

Long noncoding RNA LGALS8-AS1 promotes angiogenesis and brain metastases in non-small cell lung cancer

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Brain metastases (BM) are associated with poor prognosis in patients with non-small cell lung cancer (NSCLC). Considering that, LGALS8-AS1-mediated progression of BM was probed in NSCLC. The clinical characteristics of 60 NSCLC patients (30 without BM and 30 with BM) were analyzed. NSCLC patients with BM had higher levels of LGALS8-AS1 than NSCLC patients without BM. Depleting LGALS8-AS1 prevented NSCLC cell proliferation, migration, invasion, and angiogenesis *in vitro*, and NSCLC tumorigenesis and BM *in vivo*. LGALS8-AS1 targeted miR-885-3p to mediate Fascin actin-bundling protein 1 (FSCN1) expression. Restoring miR-885-3p inhibited NSCLC growth, angiogenesis, and BM, and FSCN1 induction rescued the performance of LGALS8-AS1 depletion on NSCLC cells. Our results provide new insights into LGALS8-AS1-mediated NSCLC metastasis and suggest that LGALS8-AS1 may be a useful biomarker for identifying NSCLC with metastatic potential.

Keywords: LGALS8-AS1, miR-885-3p, Fascin actin-bundling protein 1, non-small cell lung cancer, angiogenesis, brain metastases

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Abbreviations: BM, Brain metastases; CCK-8, Cell counting kit; FSCN1, Fascin actin-bundling protein 1; GAPDH, Glyceraldehyde-3-phosphate dehydrogenase; HUVECs, Human umbilical vein endothelial cells; lncRNAs, Long non-coding RNAs; miRNAs, MicroRNAs; MUT, Mutant type; NC, Negative control; NSCLC, Non-small cell lung cancer; RPMI, Roswell Park Memorial Institute; RT-qPCR, Real-time reverse transcriptase-polymerase chain reaction; 3'-UTR, 3' untranslated region; WT, Wild type

INTRODUCTION

Non-small cell lung cancer (NSCLC) is one of the deadliest malignancies, accounting for more than 80% of lung cancer cases worldwide, with a current 5-year survival rate of only 15% (Salehi *et al.*, 2020). Patients with advanced NSCLC generally die within 18 months of diagnosis mainly due to metastatic spread (Wood *et al.*, 2014). Brain metastases (BM) are a common complication of advanced NSCLC (Chen *et al.*, 2012), for which the management always involves systemic therapy (Fang *et al.*, 2021). Since angiogenesis is associated with aggressiveness in NSCLC (Schettino *et al.*, 2012), targeting inhibition of angiogenesis is promising in the treatment of NSCLC and many clinical trials have evaluated the addition of anti-angiogenic therapy to standard therapy in patients with NSCLC (Alshangiti *et al.*, 2018). Therefore, exploring the regulatory mechanism of angiogenesis is helpful for understanding the pathogenesis of NSCLC and developing new therapeutic drugs.

Long non-coding RNAs (lncRNAs) are of significance in the physiological and pathological processes of diseases (Xie *et al.*, 2018; Zhang *et al.*, 2019). The occurrence of human malignant tumors is often accompanied by the deregulation of lncRNA (Kondo *et al.*, 2017). More and more studies have shown that lncRNAs play a key role in tumorigenesis and metastasis (Hanniford *et al.*, 2020; Yang *et al.*, 2018; Gupta *et al.*, 2010). lncRNAs usually regulate their downstream target genes by competing endogenous RNAs with microRNAs (miRNAs), which can affect the proliferation and metastasis of various cancer types (Zheng *et al.*, 2019; Wang *et al.*, 2018; Hao *et al.*, 2019). For example, lncRNA GAN1 inhibits tumor progression in NSCLC *via* decoying miR-26a-5p; lncRNA LINC00473 promotes proliferation, migration, invasion, and inhibition of apoptosis of NSCLC cells by acting as a sponge of miR-497-5p (Xu *et al.*, 2021). LGALS8-AS1 has been confirmed to be highly expressed in breast cancer and promotes breast cancer metastasis by targeting miR-125b-5p (Zhai *et al.*, 2021). However, the expression and role of LGALS8-AS1 in NSCLC remain unclear.

MiRNAs are a group of short endogenous non-coding RNAs with a length of about 18-22 nucleotides, which can regulate post-transcriptional gene expression by binding to the 3'-UTR of target gene mRNA to inhibit mRNA translation and reduce mRNA stability (Ahn *et al.*, 2020). MiRNAs are involved in almost all biological processes, including tumor angiogenesis (Mao *et al.*, 2015). For example, miR-543 promotes tumorigenesis and angiogenesis in NSCLC (Wang *et al.*, 2020) whereas miR-20a-5p inhibits tumor angiogenesis (Han *et al.*, 2021). It is studied that miR-885-3p can architect cell autophagy and apoptosis in squamous cell carcinoma cells (Huang *et al.*, 2011). However, the biological function of miR-885-3p in NSCLC has not been fully elucidated.

