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ORIGINAL RESEARCH

Circular RNA SMARCA5 inhibits the proliferation, migration, and invasion of non-small cell lung cancer by miR-19b-3p/HOXA9 axis

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Background: Non-small cell lung cancer (NSCLC) is the major type of lung cancer, remaining a leading cause of cancer-related mortality around the world. Circular RNA SMARCA5 (circSMARCA5) is a novel circular RNA associated with the pathogenesis of several cancers. However, the role of circSMARCA5 [illegible] NSCLC remains unknown. In the present study, we aimed to evaluate the functions of circSMARCA5 in NSCLC and the underlying mechanism.

Methods: The expression pattern of circSMARCA5 was determined using qRT-PCR in NSCLC samples and cell lines. The correlation between miR-19b-3p and circSMARCA5 in NSCLC tissues was detected by qRT-PCR. Cell proliferation was examined utilizing CCK-8 assay. Cell migration and invasion was evaluated using Transwell assay. We used the bioinformatics software TargetScan and miRanda to predict circRNA-miRNA and miRNAi-mRNA interactions. Further, the regulatory role of circSMARCA5 in the malignant development of NSCLC in vivo was examined.

Results: The results showed that circSMARCA5 was down-regulated in NSCLC tissues as compared to the adjacent normal tissues. Overexpression of circSMARCA5 in NSCLC cell lines significantly inhibited the proliferation, migration, and invasion. Furthermore, circSMARCA5 exerted its tumor-suppressive activity through acting as a sponge for microRNA (miR)-19b-3p. Suppression of miR-19b-3p exhibited inhibitory effects on proliferation, migration, and invasion of NSCLC cell lines, which could be attributed to the regulation of homeobox A9 expression. Finally, overexpression of circSMARCA5 inhibited tumor growth in vivo.

Conclusion: Collectively, circSMARCA5 executed its inhibitory effects on NSCLC cell lines through miR-19b-3p/HOXA9 axis. The results indicated that circSMARCA5 might be a therapeutic target for the treatment of NSCLC.

Keywords: non-small cell lung cancer, NSCLC, circSMARCA5, miR-19b-3p, HOXA9, tumor suppressor

Introduction

Lung cancer is one of the leading causes of cancer-related mortality around the world.[1] There are two main types of lung cancer are small cell lung cancer and non-small cell lung cancer (NSCLC). NSCLC accounts for approximately 85% of all lung cancer cases.[2] Despite advances in clinical and experimental oncology have been made in recent years, the 5-year overall survival (OS) rate of NSCLC is still limited. That is because many NSCLC patients are diagnosed at advanced stages

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due to the insensitivity of diagnostic techniques.[2,3] Besides, NSCLC patients diagnosed at early-stage always suffer from tumor metastasis, even after surgical resection.[2] Consequently, better understanding of molecular mechanisms underlying the development and progression of NSCLC is essential for making early diagnosis and achieving improvements in NSCLC treatments.

Circular RNAs (circRNAs) are a newly identified class of endogenous RNAs extensively in mammalian cells that contain a covalently closed continuous loop without 5′ caps and 3′ tails.[4] Previously, circRNAs have been thought to be incorrect products resulted from splicing errors with no function. However, a large number of circRNAs have been discovered because of the rapid development of high-throughput sequencing.[5,6] In recent years, several properties of circRNAs have been uncovered. They function as miRNA sponges, regulators of transcription, and RNA-binding protein sponges. In addition, few circRNAs have been observed to be translated into proteins/peptides.[7]

Emerging evidence suggests that circRNAs have the ability to facilitate or inhibit the development and progression of various tumors, including NSCLC.[8–10] Circular RNA SMARCA5(CircSMARCA5) is a novel circRNA that has been demonstrated to be implicated in several types of cancers, such as prostate cancer,[11] gastric cancer,[12] cervical cancer,[13] glioblastoma multiforme,[14] and hepatocellular carcinoma.[15] However, the role of circSMARCA5 in NSCLC has not been elucidated. In the present study, we investigated the functions of circSMARCA5 in NSCLC and explored the underlying mechanism.

Materials and methods

Tissue collection

The clinical specimens including 45 pairs of NSCLC tumor tissues and adjacent normal tissues from patients who were diagnosed as NSCLC were collected from the patients underwent surgery from May 2016 to June 2017 at the Department of Oncology, Huaihe Hospital of Henan University (Kaifeng, China). The patients have signed the informed consents before the study. The study was approved by the ethics committee of Huaihe Hospital of Henan University. Clinical parameters of NSCLC patients enrolled in this study are shown in Table 1.

Table 1 Clinical parameters of NSCLC patients enrolled in this study

Variables	**Number**
All cases	45
Age	
≥60	29
<60	16
Gender	
Male	36
Female	9
Tumor size (cm)	
≤3	14
>3	31
Lymph nodes metastasis	
Positive	27
Negative	18
TNM stage	
I	16
II	21
III	8

Abbreviation: NSCLC, non-small cell lung cancer.

Cell culture and transfection

Normal human airway epithelial cell line 16HBE and NSCLC cell lines (A549, H1299, H1975, and H358) were purchased from Cell Bank of Chinese Academy of Sciences (Shanghai, China). The cells were cultured in RPMI-1640 medium (Hyclone, Logan, UT, USA) containing 10% fetal bovine serum (FBS, Gibco Laboratories, Grand Island, NY, USA) and incubated under an atmosphere containing 5% CO_2 at 37 °C.

