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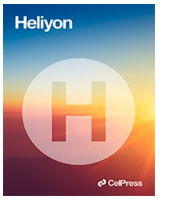

LINC00943 regulates miR-1252-5p/YWHAH axis to promote tumor proliferation and metastasis in lung adenocarcinoma

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ABSTRACT

Lung cancer is the most common malignant tumor worldwide. In recent years, the incidence of lung adenocarcinoma (LAD) has increased significantly, with an unfavorable 5-year survival rate. Long non-coding RNAs (lncRNAs) have been shown to play a significant role in the emergence, growth, and metastasis of tumors. However, the functional role and mechanism of LINC00943 in LAD progression have not yet been investigated. Aberrant expressions of LINC00943, miR-1252-5p, and YWHAH were determined by RT-qPCR and Western blot analyses. The binding relationship between miR-1252-5p and LINC00943 or YWHAH was examined by Pearson's correlation analysis, RNA pull-down, and dual-luciferase reporter assays. MTT assay was conducted to measure cell viability and colony formation assay was performed to evaluate cell proliferation potential. Transwell assay was used to investigate cell migration and invasion and flow cytometry was applied to evaluate cell apoptosis. We found that LINC00943 was highly expressed in LAD tissue samples and cell lines and was a reliable biomarker with high sensitivity, and specificity ($P < 0.0001$; AUC: 0.8966) for LAD detection. LINC00943 was mainly localized in the cytoplasm. In vitro, LINC00943 promoted LAD cell proliferation, migration, and invasion; however, silencing LINC00943 inhibited LAD tumor metastasis. Mechanistically, LINC00943 was competitively bound with miR-1252-5p to enhance YWHAH expression. Moreover, LINC00943 silencing sponged miR-1252-5p to inhibit YWHAH, thereby retraining LAD cell malignant behaviors. In summary, LINC00943 facilitates LAD cell malignancy through sponging miR-1252-5p to upregulate YWHAH. LINC00943 is a novel lncRNA that serves as an oncogene and might be used as a prognostic biomarker for LAD.

1. Introduction

Lung cancer (LC) is a frequently diagnosed malignancy in the respiratory system [1]. The most common pathological form of LC is non-small cell lung cancer (NSCLC), and the majority of tumors are lung adenocarcinomas (LAD), with insidious onset and early symptoms [2]. The options for lung adenocarcinoma treatment include targeted therapies, immunotherapy, radiotherapy, and chemotherapy [3]. However, due to diagnostic limitations, most lung adenocarcinoma patients are diagnosed at an advanced stage, and the 5-year survival rate is disappointing [4]. Therefore, the exploration of potential biomarkers and therapeutic targets is of great

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clinical significance for the early diagnosis and clinical treatment of LAD patients.

In recent years, an increasing number of novel and functional molecules have been discovered and characterized. Long non-coding RNAs (lncRNAs) are endogenous non-coding single-stranded RNA molecules that can regulate the expression of downstream target genes at epigenetic, transcriptional, and post-transcriptional levels, thus participating in cell differentiation, proliferation, invasion, and migration [5]. Substantial literature has demonstrated the aberrant expression of lncRNAs in various malignancies and demonstrated its correlation with cancer development [6,7]. More importantly, several lncRNAs have been shown to regulate the growth and metastasis of lung adenocarcinoma, and their expression levels are related to the pathological stage and prognosis in lung adenocarcinoma. For instance, Deng et al. discovered that LINC00472 could suppress lung adenocarcinoma cell migration ability and invasiveness [8]. Similarly, lncRNA DUXAP8 was found to be overexpressed in lung adenocarcinoma patients and was demonstrated to sponge miR-26b-5p activity thus enhancing lung adenocarcinoma progression [9].

LINC00943, which has a 2261 bp and located at chr12, is a member of the class of long intergenic non-coding RNAs (lncRNA) and is a recently discovered lncRNA. The information regarding LINC00943 is currently scant. Previous reports on Parkinson's disease have shown that LINC00943 was highly expressed in the brain tissues of MPTP-treated mice and MPP^+-induced SK-N-SH cells [10,11]. Interestingly, knock down of LINC00943 promoted the proliferation while inhibiting apoptosis and the inflammatory response in MPP + -treated SK-N-SH cells to alleviate cell injury by regulating the miR-338-3p/SP1 and miR-7-5p/CXCL12 axis. In another study, LINC00943 was shown to promote gastric cancer cell proliferation via hsa-miR-101-3p [12]. To the best of our knowledge, there is not a single study that has investigated whether LINC00943 is associated with lung cancer progression.

The present study was conducted to explore the impact of LINC00943 on the biological properties of lung adenocarcinoma cells. We also intended to investigate the underlying mechanism of LINC00943 in lung adenocarcinoma, which may provide a novel candidate target for LAD diagnosis as a new biomarker and a novel therapeutic target for lung adenocarcinoma treatment.

2. Materials and methods

2.1. Patient enrollment

126 cases of surgically resected lung adenocarcinoma tissues and adjacent paracancerous tissues from patients admitted to Zhongda Hospital, School of Medicine, Southeast University from March 2017 to July 2019 were collected and preserved in liquid nitrogen immediately after surgery. All specimens were confirmed as lung adenocarcinoma or paracancerous tissues by pathological biopsy. The experimental procedures were approved by the Ethics Committee of Zhongda Hospital, School of Medicine, Southeast University, and signed informed consent forms were obtained from all patients. Inclusion criteria was (1) histopathologically confirmed lung adenocarcinoma; (2) no other malignant tumors except lung adenocarcinoma; (3) complete tumor resection with negative margins and no distant metastases; (4) no radiotherapy or chemotherapy before surgery.

2.2. Follow-up survey

Patients were followed up by our thoracic surgeons by telephone and micro-mail after discharge from the hospital, together with regular outpatient review, with local or distant organ metastasis of the primary tumor suggested by imaging and pathology as recurrence and metastasis. Progression-free survival (PFS) refers to the time interval between initial surgical resection date to the tumor recurrence or metastasis date, death, or the date of last known follow-up contact.