Therefore, this study aims to explore the role of LGALS8-AS1 in NSCLC metastasis and angiogenesis through regulating miR-885-3p expression and its potential mechanism. The study identified a lncRNA, LGALS8-AS1, which is associated with NSCLC metastasis and angiogenesis and elucidated the molecular regulatory mechanism of LGALS8-AS1 in NSCLC and provides a new reference for the treatment of NSCLC patients.

METHODS

Tissue sampling

This study was approved by the ethics committee of Affiliated Brain Hospital of Nanjing Medical University. Written informed consent was obtained from all sub-

jects, 60 patients who were histologically confirmed with NSCLC. BM are confirmed by whole-brain CT scan or MRI. Tumor tissues and adjacent normal tissues were collected during surgery.

Cell culture

Human lung cancer cells (A549) and human umbilical vein endothelial cells (HUVECs) were purchased from the Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences. Under humidified air conditions of 37°C and 5% CO₂, cells were cultured in Roswell Park Memorial Institute (RPMI)-1640 (Gibco, USA) medium supplemented with 10% fetal bovine serum, 100 U/ml penicillin, and 100 mg/ml streptomycin (Invitrogen, USA).

Cell transfection

Both miR-885-3p mimic and mimic negative control (NC) were purchased from RiboBio (Guangzhou, China). The shRNA sequence targeting LGALS8-AS1 was purchased from Genepharma (Shanghai, China). Fascin actin-bundling protein 1 (FSCN1) was cloned into the pcDNA3.1 vector (Invitrogen). Cells were transiently transfected with RNAiMax and Lipofectamine 3000 with Plus reagent (Thermo Fisher Scientific).

Cell counting kit (CCK)-8

Cells growing on the 96-well plates were tested by a CCK-8 kit (Liji, Shanghai, China) to determine proliferative activity. Quantitative results were obtained on a microplate reader (SAFAS Xenius XL, Ruixuan, Shanghai, China) at 450 nm.

Flow cytometry

Cells were stained with annexin V-fluorescein isothiocyanate and propidium iodide according to the manufacturer's instructions (Bioscience, Shanghai, China). Then the apoptosis rate was detected by flow cytometry (Beckman, USA). The percentage of cells in the Q3 quadrant represents early apoptosis, and the percentage of cells in the Q2 quadrant represents late apoptosis (Wang *et al.*, 2020).

Transwell assay

Transwell chambers (8- μ m pore size; Corning Costar, Cambridge, MA, USA) measure cell migration and invasion capacity. For cell invasion, cells suspended in serum-free RPMI-1640 medium were seeded into the upper chamber pre-coated with Matrigel. The lower chamber was PMI-1640 medium with 20% serum as a chemotactic agent. After 24 h culture, the cells were fixed with 90% formaldehyde and stained with 0.1% crystal violet. The cells were photographed under a microscope and counted. In the cell migration experiment, there was no matrigel coating, and the other operations were the same as the invasion experiment (Yang *et al.*, 2018).

Tube formation

Matrigel (0.5mmol/L) was coated with pre-cooled 96-well plates. HUVECs were starved without serum for 1 h and then re-suspended in Dulbecco's modified Eagle medium to make a cell suspension. Next, cell suspensions (1 \times 10⁵ cells/mL) were inoculated into a matrigel coating containing cell conditioned culture-medium with 3 repeat wells per treatment. The plates were then incubated at 37°C for 6 to 8 h. The tube formation was

observed under a microscope (Olympus). The number of tubes in a branch (a branch point is a skeleton part where three or more tubes meet) and the number of rings (a ring is a background area surrounded by [or almost] tubular structures) were counted.

Real-time reverse transcriptase-polymerase chain reaction (RT-qPCR)

Total RNA was extracted using a Trizol reagent according to the manufacturer's instructions (Invitrogen, Carlsbad, CA, USA). RNA purity was determined using a NanoDrop spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). cDNA reverse transcription kit (Promega, Madison, WI, USA) was used to reverse transcribed 1 μ g total RNA into the first strand cDNA. Power SYBR Green PCR Master Mix (Promega) quantitative PCR was used to detect RNA levels. LncRNA and protein-coding gene were normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA, and miRNA was normalized to U6. The primer sequences used are presented in Table 1.

Western blot

Total protein lysates were subjected to 10% or 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis, loading on a polyvinylidene fluoride membrane (Millipore, USA), and blocking the membrane with 5% non-fat milk. After that, primary antibodies FSCN1 (1:1000, sc-21743, Santa Cruz Biotechnology) and GAPDH (1:1000, ab8245, Abcam) were supplemented, and Immunoreactive bands were visualized after incubation with secondary antibody (Invitrogen) by enhanced chemiluminescent detection system (Thermo Fischer Scientific).