The packaged lentivirus-containing circSMARCA5-overexpressing vector (LV-circSMARCA5) or empty vector (LV-NC), homeobox A9 (HOXA9)-overexpressing plasmid (pcDNA3.1-HOXA9) or control plasmid (pcDNA3.1) was purchased from GeneChem (Shanghai, China). The miR-19b-3p mimics, miR-NC, miR-19b-3p inhibitor, and control inhibitor were obtained from GenePharma (Shanghai, China). The transfections were performed using Lipofectamine 3000 (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. The A549 cells were infected with LV-circSMARCA5 or LV-NC, and the stable cells were selected.

Cell proliferation assay

Cell proliferation was measured using the cell counting kit-8 assay (CCK-8; Dojindo, Kumamoto, Japan). Cells were seeded into 96-well plates at a density of 2×10^3 cells/well and incubated for 0, 24, 48, or 72 hrs. Then, CCK-8 solution was added to each well and incubated for

4 hrs. After that, the absorbance at 450 nm was measured on a microplate reader (Bio-Tek, Winooski, VT, USA).

Cell migration and invasion assays

Migrative and invasive abilities were evaluated using transwell assay with transwell chambers (Corning, NY, USA). A549 cells with different transfections were, respectively, seeded into the upper chambers with serum-free medium. The chambers used in the invasion assay were coated with matrigel. The lower chambers were loaded with normal medium. After incubation for 24 hrs, the cells moved to the lower surfaces of the membranes were fixed with methanol, followed by staining with crystal violet. The cell number on five fields was counted under a light microscope.

Quantitative real-time PCR (qRT-PCR)

Total RNA of tissues and cells was extracted by Trizol reagent (Invitrogen) following the manufacturer's directions. The cDNA was synthesized with the PrimeScript RT reagent Kit (Takara Bio, Shiga, Japan). And the PCR amplification was conducted using the TB Green Premix Ex Taq II (Takara). For the determination of miR-19b-3p expression, total RNA was isolated using a High Pure miRNA isolation kit (Roche, Mannheim, Germany) and qRT-PCR was performed using a TaqMan MicroRNA Reverse Transcription kit (Life Technologies, Grand Island, NY, USA). The relative gene expressions were calculated by $2^{-\Delta\Delta Ct}$ method relative to β-actin or U6.

Luciferase reporter assay

The luciferase plasmids including pGL3-circSMARCA5-WT, pGL3-circSMARCA5-Mut, pGL3-HOXA9-WT, and pGL3-HOXA9-Mut were co-transfected with miR-19b-3p mimics or control mimics into A549 cells. At 48 hrs after transfection, the luciferase activity in each group was detected by using Dual-Luciferase Assay System (Promega, Madison, WI, USA).

Mouse xenograft model

The animal experiments were approved by the Animal Ethics Committee of Huaihe Hospital of Henan University. All protocols were conducted according to the institutional guidelines. Four-week-old female nude mice were purchased from Shanghai SIPPR-BK Laboratory Animal Co. Ltd. (Shanghai, China). The mice were subcutaneously inoculated with A549 cells (5×10^7 cells) infected with LV-circSMARCA5 or LV-NC. The tumor sizes were measured every 7 days. After 4 weeks, the mice were sacrificed, and the tumors were separated and weighed. The tumor volume was calculated using the formula: length × $\text{width}^2/2$.

Statistical analysis

All data analyses were conducted using SPSS 22.0 (SPSS Inc., Chicago, IL, USA). Student's *t*-test (two groups) or one-way ANOVA (more than two groups) was performed to compare the differences. *p*-values lower than 0.05 were considered statistically significant.

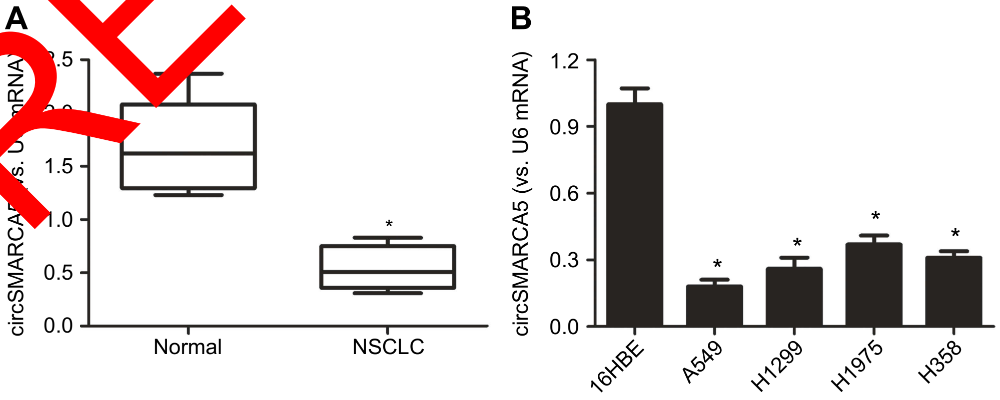

Figure 1 Downregulation of circSMARCA5 in NSCLC tissues and cell lines.

Notes: (**A**) Expression levels of circSMARCA5 in 45 pairs of NSCLC tumor tissues and adjacent normal tissues. (**B**) Expression levels of circSMARCA5 in human airway epithelial cell line 16HBE and four NSCLC cell lines including A549, H1299, H1975, and H358 cells. *p<0.05.

Abbreviations: circSMARCA5, circular RNA SMARCA5; NSCLC, non-small cell lung cancer.