2.3. Cell culture and transfection

Human lung adenocarcinoma cell lines A549, H1299, H1975, and normal bronchial epithelial cell line BEAS-2B were purchased from the Typical Culture Collection Committee Cell Bank of Chinese Academy of Sciences (Shanghai, China). Cells were maintained in Dulbecco's modified Eagle medium containing 10% FBS (Sigma-Aldrich) and incubated at 37 °C with 5% CO_2 in a saturated humidity incubator.

Following 80% confluence in plate culture, A549 cells were transfected with the appropriate plasmids using Lipofectamine 3000 transfection reagent (Invitrogen) in accordance with the manufacturer's instructions. To silence LINC00943, cells were transfected with LINC00943 siRNA (si-LINC00943), and the negative control si-RNA (si-NC) provided by Oligobio (Shanghai, China). LINC00943 and YWHAH overexpression plasmids ov-LINC00943 and ov-YWHAH were based on pcDNA3.1 plasmids (Invitrogen, USA). The miRNA mimics/inhibitor for miR-1252-5p and negative control (NC) mimics/inhibitor were purchased from Genechem Company (Shanghai, China). After transfecting the cells for 48 h, cells were harvested for assays.

2.4. RT-qPCR analysis

Total RNA was extracted from tissue samples and cells using a TRIzol kit and reverse transcribed into cDNA. qRT-PCR reaction conditions were set according to the instructions of qRT-PCR kit: pre-denaturation at 95 °C for 5 min, 62 °C for 35 s, 72 °C for 35 s, 35 cycles in total. The expression of miR-1252-5p was detected using U6 as an internal reference, and the expression of LINC00943 and YWHAH mRNA was detected using GAPDH as an internal reference. Gene expression was analyzed by the $2^{-\triangle\triangle Ct}$ method.

2.5. MTT assay

A549 cells after indicated transfection were harvested and plated in a 96-well plate at 2×10^4 cells/mL in each well. Then, 100 μL of MTT reagent was supplemented into each well and thoroughly shaken for 20 min. Cell viability was measured with OD value at 570 nm on an enzyme marker, and the cell growth curve was plotted.

2.6. Cell proliferation measurement

A549 cells were inoculated into a six-well plate at 5000 cells per well, followed by culturing at 37 °C for two weeks. The culture medium was exchanged every other day. After fixing for 20 min with 4% paraformaldehyde (Sigma-Aldrich) (−20 °C), cells were dyed for 15 min using 1% crystal violet.

2.7. Cell apoptosis

Transfected A549 cells were rinsed using precooled PBS and centrifugated for 5 min at 1000×*g*. An Annexin V-fluorescein isothiocyanate (FITC)/propidium iodide (PI) apoptosis kit (BestBio, Shanghai, China) was applied to detect cell apoptosis in accordance with manufacturer's protocol. 2×10^5 A549 cells were resuspended in binding buffer and stained with 5 μL Annexin-V and 5 μL PI for 15 min in dark. The ratio of apoptotic cells was evaluated using flow cytometry (Thermo Fisher).

2.8. Transwell assays

The 8-mm pore size Transwell chambers were applied for migration and invasion measurement (the top layer of the chambers was uniformly pre-coated with Matrigel for invasion measurement). A549 cells were cultured and digested and resuspended in serum-free medium. The top layer was supplemented with cell suspension, while 500 μL 100% cell culture medium was supplemented into the bottom and incubated for 36 h. The Transwell chambers were removed, fixated by methanol, and dyed by 0.1% crystal violet for 20 min, and the remaining cells were removed from the upper layer of the chambers by cotton swabs, and counted under a random field of view under a microscope.

2.9. Subcellular localization assay

Using the RNA Isolation Kit, ThermoFisher System, the collected cells were resuspended in pre-cooled Cell Disruption Buffer according to the instruction manual. The cells were centrifuged at 750 g for 5 min at 4 °C and the supernatant was carefully aspirated and added to a new Eppendorf tube to obtain the cytoplasmic lysis product. The precipitate was resuspended with Cell Disruption Buffer, and the nucleus lysis product was obtained after vigorous shaking. The subcellular localization of LINC00943 was then detected using RT-qPCR assay.

2.10. Western blotting analysis

BCA assay was conducted for protein concentration determination. For each group, 40 μg of protein was loaded onto 10% SDS-PAGE gel, and then electro-transferred onto PVDF membranes. The membranes were then incubated with primary antibodies against YWHAH (1:1000) and GAPDH (1:2000) overnight at 4 °C. Next morning, after washing the membranes, secondary antibody was added and incubated the membranes at room temperature for 2 h. Finally, ECL luminescence reagent was used to probe the proteins, and GAPDH was applied as a loading control.

2.11. Dual-luciferase reporter system

LINC00943 and YWHAH 3′UTR mutant (MUT) or wild-type (WT) were constructed through miR-1252-5p binding site on LINC00943 and YWHAH with pmirGLO vectors (Promega, Madison, WI). The corresponding sequences were then subcloned into the vectors. A549 cells (4 × 104 cells/well) were grown at appropriate density in thin-bottomed opaque 96-well plates and the indicated luciferase reporter vectors were then co-transfected with miR-125-5p mimics or mimics NC using the Lipofectamine® 2000 Transfection Reagent. 24 h after the transfection, a Dual-Luciferase Reporter Assay System (Promega) was utilized to assess luciferase activity.

2.12. Statistical analysis

GraphPad Prism 8.0 was applied for data analysis and expressed as the mean ± standard deviation (SD) from at least 4 independent replicates. The student's t-test or ANOVA followed by Tukey's post-hoc test was applied for data comparison in different groups. $P < 0.05$ was considered as statistically significant difference.