Luciferase reporter gene assay

pGL3 luciferase reporters for LGALS8-AS1 and FSCN1 were designed by Genomeditech (Shanghai, China), named LGALS8-AS1-wild type (WT), LGALS8-AS1-mutant type (MUT), FSCN1-WT, and FSCN1-MUT. These reporters and miR-885-3p mimic or mimic NC were co-transfected into A549 cells, thus measuring luciferase activity with a luciferase assay kit (Promega) (Wu *et al.*, 2021).

Table 1. Primer sequences

Genes	Primer sequences (5'- 3')
LGALS8-AS1	F: ACATCCGAATGCCATCCTCC
	R: AGGACTGACTCCTGTCCGCTT
miR-885-3p	F: AGGCAGCGGGTGTAGTGGATAG
	R: CCAAGTGCAGGGTCCGAGGTATTC
FSCN1	F: ACAGCAGGGGACTCAG
	R: CCCACCGTCCAGTATTT
U6	F: CTCGCTTCGGCAGCACCA
	R: AACGCTTCACGAATTTGCGT
GAPDH	F: CACCACTCCTCCACCTTTG
	R: CCACCACCTGTTGCTGTAG

Note: LGALS8-AS1, long noncoding RNA LGALS8-AS1; miR-885-3p, microRNA-885-3p; FSCN1, Fascin actin-bundling protein 1; GAPDH, glyceraldehyde 3-phosphate dehydrogenase

Table 2. Analysis of clinical characteristics of NSCLC patients with and without BM

Characteristics	NSCLC with BM	NSCLC without BM
	(n=30)	(n=30)
Age (years), median (range)	56 (50-63)	59 (55-66)
Gender		
Male	12 (40.0%)	23 (76.7%)
Female	18 (60.0%)	7 (23.3%)
Tumor histology		
Adenocarcinoma	26 (86.7%)	18 (60.0%)
Squamous cell carcinoma	4 (13.3%)	6 (20.0%)
Carcinosarcoma	–	2 (6.7%)
Large cell carcinoma	–	1 (3.3%)
Neuroendocrine carcinoma	–	3 (10.0%)
Disease stage at diagnose		
I	–	3 (10.0%)
II	–	6 (20.0%)
III	–	7 (23.3%)
IV	30 (100.0%)	14 (46.7%)
Smoking history		
Smoker	8 (26.7%)	5 (16.7%)
Former smoker	4 (13.3%)	10 (33.3%)
Never	18 (60.0%)	15 (50.0%)
Lymph node metastatic status		
N0	2 (6.7%)	4 (13.3%)
N1	4 (13.3%)	3 (10.0%)
N2	9 (30.0%)	9 (30.0%)
N3	15 (50.0%)	14 (46.7%)

Xenograft models in nude mice

For xenograft models, 4-week-old BALB/c nude mice (Cyagen Biosciences) bred under pathogen-free conditions were subcutaneously injected with A549 cells stably transfected with sh-LGALS8-AS1 and sh-NC (5×10^6 , $n=6$ /group). A549 cells were pre-diluted in 200 μ L phosphate-buffered saline (PBS) + 200 μ L Matrigel (BD Biosciences). During 28-h housing, mice were measured for longitudinal diameter and lateral diameter at an interval of 7 days to calculate tumor volume as $0.5 \times L \times D^2$. On day 28, xenograft tumors were dissected from mice and weighed.

A lung cancer BM model was established according to previous studies (Li *et al.*, 2017; Nguyen *et al.*, 2009). A549 cells after transfection were resuspended in 100 μ L of PBS and injected into the right ventricle of mice (1×10^6 , $n=6$ /group). Finally, brain tissues were collected and prepared for HE staining and observations of metastatic nodules.

Statistical analysis

The selected way to perform statistical analysis was SPSS 19.0, and that to construct graphs was GraphPad Prism 6. Two-tailed paired Student's *t*-test and one-way analysis of variance were of utility for data comparison of two groups and more than two groups, respectively. Tukey's post hoc test validated pairwise comparisons.

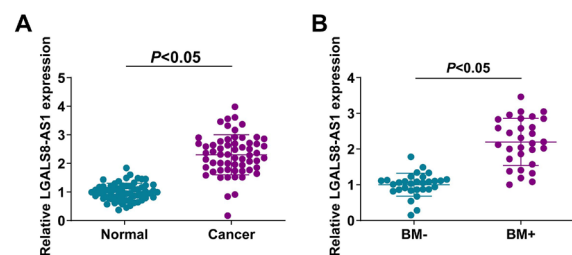


Figure 1. LGALS8-AS1 is more expressed in NSCLC patients with BM

LGALS8-AS1 expression in clinical samples (A–B); the values were expressed as mean \pm standard deviation ($n=60$).

Pearson correlation analysis assessed gene correlation. $P < 0.05$ was considered to indicate a statistically significant difference.