Results

CircSMARCA5 was lowly expressed in NSCLC tissues and cell lines

To identify the importance of circSMARCA5 in NSCLC, we first analyzed the expression levels of circSMARCA5 in 45 pairs of NSCLC tumor tissues and adjacent normal tissues. As shown in Figure 1A, the circSMARCA5 was markedly down-regulated in NSCLC tissues as compared to the normal tissues. Next, the circSMARCA5 expressions in cultured cell lines were also detected. Compared with the human airway epithelial cell line 16HBE, the circSMARCA5 expressions were significantly decreased in NSCLC cell lines including A549, H1299, H1975, and H358 cells (Figure 1B).

Overexpression of circSMARCA5 inhibited the proliferation, migration, and invasion of NSCLC cells

To further investigate the role of circSMARCA5 in NSCLC, A549 cells were infected with LV-circSMARCA5 or LV-NC. As shown in Figure 2A, the circSMARCA5 expression level was dramatically increased in LV-circSMARCA5-infected A549 cells. Through CCK-8 assay, we found that circSMARCA5 overexpression significantly inhibited the cell proliferation of A549 cells (Figure 2B). Transwell assay illustrated that cell migration and invasion of A549 cells were also reduced by circSMARCA5 overexpression (Figure 2C and D).

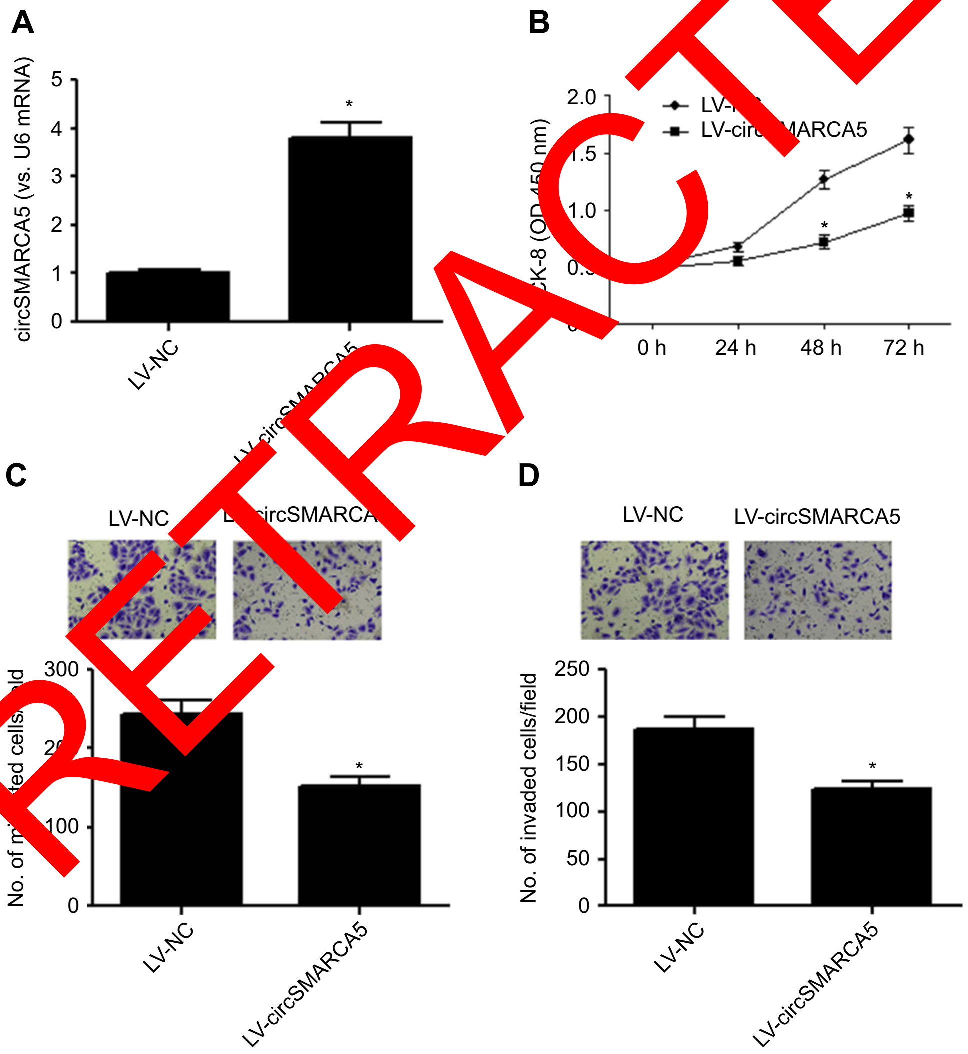

Figure 2 CircSMARCA5 overexpression inhibited the proliferation, migration, and invasion in NSCLC cells.
Notes: (**A**) CircSMARCA5 expression levels after infection with LV-circSMARCA5 or LV-NC. (**B**) CCK-8 assay was performed to evaluate cell proliferation. (**C** and **D**) Transwell assay was carried out to examine cell migration and invasion. *$p<0.05$.
Abbreviations: circSMARCA5, circular RNA SMARCA5; NSCLC, non-small cell lung cancer.