3. Results

3.1. Expression characteristics of LINC00943 and miR-1252-5p in lung adenocarcinoma

We first investigated the differential expression profiles of LINC00943 and miR-1252-5p in clinical specimens. As shown in Fig. 1A, LINC00943 was remarkably increased in lung adenocarcinoma tumor tissues in comparison to its adjacent non-tumor paracarcinoma tissues. We also found that LINC00943 could discriminate lung adenocarcinoma tumor tissues from para-carcinoma tissues with high sensitivity, specificity, and accuracy (AUC: 0.8966, $P < 0.0001$) (Fig. 1B). On the contrary, miR-1252-5p was significantly reduced in lung adenocarcinoma tissue samples and also showed the potential to distinguish lung adenocarcinoma tumor tissues from non-tumor specimens with the AUC of 0.9007 (Fig. 1C and D). The Pearson's correlation analysis further revealed that there was an inverse correlation between LINC00943 and miR-1252-5p in lung adenocarcinoma specimens (Fig. 1E).

We further verified the expressions of LINC00943 and miR-1252-5p in lung adenocarcinoma cell lines. As depicted in Fig. 1F and G, in comparison with the normal BEAS-2B cells, LINC00943 was overtly elevated while miR-1252-5p was less expressed in A549, H1299 and H1975 lung adenocarcinoma cell lines. Notably, these differences were more apparent in A549 cells, thus this cell line was chosen for following assays.

3.2. LINC00943 silencing restrains lung adenocarcinoma cell growth

Given that LINC00943 expression was noticeably high in LAD cells, we hypothesized that LINC00943 might play a role as an oncogene in the cellular activities of LAD cells. Thus, we conducted loss-of-function assays. We knocked down LINC00943 by transfecting A549 cells with si-LINC00943. After transfection, we found that LINC00943 expression level was prominently reduced, suggesting that the transfection was successful (Fig. 2A). Then, the MTT, colony formation and flow cytometry analysis were performed to measure cell viability, proliferation, and apoptosis in LAD cells. The results (Fig. 2B-D) depicted that in relation to si-NC group, si-LINC00943 group presented a remarkable decline in cell viability, restrained cell proliferation, and promoted cell apoptosis. Moreover, the Transwell assays suggested that LINC00943 silencing could suppress A549 cell migration and invasion compared with si-NC group (Fig. 2E and F). These findings show that LINC00943 promotes LAD cell malignant behaviors.

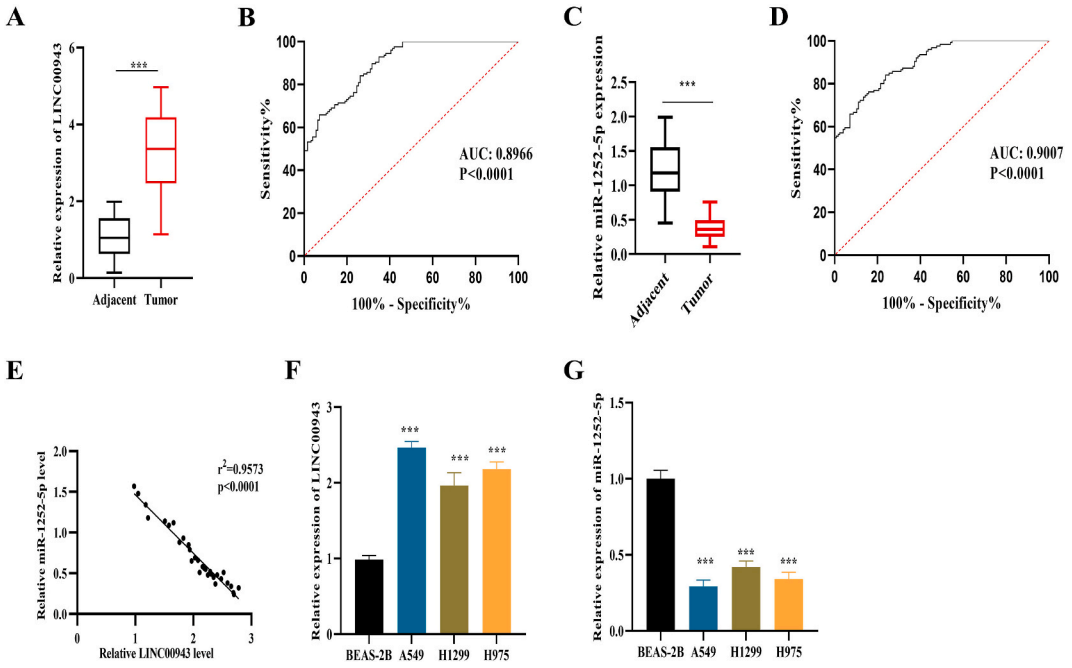

Fig. 1. Aberrant expression of LINC00943 **and miR-1252-5p in lung adenocarcinoma tumor tissues.** (A) LINC00943 expression in lung adenocarcinoma tissues. (B) Diagnostic value of LINC00943 in discriminating lung adenocarcinoma tumor tissues from non-tumor adjacent tissues. (C) miR-1252-5p expression in lung adenocarcinoma tissues. (D) Diagnostic value of miR-1252-5p in discriminating lung adenocarcinoma tumor tissues from non-tumor adjacent tissues. (E) Correlation analysis between LINC00943 and miR-1252-5p level in lung adenocarcinoma tissues. (F) LINC00943 level in normal BEAS-2B cells and lung adenocarcinoma cell lines. (G) miR-1252-5p level in normal BEAS-2B cells and lung adenocarcinoma cell lines. ***$P < 0.001$.