RESULTS

LGALS8-AS1 is more expressed in NSCLC patients with BM

A total of 60 patients with NSCLC were included in the study, of which 30 had BM and the remaining 30 did not. The clinical characteristics are shown in Table 2. Examinations of LGALS8-AS1 found that LGALS8-AS1

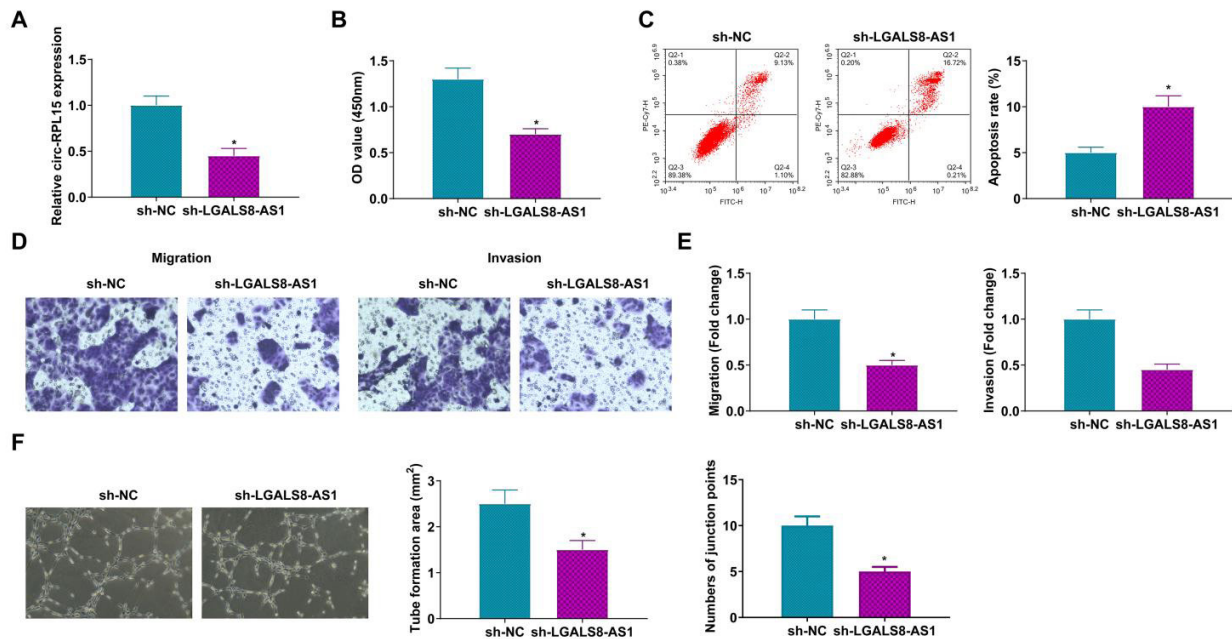


Figure 2. Targeting suppression of LGALS8-AS1 blocks NSCLC development

Based on sh-LGALS8-AS1-induced knockdown of LGALS8-AS1 (A), assays were to determine proliferation (B), apoptosis (C), migration (D), invasion (E), and angiogenesis (F); values are presented as mean \pm standard deviation (N=3) * P <0.05 vs. sh-NC.

was increased in tumor tissues, and it was higher in tumor tissues of NSCLC patients with BM (Fig. 1A, B).

Targeting suppression of LGALS8-AS1 blocks NSCLC development

Firstly, the effect of LGALS8-AS1 on the progression of NSCLC cells was investigated. A549 cells were transfected with sh-LGALS8-AS1 to silence the expression of LGALS8-AS1, and the transfection was verified by RT-qPCR (Fig. 2A). Cell proliferation was then detected by CCK-8, and it was found that A549 cell proliferation decreased after down-regulating LGALS8-AS1 (Fig. 2B). Apoptosis was detected by flow cytometry, and the results showed that apoptosis of A549 cells increased after downregulation of LGALS8-AS1 (Fig. 2C). Transwell was used to detect cell migration and invasion,

and it was found that A549 cell migration and invasion decreased after downregulating LGALS8-AS1 (Fig. 2D, E). Angiogenesis was also examined by tube formation assays, which showed that angiogenesis was attenuated after down-regulating LGALS8-AS1 (Fig. 2F). These results indicate that down-regulation of LGALS8-AS1 inhibits proliferation, migration, invasion, and angiogenesis of NSCLC cells, and promotes cell apoptosis.