CircSMARCA5 exerted its tumor-suppressive activity via sponging miR-19b-3p in NSCLC cell lines

According to the predicted results, miR-19b-3p might be target miRNA of circSMARCA5 (Figure 3A). Luciferase reporter assay proved that luciferase activity was significantly reduced in the cells co-transfected with pGL3-circSMARCA5-WT and miR-19b-3p mimics (Figure 3B). The miR-19b-3p expression was obviously suppressed by circSMARCA5 overexpression (Figure 3C). Then, we

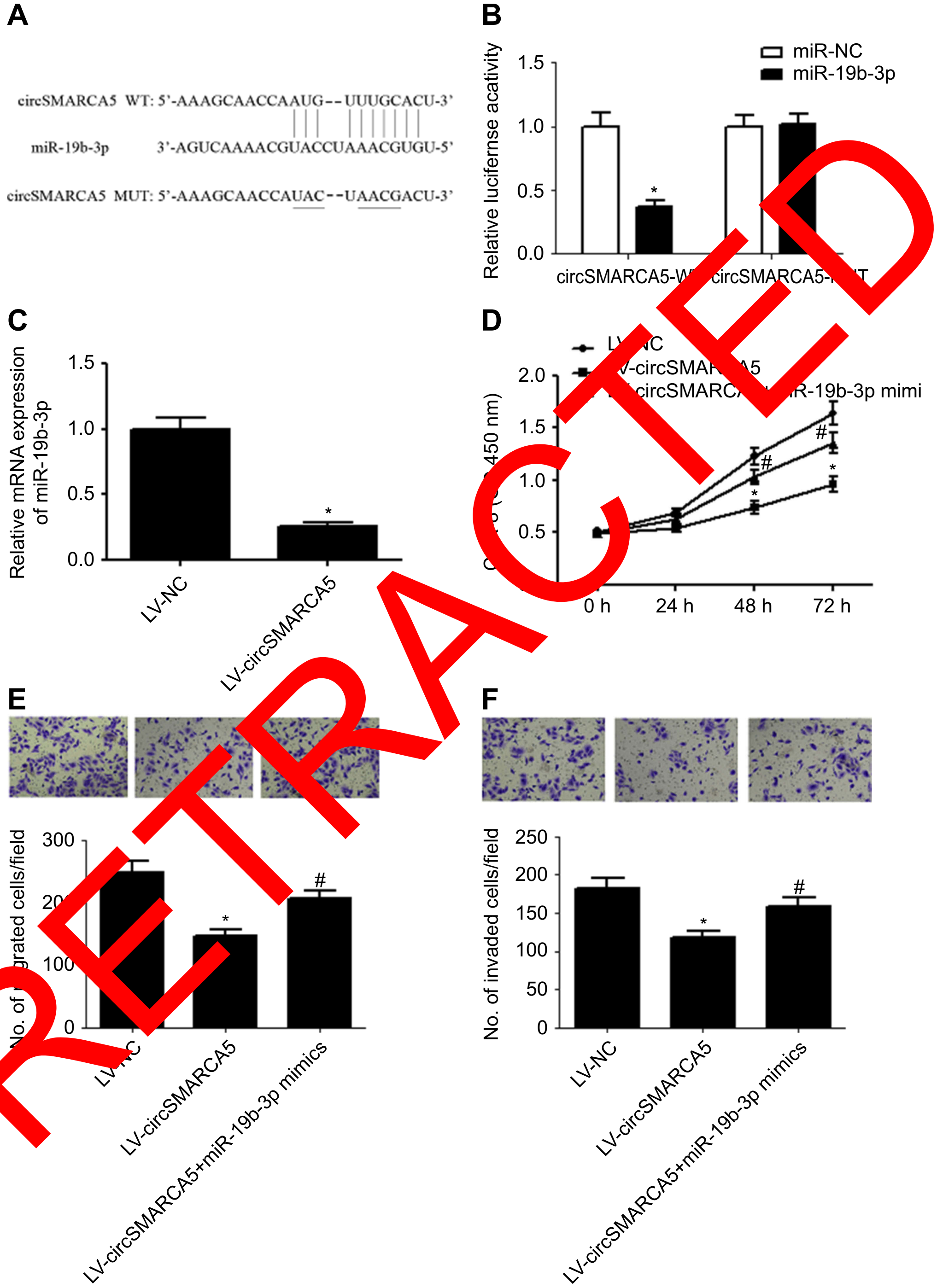

Figure 3 MiR-19b-3p mimics reversed the inhibitory effects of circSMARCA5 on NSCLC cells.

Notes: (**A**) Predicted results of the binding sites between circSMARCA5 and miR-19b-3p. (**B**) Luciferase reporter assay was performed to confirm the correlation between circSMARCA5 and miR-19b-3p. *p<0.05 vs control group. (**C**) Effect of circSMARCA5 overexpression on miR-19b-3p expression. *p<0.05 vs LV-NC group. (**D**–**F**) Effects of miR-19b-3p mimics on cell proliferation, migration, and invasion in circSMARCA5-overexpressed NSCLC cells. *p<0.05 vs LV-NC group; $^{\#}p$<0.05 vs LV-circSMARCA5 group.

Abbreviations: circSMARCA5, circular RNA SMARCA5; NSCLC, non-small cell lung cancer.

found that the expression of miR-19b-3p was negatively correlated with that of circSMARCA5 in NSCLC tissues (Figure S1). Furthermore, the inhibitory effects of circSMARCA5 overexpression on cell proliferation, migration, and invasion were mitigated by miR-19b-3p mimics (Figure 3D–F).