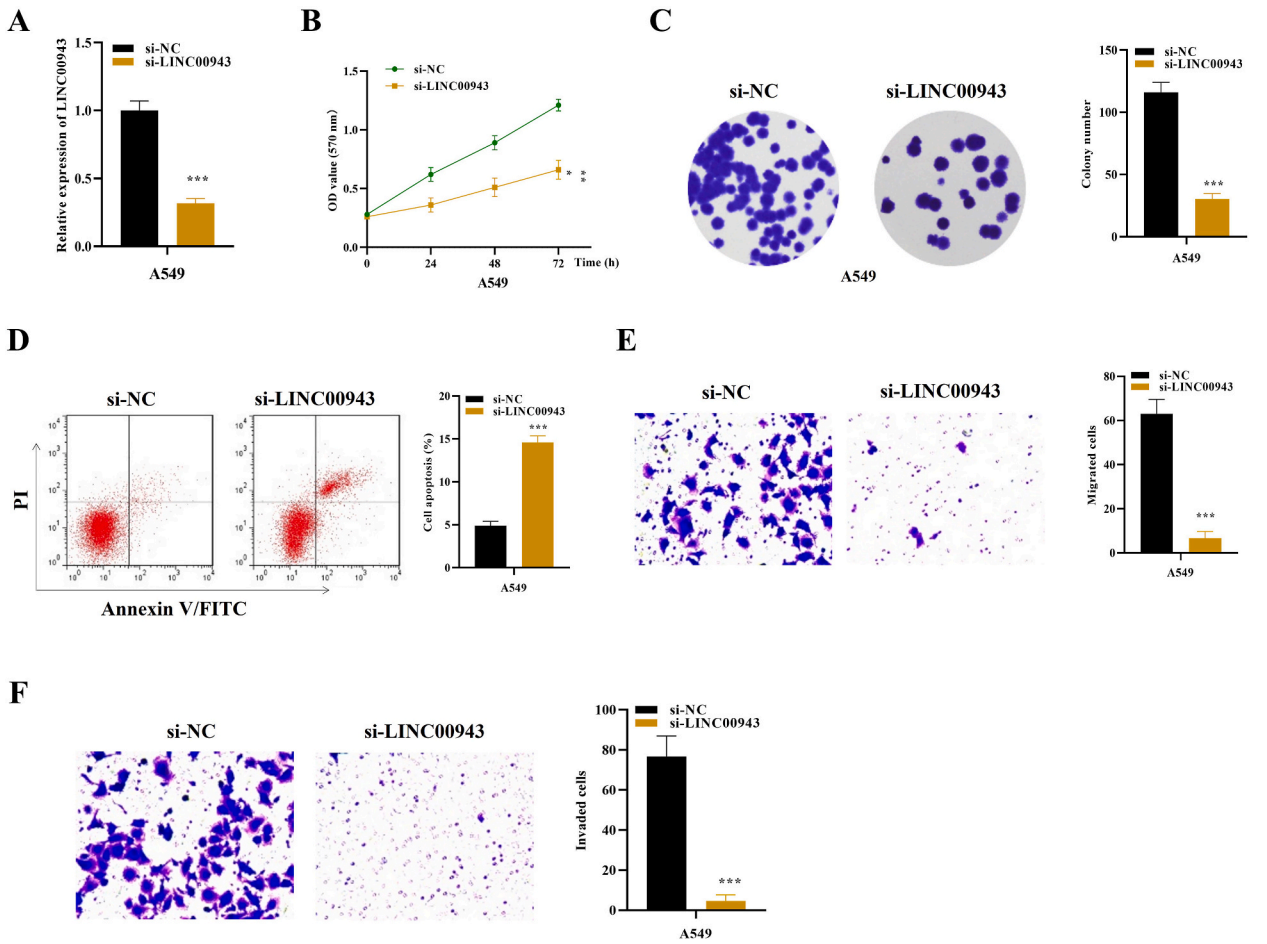

Fig. 2. LINC00943 facilitates lung adenocarcinoma cell proliferation, migration, and invasion. (A) The transfection efficacy of LINC00943 was determined by RT-qPCR analysis. (B) MTT assay was used to detect cell viability. (C) Colony formation analysis confirmed the cell proliferation rate. (D) Flow cytometry analysis was used to measure the cell apoptosis rate. (E) Cell migration and (F) Cell invasion was measured by Transwell assays. $^{***}P < 0.001$.

3.3. LINC00943 acts as a sponge for miR-1252-5p

Since circRNAs mainly function as miRNA "sponges", we therefore proposed that LINC00943 might perform its role in LAD cells in this way. First, the subcellular location of LINC00943 in A549 cells was verified. As demonstrated in Fig. 3A, LINC00943 was mainly located in the cytoplasm. By using Starbase online tool, a complementary sequence between LINC00943 and miR-1252-5p was found, which suggested that LINC00943 may bind with miR-1252-5p (Fig. 3B). Then, we observed the abundant enrichment of LINC00943 in Bio-miR-1252-5p group (Fig. 3C). Consequently, we conducted luciferase reporter experiments to support the notion that there is a direct relationship between miR-1252-5p and LINC00943. Luciferase activity assay demonstrated that miR-1252-5p overexpression dramatically decreased the luciferase activity of LINC00943-WT and that of LINC00943-MUT exhibited no evident change (Fig. 3D). Additionally, the expression of LINC00943 showed significant elevation in A549 cells transfected with LINC00943 overexpression plasmids (Fig. 3E). Furthermore, after knocking down or overexpressing LINC00943 expression in A549 cells, miR-1252-5p was raised in si-LINC00943 while restrained in ov-LINC00943 groups (Fig. 3F). Finally, miR-1252-5p level was enhanced after silencing LINC00943; however, the up-regulation was partially counteracted with the introduction of miR-1252-5p inhibitor (Fig. 3G). Collectively, the above results suggest that LINC00943 functions as a sponge for miR-1252-5p in A549 cells.