LGALS8-AS1 deficiency suppresses tumor growth and BM *in vivo*

Based on our findings that LGALS8-AS1 is involved in NSCLC cell progression, the study further explored its role in tumor growth and brain metastasis *in vivo*. Tumor growth was studied by subcutaneous injection of A549 cells stably transfected with sh-LGALS8-AS1 into

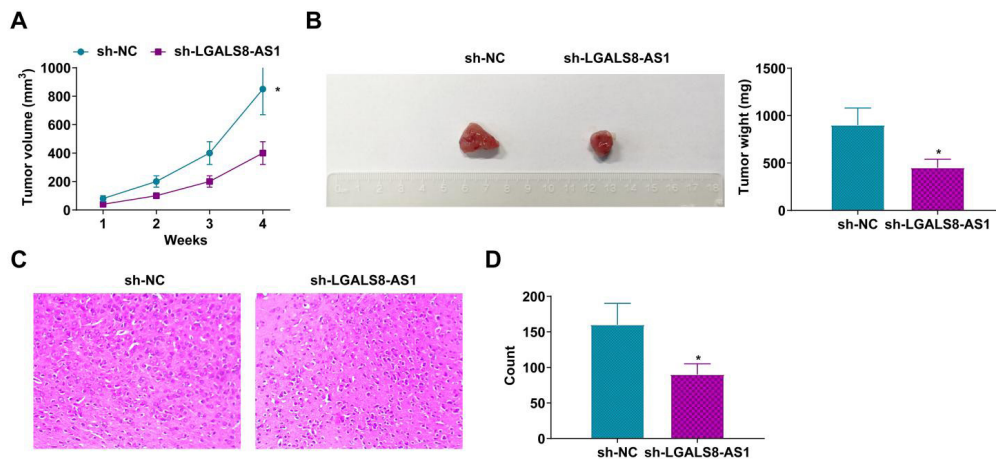


Figure 3. LGALS8-AS1 deficiency suppresses tumor growth and BM *in vivo*

In nude mice injected with sh-LGALS8-AS1, tumor volume and weight were measured (A–B) and BM was evaluated (C–D); values are presented as mean \pm standard deviation (n=6) * P <0.05 vs. sh-NC.

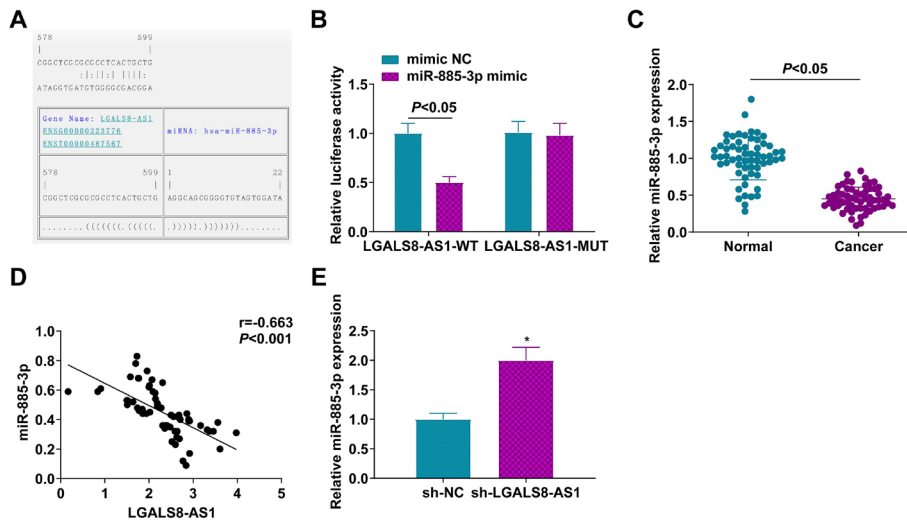


Figure 4. miR-885-3p expression is controlled by LGALS8-AS1

A potential binding relationship between LGALS8-AS1 and miR-885-3p was predicted (A) and confirmed by detecting luciferase activity (B) miR-885-3p expression in clinical samples (C) and its correlation with LGALS8-AS1 expression (D) miR-885-3p expression was altered by sh-LGALS8-AS1 in A549 cells (E); values are presented as mean \pm standard deviation (N=3) * P <0.05 vs. sh-NC.

nude mice. As measured, LGALS8-AS1 down-regulation reduced tumor volume and weight (Fig. 3A, B). BM were explored by injecting A549 cells stably transfected with sh-LGALS8-AS1 into the right ventricle of nude mice. As presented in HE staining results, the number of brain metastatic nodules was reduced in sh-LGALS8-AS1-treated mice (Fig. 3C, D).

miR-885-3p expression is controlled by LGALS8-AS1

Next, LGALS8-AS1 was predicted to have binding sites with miR-885-3p through the RNA22 database (Fig. 4A). Subsequently, dual luciferase assay was performed to verify their targeting relationship, and the results showed that the luciferase activity of A549 cells could be significantly reduced by co-transfection of LGALS8-AS1-WT with miR-885-3p mimic (Fig. 4B). In addition, the expression of miR-885-3p in clinical samples was detected, and it was found that miR-885-3p in lung cancer tissues was down-regulated (Fig. 4C) and negatively correlated with LGALS8-AS1 expression (Fig. 4D). Moreover, after downregulating LGALS8-AS1, it was found that miR-885-3p expression was increased in A549 cells (Fig. 4E). The results showed that LGALS8-AS1 inhibited miR-885-3p expression by targeting miR-885-3p.