MiR-19b-3p executed its roles via targeting HOXA9 in NSCLC cell lines

Next, we found that HOXA9 might be a target gene of miR-19b-3p, which was screened by the online software (Figure 4A). To confirm the interaction between miR-19b-3p and HOXA9, luciferase reporter assay was performed. The results showed that A549 cells co-transfected with miR-19b-3p mimics and pGL3-HOXA9-WT exhibited lower luciferase activity than the cells with other transfections (Figure 4B). Moreover, qRT-PCR analysis demonstrated that transfection with miR-19b-3p mimics in A549 cells caused a significant decrease in HOXA9 expression (Figure 4C). In addition, miR-19b-3p inhibitor resulted in remarkable decrease in cell proliferation, migration, and invasion. However, the tumor-suppressive effects were enhanced by overexpression of HOXA9 (Figure 4D–F). Furthermore, we found that miR-19b-3p mimics significantly promoted cell proliferation, migration, and invasion of A549 cells (Figure S2).

Overexpression of circSMARCA5 inhibited tumor growth in vivo

To investigate the role of circSMARCA5 in NSCLC in vivo, a xenograft mouse model was established. The results showed that the circSMARCA5 overexpressing group exhibited significantly decreased tumor volume and weight than the control group (Figure 5A and B).

Discussion

CircSMARCA5 has been found to be associated with the tumorigenesis of many types of cancers. Cai et al,[12] proved that circSMARCA5 expression is decreased in gastric cancer tissues. Low circSMARCA5 expression is correlated with poor OS and disease-free survival. Upregulation of circSMARCA5 inhibits the proliferation, migration, and invasion of gastric cancer cells.[12] CircSMARCA5 is down-regulated in cervical cancer, while overexpression of circSMARCA5 suppresses cell proliferation, migration, and invasion, and induces cell cycle arrest in cervical cancer cell lines.[13] These findings reveal that circSMARCA5 exerts tumor-suppressive effects and functions as a potential biomarker for the prognosis and diagnosis for several cancers. Additionally, circSMARCA5 was found to act as an oncogene in other cancers. For instance, Kong et al,[11] demonstrated that circSMARCA5 expression is up-regulated in prostate cancer tissue samples as compared to match normal tissues. Functional experiments denote that circSMARCA5 promotes cell cycle and inhibits cell apoptosis of prostate cancer cell lines, indicating that circSMARCA5 acts as an oncogene in prostate cancer. In the present study, we found that circSMARCA5 was lowly expressed in NSCLC tissues and cell lines. Overexpression of circSMARCA5 in NSCLC cell lines significantly inhibited the proliferation, migration, and invasion.

Many circRNAs may act as competitive endogenous RNAs and modulators of miRNA activity by competing for miRNA-binding sites.[5] CircSMARCA5 suppresses proliferation and invasion of cervical cancer cells through binding to miR-620 and suppressing its expression.[13] We used online software to predict the target miRNA of circSMARCA5. The results showed that circSMARCA5 acted as a sponge of miR-19b-3p, which has been found to be involved in many types of cancers. For instance, miR-19b-3p is significantly up-regulated in colon cancer samples.[16] High expression of miR-19b-3p is significantly associated with high N stage, high AJCC stage, poor histologic grade, frequent venous and lymphatic invasion, and liver metastasis. Besides, miR-19b-3p is an independent prognostic factor associated with OS and disease-free survival in colon cancer patients.[16] The miR-19b expression level is significantly down-regulated in breast cancer and may function as a tumor suppressor.[17] In addition, plasma miR-19b-3p level is significantly higher in the lung cancer patients, compared with the healthy control group.[18] Our results proved that miR-19b-3p inhibitor resulted in remarkable decrease in cell proliferation, migration, and invasion. In addition, the inhibitory effects of circSMARCA5 overexpression on cell proliferation, migration, and invasion were mitigated by miR-19b-3p mimics in NSCLC cells, indicating that circSMARCA5 exerted its tumor-suppressive activity via sponging miR-19b-3p.

MiRNAs are known to be involved in multiple biological and pathological processes through modulating gene expression via direct base pairing to target sites within mRNAs.[19,20] According to our predicted results, HOXA9 might be a target gene of miR-19b-3p. Previous study has reported that