3.4. LINC00943 knockdown increases miR-1252-5p expression to suppress the migration and invasion of lung adenocarcinoma cells

In the next step, we carried out rescue experiments to better understand LINC00943-miR-1252-5p regulatory pattern in LAD cellular processes. As shown in Fig. 4A–E, MTT, colony formation, cytometry analysis, and Transwell assays revealed that LINC00943 silencing promoted A549 cell apoptosis while hindered LAD cell proliferation potential, migration ability, and invasiveness, whereas the effect of si-LINC00943 on A549 cells was partially restored by miR-1252-5p silencing. These findings suggest that sponging of miR-

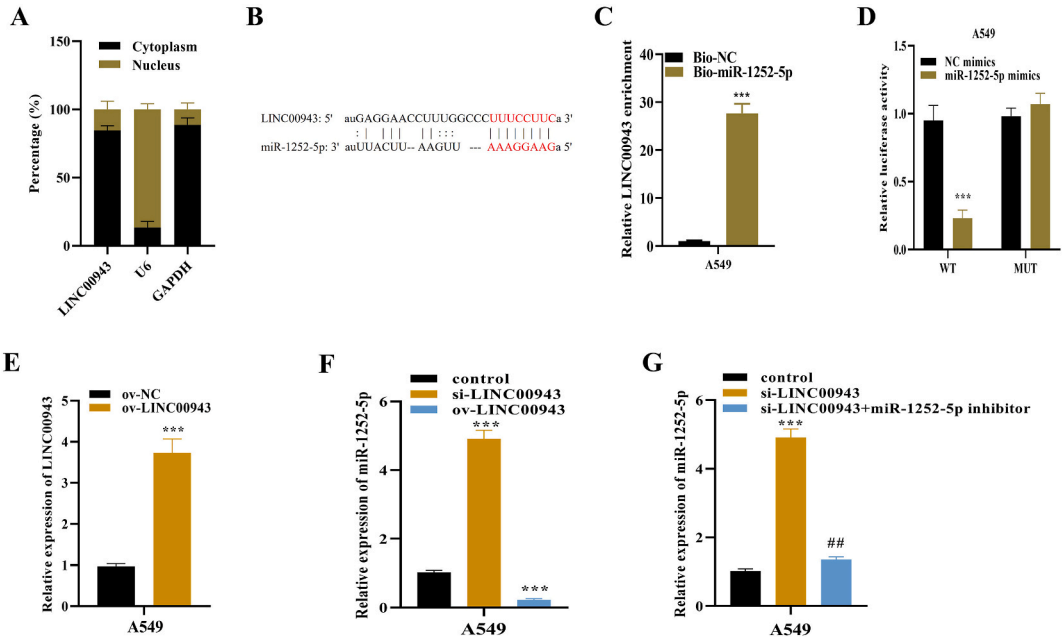

Fig. 3. Targeting relationship between LINC00943 and miR-1252-5p. (A) Subcellular location of LINC00943 in lung adenocarcinoma cells. (B) Starbase online tool predicted the potential binding sites between LINC00943 and miR-1252-5p. (C) RNA pull-down assay verified the binding association between LINC00943 and miR-1252-5p. (D) Dual-luciferase reporter assay was used to explore the interaction between LINC00943 and miR-1252-5p. (E) RT-qPCR analysis was used to assess the miR-1252-5p overexpression efficacy. (F) The expression of miR-1252-5p in A549 cells after LINC00943 silencing or overexpression. (G) Effect of co-transfection of LINC00943 and miR-1252-5p on miR-1252-5p expression. $^{***}P < 0.001$; $^{\#\#}P < 0.01$.

1252-5p by LINC00943 promotes the malignant phenotype of LAD cells.

3.5. MiR-1252-5p acts as a cancer-suppressor in lung adenocarcinoma

We then investigated the functional role of miR-1252-5p in LAD progression. For this purpose, we first overexpressed miR-1252-5p in A549 cells with miR-1252-5p mimics. As exhibited in Fig. 5A, miR-1252-5p exhibited significant raise in miR-1252-5p mimics-transfection group compared to its control mimic group. Then, a series of functional experiments were performed to investigate miR-1252-5p overexpression effects on LAD cells malignant behaviors. The results suggested that miR-1252-5p mimics significantly promoted lung adenocarcinoma cell apoptosis while depleted cell proliferation, migration, and invasion (Fig. 5B-F). Comprehensively, the above-mentioned experiments implies that miR-1252-5p function as an anti-cancer miRNA in lung adenocarcinoma.

3.6. LINC00942 downregulates YWHAH expression by sponging miR-1252-5p

To explore the target of miR-1252-5p, we utilized TargetScan online tool, and we found the binding sequences of YWHAH and miR-1252-5p (Fig. 6A). We then verified the interaction between YWHAH and miR-1252-5p by performing the RNA pull-down and dual-luciferase reporter assays. We found that biotinylated miR-1252-5p in LAD cells abundantly enriched YWHAH in pull-down products, and miR-1252-5p overexpression dramatically decreased the luciferase activity of YWHAH-WT and that of YWHAH-MUT exhibited no evident change (Fig. 6B and C), which suggests that YWHAH binds to miR-1252-5p in LAD cells. Furthermore, transfecting A549 cells with miR-1252-5p mimics hindered YWHAH expression while miR-1252-5p inhibitor facilitated YWHAH expression level in A549 cells (Fig. 6D and E). Finally, miR-1252-5p mimics and ov-YWHAH were co-transfected in A549 cells, and we noticed that the inhibitory effect of miR-1252-5p mimics on YWHAH expression was partially counterbalanced by YWHAH overexpression (Fig. 6F and G). In sum, these findings suggest that YWHAH directly interacts with miR-1252-5p.

Lastly, we confirmed the regulatory function of miR-1252-5p/YWHAH axis in modulating lung adenocarcinoma cell development by performing functional assays. As illustrated in Fig. 6H-L, in relation to the control group, lung adenocarcinoma cell malignant behaviors were markedly hindered in miR-1252-5p mimics group; whereas the inhibitory effect exerted by miR-1252-5p mimics was partially reversed by YWHAH overexpression. These outcomes illustrate that YWHAH downregulation by miR-1252-5p inhibits the malignant phenotype of LAD cells.