miR-885-3p represses NSCLC development *in vitro*

In order to investigate the effect of miR-885-3p on NSCLC cells, miR-885-3p mimic or mimic NC was transfected into A549 cells, and successful transfection was verified by RT-qPCR (Fig. 5A). In A549 cells over-expressing miR-885-3p, it could be recognized that proliferative, invasive, migratory, and an-apoptotic activities were all in a weakened status, and the same was true for angiogenic capacity (Fig. 5B–F). These results suggest that up-regulation of miR-885-3p inhibits proliferation, migration, invasion, and angiogenesis of NSCLC cells, and promotes cell apoptosis.

miR-885-3p targets FSCN1

Subsequently, the RNA22 database predicted that miR-885-3p and FSCN1 had binding sites (Fig. 6A).

Then, dual luciferase assay was performed to verify the targeting relationship between them. The results showed that the luciferase activity of A549 cells could be significantly reduced by co-transfection of FSCN1-WT with miR-885-3p mimic (Fig. 6B). RT-qPCR detected FSCN1 mRNA expression in lung cancer tissues, and the results showed that FSCN1 mRNA expression was up-regulated (Fig. 6C) and was negatively correlated with the expression of miR-885-3p (Fig. 6D). Moreover, mRNA and protein expression of FSCN1 in A549 cells decreased after up-regulation of miR-885-3p (Fig. 6E). These results suggest that targeting miR-885-3p regulates FSCN1 expression.

LGALS8-AS1-mediated impairments of A549 cell activities can be rescued by FSCN1

To verify the regulatory role of LGALS8-AS1/miR-885-3p/FSCN1 axis in lung cancer cells, sh-LGALS8-AS1 + pcDNA3.1-FSCN1 and sh-LGALS8-AS1 + pcDNA3.1-NC were transfected into A549 cells. RT-qPCR and Western blot results showed that pcDNA3.1-FSCN1 reversed the inhibition effect of sh-LGALS8-AS1 on FSCN1 expression (Fig. 7A). The results of CCK-8, flow cytometry, Transwell and tube formation experiments showed that up-regulation of FSCN1 mitigated the impact of down-regulation of LGALS8-AS1 on proliferation, apoptosis, migration, invasion, and angiogenesis of A549 cells (Fig. 7B–F). In conclusion, LGALS8-AS1 promotes NSCLC cell progression by regulating the miR-885-3p/FSCN1 axis.

DISCUSSION

As studies indicate, 90% of lung cancer deaths are caused by distant metastasis, and BM is a common site of distant metastasis in NSCLC (Rybarczyk-Kasiuchnicz *et al.*, 2021). LncRNA dysregulation is fundamental for tumorigenesis and distant metastasis of NSCLC and serves as a biomarker for NSCLC (Pan *et al.*, 2020). In the present study, LGALS8-AS1 was up-regulated in NSCLC patients' tumor tissues and was more expressed in NSCLC patients with BM. Silencing LGALS8-AS1

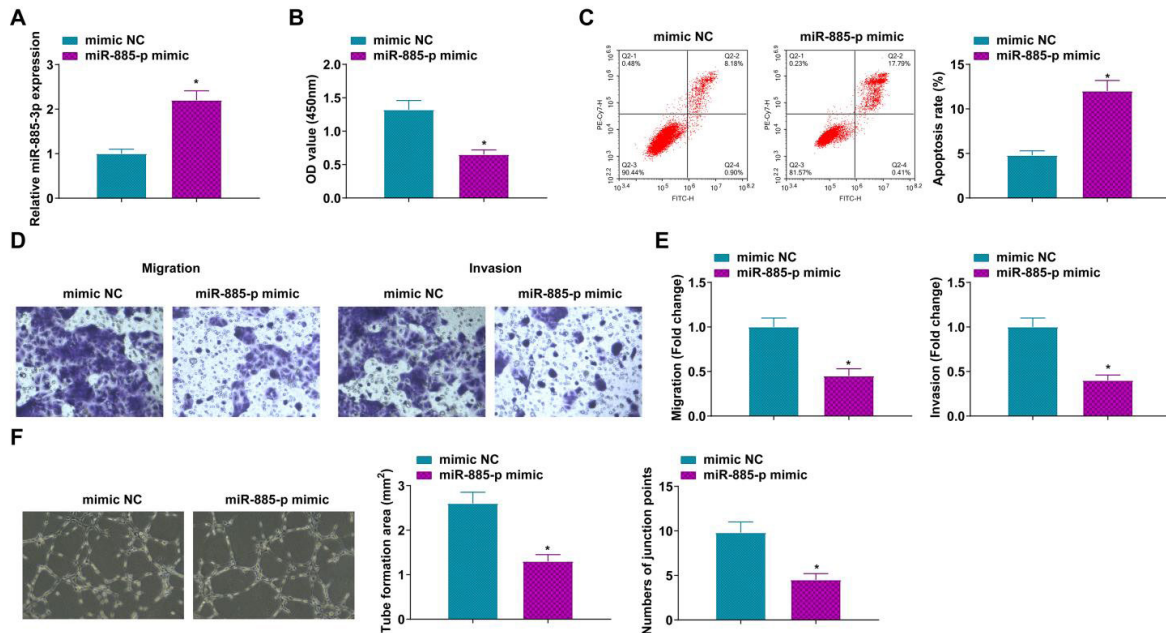


Figure 5. miR-885-3p represses NSCLC development *in vitro*.