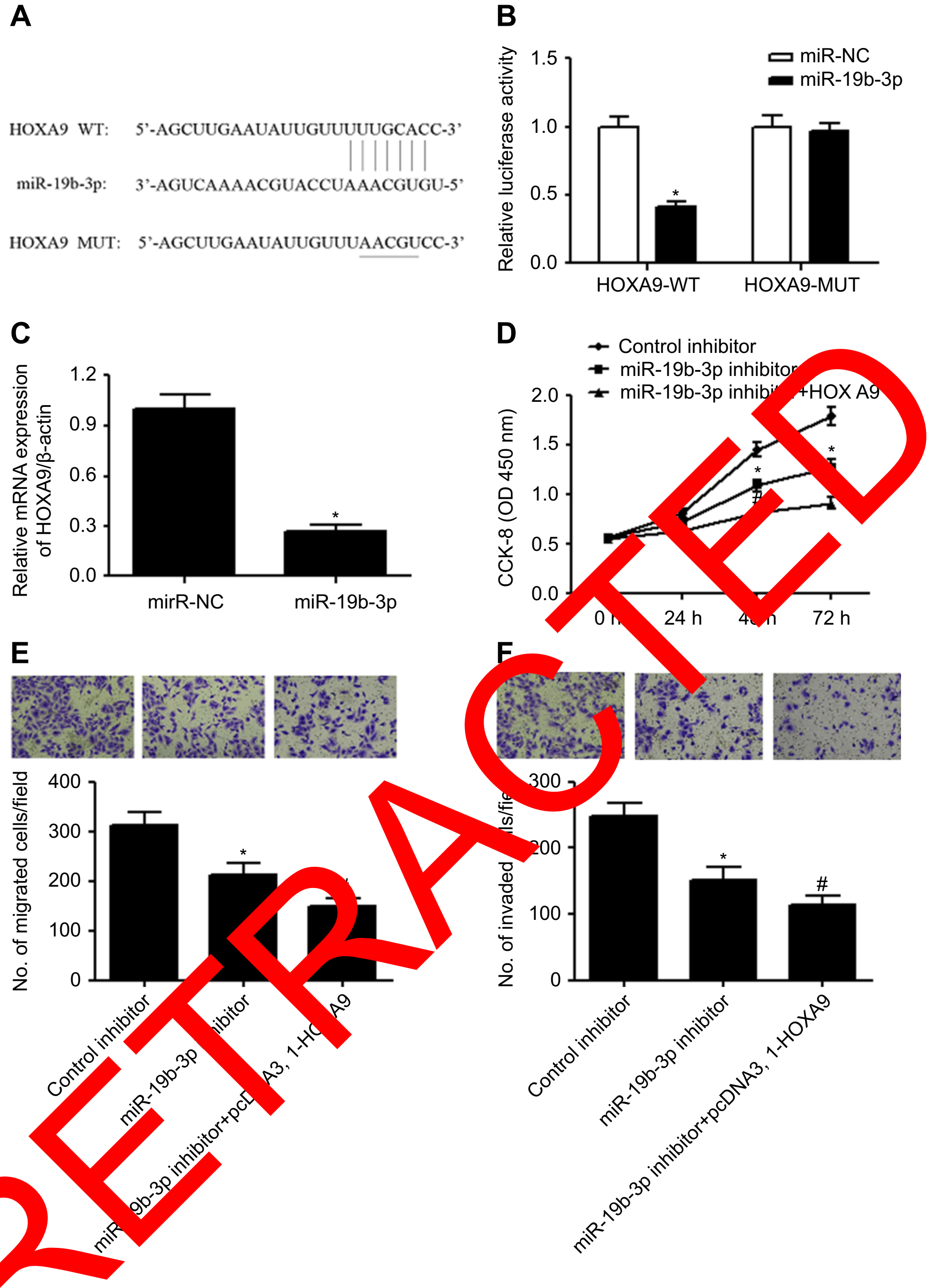

Figure 4 Overe[illegible]ession of HOXA9 enhanced the tumor-suppression effects of miR-19b-3p inhibitor in NSCLC cells.

Notes: (**A**) HOXA[illegible]ight be a target gene of miR-19b-3p. (**B**) MiR-19b-3p directly bound to the 3′-UTR of HOXA9. *$p<0.05$ vs miR-NC group. (**C**) MiR-19b-3p mimics suppressed HOXA9 e[illegible]ssion. *$p<0.05$ vs miR-NC group. (**D**–**F**) Overexpression of HOXA9 enhanced the inhibitory effects of miR-19b-3p inhibitor on cell proliferation, migration, and invasion. *$p<0.05$ vs control inhibitor group, $^{\#}p<0.05$ vs miR-19b-3p inhibitor group.

Abbreviation: NSCLC, non-small cell lung cancer.

HOXA9 is markedly expressed in osteosarcoma tissues. HOXA9 is implicated in the progression of osteosarcoma and validated as a direct target gene of miR-873.[21] Yu et al,[22] demonstrated that recombinant R10-HOXA9 protein significantly reduces the invasion and migration rate of the NSCLC cells. Treatment of NSCLC cells with recombinant R10-HOXA9 protein results in a significant increase in E-cadherin expression, indicating that R10-HOXA9 suppresses the epithelial-mesenchymal transition. Additionally, recombinant R10-HOXA9 protein effectively reduces the rate of lung cancer cell motility in an experimental metastatic mouse model.[22] Our results revealed that miR-19b-3p

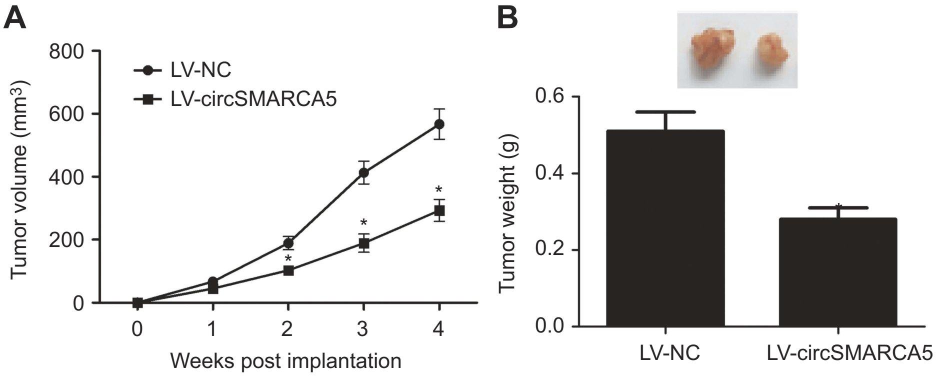

Figure 5 Overexpression of circSMARCA5 inhibited tumor growth in vivo. (**A** and **B**) Tumor volume and weight were decreased in circSMARCA5 overexpressing group. **p*<0.05.

directly bound to HOXA9 and inhibited its expression. Moreover, the tumor-suppressive effects of miR-19b-3p inhibitor were enhanced by overexpression of HOXA9. The results implied that miR-19b-3p executed its role via targeting HOXA9 in NSCLC cell lines.