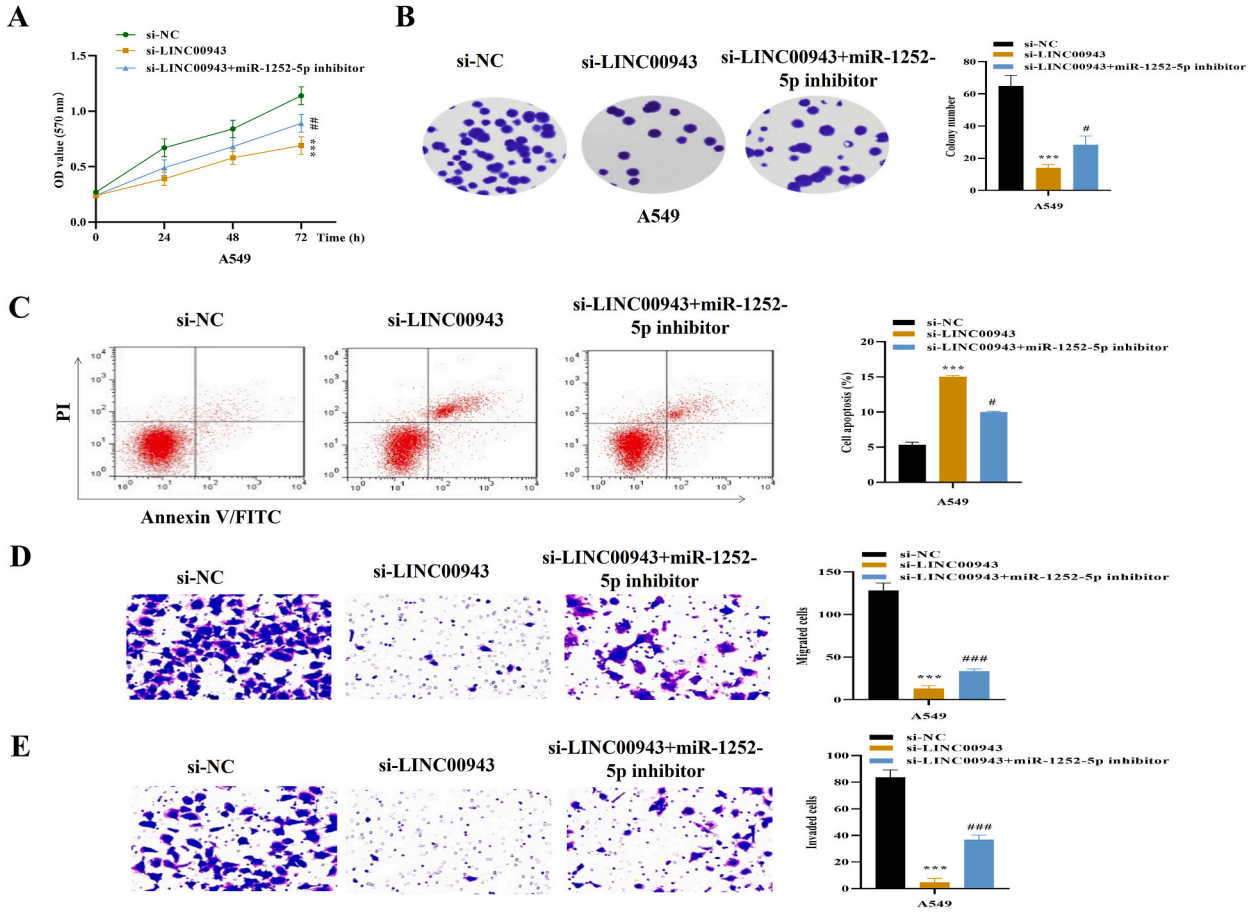

Fig. 4. Silencing of LINC00943 sponged miR-1252-5p to suppress lung adenocarcinoma cell proliferation, migration, and invasion but promoted apoptosis. (A) MTT assay detected cell viability. (B) Colony formation analysis confirmed the cell proliferation rate. (C) Flow cytometry analysis was used to measure the cell apoptosis rate. (D) Cell migration and (E) Cell invasion was measured by Transwell assays. $^{***}P < 0.001$; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$.

4. Discussion

With the development of genomic research, more and more molecules, including miRNAs and lncRNAs, have been identified as potential biomarkers to evaluate tumor progression and prognosis, and as potential targets for biologically targeted therapies [13,14]. In lung adenocarcinoma, many lncRNAs have been shown to play the role of "oncogenes" or "anti-oncogenes". LINC00943 is a newly discovered lncRNA which is widely reported in Parkinson's disease and in very few tumors development. For instance, Meng et al. revealed that knockdown of LINC00943 increased cell viability and inhibited apoptosis in MPP^{+}-induced SK-N-SH cells and attenuated inflammatory and oxidative neuronal injury through the miR-15b-5p/RAB3IP axis [15]. Other studies have reported that knockdown of LINC00943 ameliorated Parkinson's disease [11,16,17]. In skin cancer realm, LINC00943 was found to be responsible for the development and outcome of cutaneous melanoma and clear cell renal cell carcinoma [18,19]. In addition, LINC00943 was highly expressed in gastric cancer and was associated with poor prognosis in patients with gastric cancer; meanwhile, knockdown of LINC00943 inhibited gastric cancer cell proliferation and regulated gastric cancer cell growth and chemosensitivity by binding with miR-101-3p [12]. In line with these studies, in the present report, we also found that LINC00943 was highly expressed in lung adenocarcinoma and acted as a cancer promoter to facilitate malignant behaviors in lung adenocarcinoma. Emerging evidence have demonstrated that lncRNA acts as "molecular sponge" for miRNAs by binding competitively to miRNAs in various diseases. For example, miR-1252 has been shown to play an important role in a variety of biological processes, including inflammation, autophagy, and tumorigenesis [20–22]. In gastric cancer and cervical cancer, miR-1252 was found to be remarkably increased [23,24]; whereas, in papillary thyroid cancer, pancreatic cancer and endometrial carcinoma, miR-1252 was reported to be significantly down-regulated [25–27]. Consistent with previous studies [28,29], our study revealed that miR-1252-5p was expressed at low levels in lung adenocarcinoma tumor tissue samples and cells. Moreover, our data also showed that LINC00943 functions as a sponge for miR-1252-5p to ameliorate lung adenocarcinoma development by promoting cell apoptosis and restraining LAD cell growth.