Based on miR-885-3p mimic-induced elevation of miR-885-3p expression (A), assays were to determine proliferation (B), apoptosis (C), migration (D), invasion (E), and angiogenesis (F); values are presented as mean \pm standard deviation (N=3) * P <0.05 vs. mimic NC.

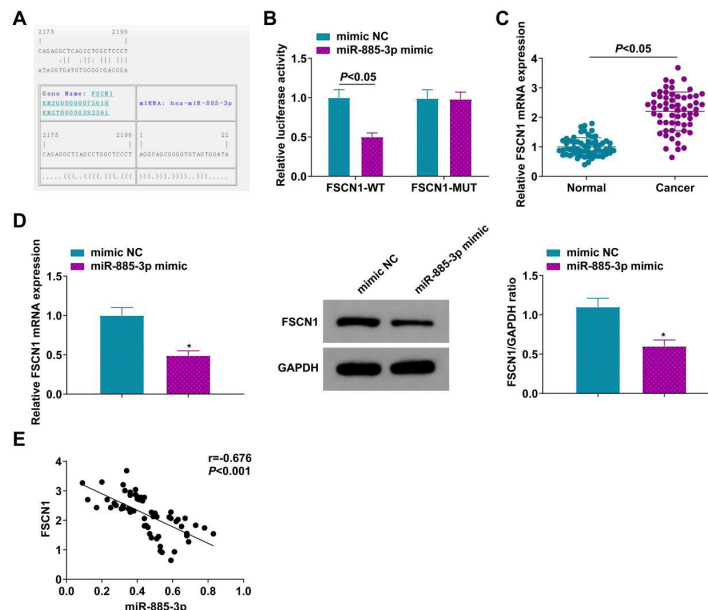


Figure 6. miR-885-3p targets FSCN1.

A potential binding relationship between FSCN1 mRNA and miR-885-3p was predicted (A) and confirmed by detecting luciferase activity (B); FSCN1 mRNA expression in clinical samples (C) and its correlation with miR-885-3p expression (D) FSCN1 mRNA and protein expression was altered by miR-885-3p mimic in A549 cells (E); values are presented as mean \pm standard deviation (N=3) * P <0.05 vs. mimic NC.

blocked the malignant phenotype and angiogenesis in NSCLC cells, as well as inhibiting tumor growth and BM *in vivo*. Overall, LGALS8-AS1 plays a critical role in NSCLC metastasis and serves as a potential therapeutic target for NSCLC metastasis.

Abnormally expressed lncRNAs are involved in regulating NSCLC development by acting as miRNA sponges. For example, lncRNA plasmacytoma variant translocation 1 promotes angiogenesis in NSCLC by competitive absorption of miR-29c (Wang *et al.*, 2018). lncRNA

DNAH17 antisense RNA 1 induces the occurrence and metastasis of NSCLC by binding to miR-877-5p (Du *et al.*, 2020). LGALS8-AS1 has only been reported in breast cancer considered an oncogenic gene regarding its potential to induce malignant phenotype and metastasis. Here, the study observed high LGALS8-AS1 expression in NSCLC, and LGALS8-AS1 expression was able to discriminate whether NSCLC patients developed BM. Cell and animal experiments consistently confirmed that LGALS8-AS1 knockdown played a negative role in