To conclude, the current study revealed that the circSMARCA5 was down-regulated in NSCLC tissues and cell lines. Overexpression of circSMARCA5 significantly inhibited the proliferation, migration, and invasion in cell lines, and suppressed tumor growth in vivo. Furthermore, circSMARCA5 exerts its inhibitory effects on NSCLC via regulating miR-19b-3p/HOXA9 axis. Based on these results, circSMARCA5 might serve as a tumor suppressor for NSCLC.

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Disclosure

The authors report no conflicts of interest in this work.

References

1. Alberg AJ, Brock MV, Samet JM. Epidemiology of lung cancer: looking to the future. *J Clin Oncol*. 2005;23(14):3175–3185. doi:10.1200/JCO.2005.10.462
2. Molina JR, Yang P, Cassivi SD, Schild SE, Adjei AA. Non-small cell lung cancer: epidemiology, risk factors, treatment, and survivorship. *Mayo Clin Proc*. 2008;83(5):584–594. doi:10.4065/83.5.584
3. Tsim S, O'Dowd CA, Milroy R, Davidson S. Staging of non-small cell lung cancer (NSCLC): a review. *Respir Med*. 2010;104(12):1767–1774. doi:10.1016/j.rmed.2010.08.005
4. Hsiao KY, Sun HS, Tsai SJ. Circular RNA – new member of noncoding RNA with novel functions. *Exp Biol Med (Maywood)*. 2017;242(11):1136–1141. doi:10.1177/1535370217708978
5. Chen I, Chen CY, Chuang TJ. Biogenesis, identification, and function of exonic circular RNAs. *Wiley Interdiscip Rev RNA*. 2015;6(5):563–579. doi:10.1002/wrna.1294
6. Ebbesen KK, Kjems J, Hansen TB. Circular RNAs: identification, biogenesis and function. *Biochim Biophys Acta*. 2016;1859(1):163–168. doi:10.1016/j.bbagrm.2015.07.007
7. Liu L, Wang J, Khanabdali R, Kalionis B, Tai X, Xia S. Circular RNAs: isolation, characterization and their potential role in diseases. *RNA Biol*. 2017;14(12):1715–1721. doi:10.1080/15476286.2017.1367886
8. Kristensen LS, Hansen TB, Veno MT, Kjems J. Circular RNAs in cancer: opportunities and challenges in the field. *Oncogene*. 2018;37(5):555–565. doi:10.1038/onc.2017.361
9. Hang D, Zhou J, Qin N, *et al*. A novel plasma circular RNA circFARSA is a potential biomarker for non-small cell lung cancer. *Cancer Med*. 2018;7(6):2783–2791. doi:10.1002/cam4.1514
10. Zhang S, Zeng X, Ding T, et al. Microarray profile of circular RNAs identifies hsa_circ_0014130 as a new circular RNA biomarker in non-small cell lung cancer. *Sci Rep*. 2018;8(1):2878–2889. doi:10.1038/s41598-018-21300-5
11. Kong Z, Wan X, Zhang Y, *et al*. Androgen-responsive circular RNA circSMARCA5 is up-regulated and promotes cell proliferation in prostate cancer. *Biochem Biophys Res Commun*. 2017;493(3):1217–1223. doi:10.1016/j.bbrc.2017.07.162
12. Cai J, Chen Z, Zuo X. circSMARCA5 functions as a diagnostic and prognostic biomarker for gastric cancer. *Dis Markers*. 2019;2019:2473652–2473663.
13. Tian JDC, Liang L. Involvement of circular RNA SMARCA5/microRNA-620 axis in the regulation of cervical cancer cell proliferation, invasion and migration. *Eur Rev Med Pharmacol Sci*. 2018;22(24):8589–8598. doi:10.26355/eurrev_201812_16622
14. Barbagallo D, Caponnetto A, Cirnigliaro M, *et al*. CircSMARCA5 inhibits migration of glioblastoma multiforme cells by regulating a molecular axis involving splicing factors SRSF1/SRSF3/PTB. *Int J Mol Sci*. 2018;19(2):pii: E480. doi:10.3390/ijms19020480
15. Li Z, Zhou Y, Yang G, *et al*. Using circular RNA SMARCA5 as a potential novel biomarker for hepatocellular carcinoma. *Clin Chim Acta*. 2019;492:49237–49244.
16. Jiang T, Ye L, Han Z, *et al*. miR-19b-3p promotes colon cancer proliferation and oxaliplatin-based chemoresistance by targeting SMAD4: validation by bioinformatics and experimental analyses. *J Exp Clin Cancer Res*. 2017;36(1):131–145. doi:10.1186/s13046-017-0602-5
17. Maleki E, Ghaedi K, Shahanipoor K, Karimi Kurdistani Z. Down-regulation of microRNA-19b in hormone receptor-positive/HER2-negative breast cancer. *APMIS*. 2018;126(4):303–308. doi:10.1111/apm.12820