YWHAH belongs to a family of highly conserved proteins that bind to a variety of protein targets such as kinases, phosphatases,

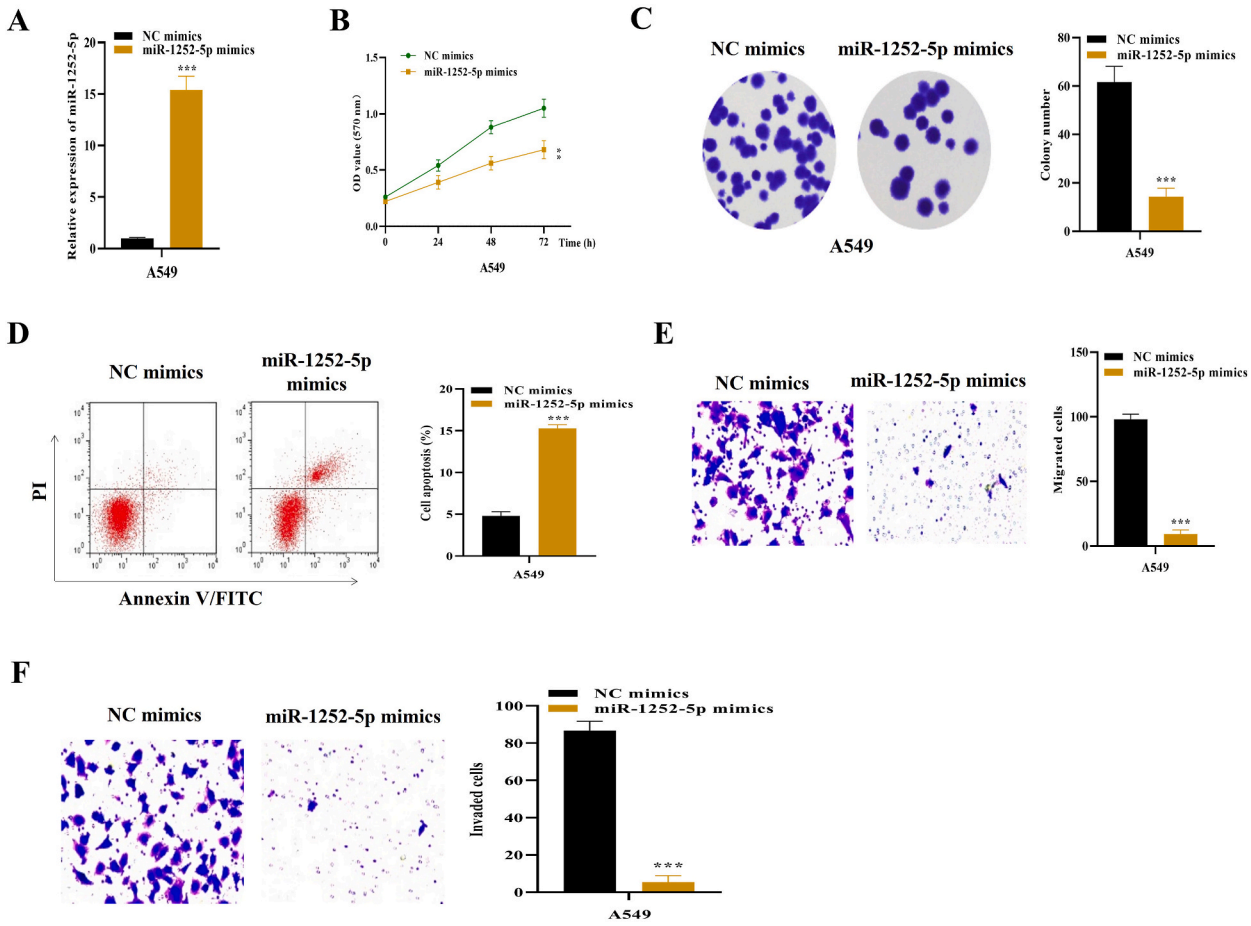

Fig. 5. miR-1252-5p overexpression inhibited lung adenocarcinoma cell malignant behaviors. (A) The transfection efficacy of miR-1252-5p was determined by RT-qPCR analysis. (B) MTT assay detected cell viability. (C) Colony formation analysis confirmed the cell proliferation rate. (D) Flow cytometry analysis verified cell apoptosis rate. (E) Cell migration and (F) Cell invasion was measured by Transwell assays. $^{**}P < 0.01$, $^{***}P < 0.001$.

transmembrane receptors, and transcription factors [30,31]. It was reported that YWHAH was involved in a large number of physiological processes including cell growth, metabolic regulation and signal transduction [32–34]. In cancer realm, YWHAH was discovered to function as an oncogene in thyroid cancer and papillary thyroid cancer [32,35]. However, in hepatocellular carcinoma, YWHAH was down-regulated and acted as a tumor-suppressor [36]. These studies suggest that YWHAH may act as tumor suppressor or promoter in various cancers. In our experiments, YWHAH was remarkably raised in lung adenocarcinoma specimens and cells, and mechanistically functioning as a target for miR-1252-5p to exacerbate tumorigenesis in lung adenocarcinoma. Nonetheless, the downstream signaling pathway of YWHAH in lung adenocarcinoma was not further examined in this study, which will be the subject of subsequent studies.

To the best of our knowledge, this is the first study to report that LINC00943 is up-regulated in lung adenocarcinoma cells and acts as an oncogene by promoting the proliferation, invasion, and migration of lung adenocarcinoma cells through the miR-1252-5p/YWHAH axis. Therefore, LINC00943 may be a potential target for lung adenocarcinoma therapy.