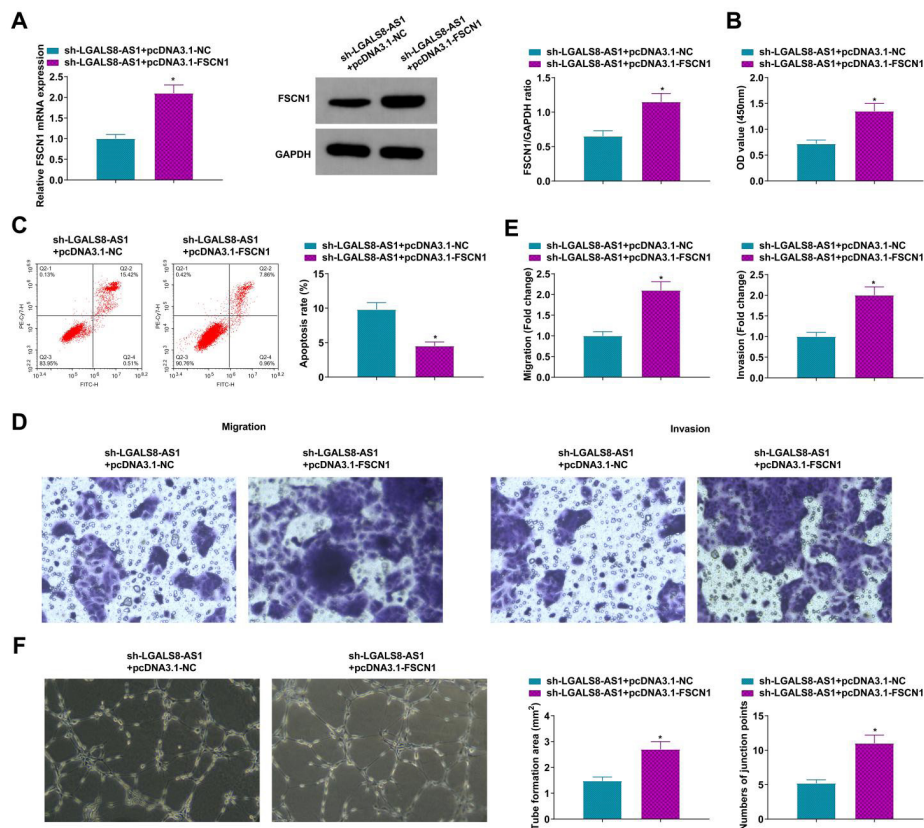


Figure 7. LGALS8-AS1-mediated impairments of A549 cell activities can be rescued by FSCN1.

Based on pcDNA3.1-FSCN1-induced elevation of FSCN1 expression in sh-LGALS8-AS1-treated A549 cells (A), assays were to determine proliferation (B), apoptosis (C), migration (D), invasion (E), and angiogenesis (F); values are presented as mean \pm standard deviation (N=3) * P <0.05 vs. sh-LGALS8-AS1 + pcDNA3.1-NC.

NSCLC for cell growth, angiogenesis, and tumor growth and metastasis. In addition, the RNA22 database predicted that LGALS8-AS1 had a binding site with miR-885-3p.

miR-885-3p was mediated by LGALS8-AS1 in this study. miR-885-3p has received academic attention in the field of cancer. miR-885-3p expression decline has previously been found in lung adenocarcinoma to be associated with pathological stage and poor survival in patients (Yang *et al.*, 2021). Functionally discussed, miR-885-3p impairs gastric cancer cell activities and oxidative stress (He *et al.*, 2021), and tumor angiogenesis in colon cancer (Xiao *et al.*, 2015). This study confirmed the reduction in miR-885-3p expression in NSCLC, and the suppressive effects of miR-885-3p on NSCLC cell growth and angiogenesis.

miRNAs usually function by binding to target gene mRNAs (Datta *et al.*, 2019). Here, FSCN1 was selected to be a target of miR-885-3p. FSCN1 is a structurally unique and highly conserved actin cross-linking protein that mediates cellular interaction (Liu *et al.*, 2019). It has been described that dysregulation of FSCN1 aggrandizes tumor motility and invasiveness by altering the structure of cell protrusions and focal extracellular matrix adhesions (Gao *et al.*, 2019). Therefore, FSCN1 is considered an oncogene in cancers, including adrenocortical carcinoma (Liang *et al.*, 2019), ovarian cancer (Li *et al.*, 2018), laryngeal squamous cell carcinoma (Gao *et al.*, 2018), and NSCLC (Xiao *et al.*, 2016). Here, FSCN1 was overexpressed in NSCLC and can mitigate the effects of LGALS8-AS1 knockdown, promoting NSCLC cell growth and angiogenesis.

This study did not address a possible direct relationship between LGALS8-AS1 expression and clinical factors, including tumor node metastasis staging and survival analysis, due to insufficient clinical sample size and lack of a long-term follow-up of all included patients. Also, this study did not further explore the effects of overexpression of LGALS8-AS1 and downregulation of miR-885-3p alone on NSCLC cells. In addition, FSCN1 has been confirmed to mediate NSCLC cell migration and invasion by altering the Mitogen-activated protein kinase pathway (Zhao *et al.*, 2018). Whether the MAPK pathway or other signaling pathways are involved in the regulation of LGALS8-AS1 in NSCLC still needs to be further explored to determine detailed and comprehensive mechanics.

CONCLUSION

Our study provides new insights into the mechanism by which lncRNAs regulate NSCLC progression. Our findings suggest that the LGALS8-AS1/miR-885-3p/FSCN1 axis is an essential signaling pathway for NSCLC cell growth, angiogenesis, and BM.

Declarations

Acknowledgments. Not applicable.

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Declaration of Conflicting Interests. Authors declared no conflict of interest.

Ethical statement. All procedures performed in this study involving human participants were in accordance

with the ethical standards of the Affiliated Brain Hospital of Nanjing Medical University research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. All subjects were approved by the Affiliated Brain Hospital of Nanjing Medical University.

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