18. Bulgakova O, Zhabayeva D, Kussainova A, *et al*. miR-19 in blood plasma reflects lung cancer occurrence but is not specifically associated with radon exposure. *Oncol Lett*. 2018;15(6):8816–8824. doi:10.3892/ol.2018.8392
19. Garzon R, Fabbri M, Cimmino A, Calin GA, Croce CM. MicroRNA expression and function in cancer. *Trends Mol Med*. 2006;12(12):580–587. doi:10.1016/j.molmed.2006.10.006
20. Bushati N, Cohen SM. microRNA functions. *Annu Rev Cell Dev Biol*. 2007;23(23):175. doi:10.1146/annurev.cellbio.23.090506.123406
21. Liu Y, Wang Y, Yang H, *et al*. MicroRNA873 targets HOXA9 to inhibit the aggressive phenotype of osteosarcoma by deactivating the Wnt/betacatenin pathway. *Int J Oncol*. 2019;54(5):1809–1820. doi:10.3892/ijo.2019.4735
22. Yu SL, Koo H, Lee HY, *et al*. Recombinant cell-permeable HOXA9 protein inhibits NSCLC cell migration and invasion. *Cell Oncol (Dordr)*. 2019. [Epub ahead of print]. doi:10.1007/s13402-019-00424-4

Supplementary materials

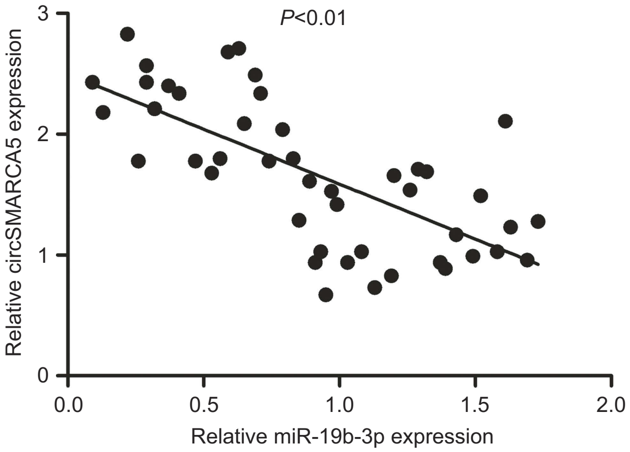

Figure S1 Expression correlation between miR-19b-3p and circSMARCA5 in NSCLC tissues by qRT-PCR.

Abbreviations: NSCLC, non-small cell lung cancer; circSMARCA5, circular RNA SMARCA5; qRT-PCR, quantitative real-time PCR.

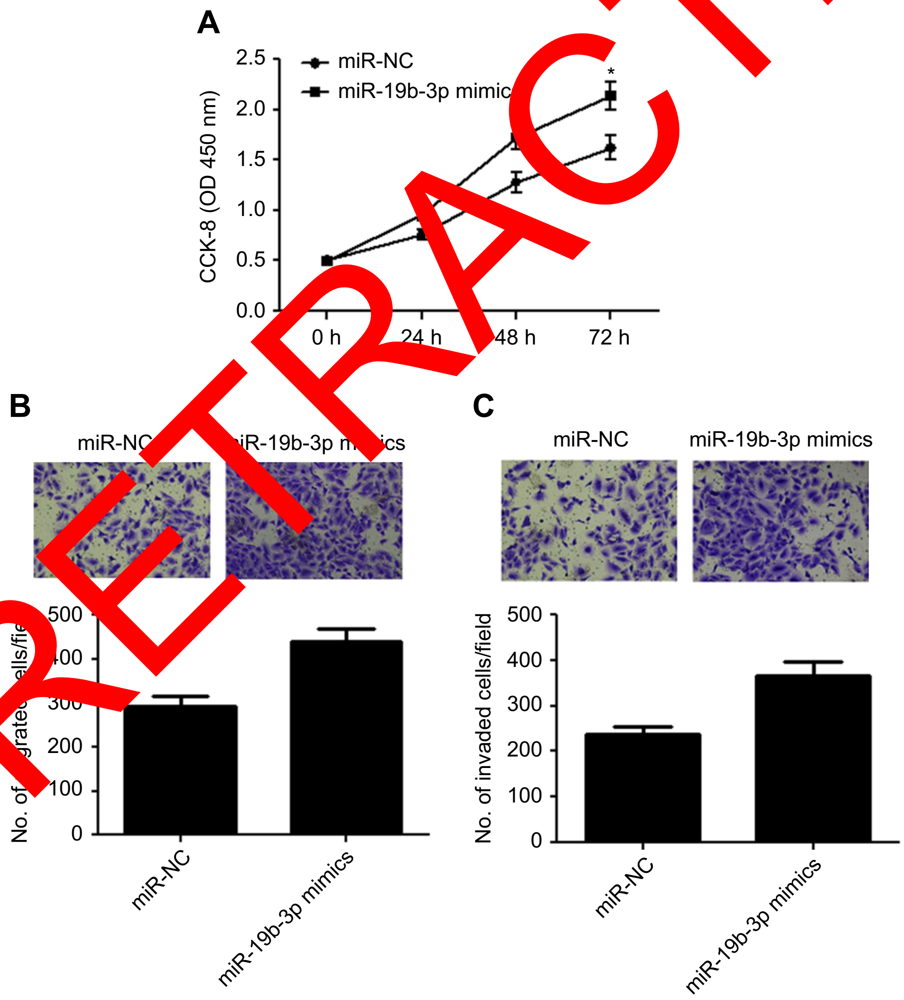

Figure S2 miR-19b-3p mimics significantly promoted cell proliferation, migration, and invasion of A549 cells. A549 cells were transfected with miR-19b-3p mimics or miR-NC for 24 hrs.

Notes: (**A**) CCK-8 assay was performed to evaluate cell proliferation. (**B** and **C**) Transwell assay was performed to evaluate cell migration and invasion. *$p<0.05$.