Ethics statement

All patients provided their written, voluntarily informed consent. All procedures were carried out in accordance with the guidelines outlined in the Helsinki Declaration and this study was approved by the Ethics Committee of Zhongda Hospital, School of Medicine, Southeast University, Nanjing, Jiangsu, China.

Consent for publication

Not applicable.

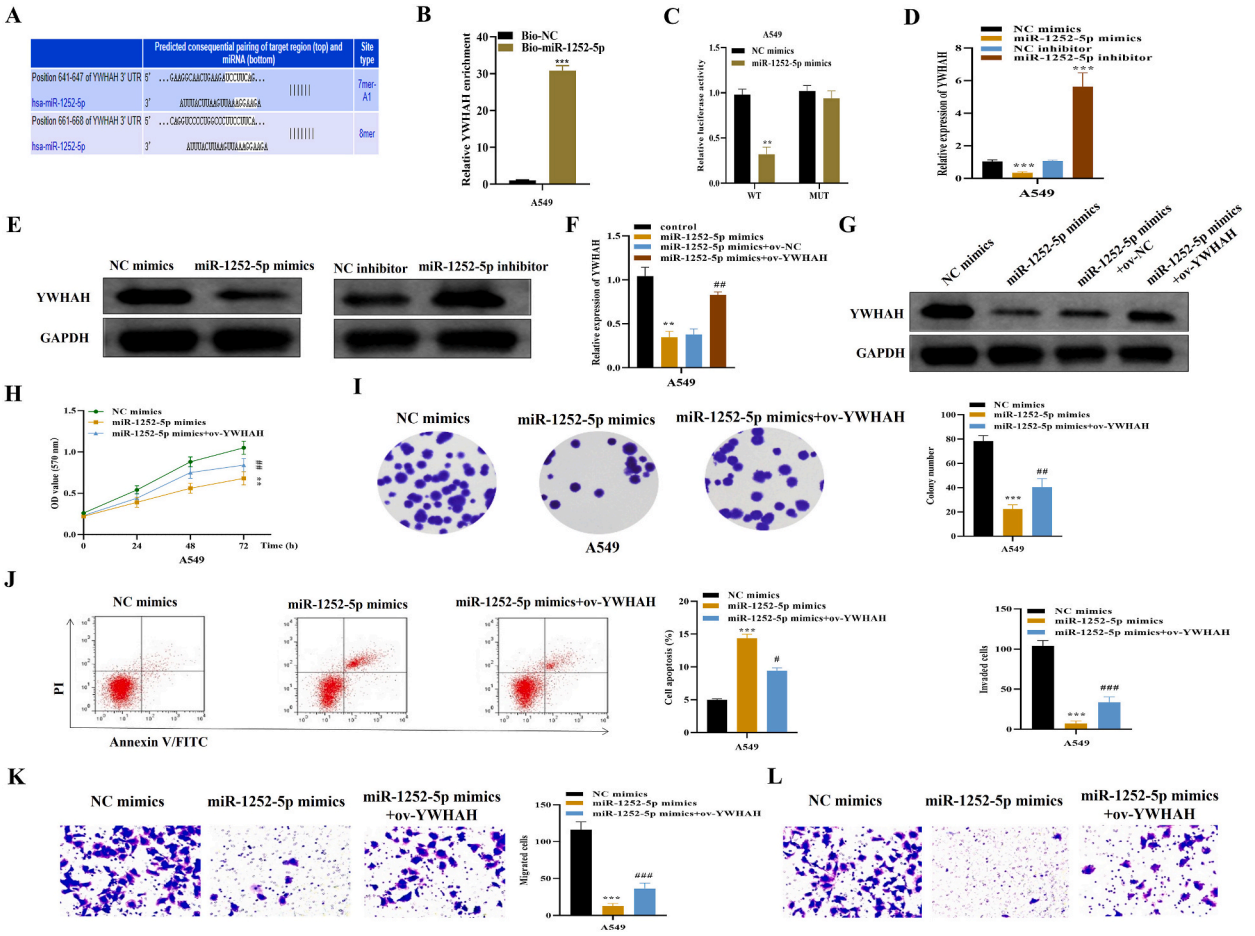

Fig. 6. miR-1252-5p directly targeted YWHAH to inhibit lung adenocarcinoma development. (A) TargetScan online tool predicted the complementary sequences between miR-1252-5p and YWHAH. (B) RNA pull-down assay verified the binding association between YWHAH and miR-1252-5p. (C) Dual-luciferase reporter assays were performed to examine the interaction between YWHAH and miR-1252-5p. (D) RT-qPCR analysis was used to detect the YWHAH expression in A549 cells after miR-1252-5p overexpression or inhibition. (E) Western blot analysis was used to detect the YWHAH expression in each group. (F) The effect of co-transfection of YWHAH and miR-1252-5p on YWHAH expression was determined by RT-qPCR analysis. (G) The effect of co-transfection of YWHAH and miR-1252-5p on YWHAH expression was determined by Western blot analysis. (H) MTT assay detected cell viability. (I) Colony formation analysis confirmed the cell proliferation rate. (J) Flow cytometry analysis was used to examine cell apoptosis. (K) Cell migration and (L) Cell invasion was measured by Transwell assays. $^{**}P < 0.01$, $^{***}P < 0.001$; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$.

Authors contributions

Bin Liu: Conceived and designed the experiments; Performed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Hongyan Li; Jianming Zhou; Lei Wang: Performed the experiments.

Jin Fang; Zhenye Pu: Analyzed and interpreted the data.

Tao Xue: Conceived and designed the experiments; Wrote the paper.

Data availability statement

Data will be made available on request.

Funding statement

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

List of abbreviations

LAD Lung adenocarcinoma
LncRNAs Long non-coding RNAs
LC Lung cancer
NSCLC Non-small cell lung cancer
LINC00943 Long intergenic non-protein coding RNA 943
miR-1252-5p MicroRNA 1252-5p
NC Negative control

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e16736.

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