crucial regulator in gene transcription, DNA repair and DNA replication, which is associated with malignant transformation [36,37]. Previously, some studies have reported abnormally high expression of SMARCA5 in breast cancer, gastric cancer, ovarian cancer and acute leukemia [38—40]. In the present work, we demonstrated that in NSCLC, SMARCA5 expression was significantly up-regulated, which was negative correlated with miR-100 expression and positively correlated with HAGLORS expression. Additionally, we validated that SMARCA5 was a target gene of miR-100 in NSCLC, which is also reported in glioblastoma and prostate cancer [16,41]. Importantly, we proved that SMARCA5 expression was up-regulated by HAGLROS overexpression in NSCLC cells, and SMARCA5 knockdown counteracted the biological effects of HAGLROS overexpression on NSCLC cells. These results suggest that HAGLROS exerts its oncogenic functions via regulating SMARCA5.

Conclusion

To conclude, the present study demonstrates that HAGLORS/miR-100/SMARCA5 axis is a novel molecular mechanism involved in NSCLC progression. Our work provides novel targets for the molecular treatment of NSCLC.

Ethics statement

Our study was approved by Medical College Review Board of the Xiangyang Central Hospital.

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Conflicts of interest

The authors declare that they have no competing interest.

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