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RESEARCH ARTICLE

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Long non-coding RNA ATB expedites non-small cell lung cancer progression by the miR-200b/fibronectin 1 axis

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Abstract

Background: Long non-coding RNA (lncRNA) ATB belongs to an active modulator in multiple cancers, but its expression along with potential underlying non-small cell lung cancer (NSCLC) is obscure. Our study aimed to investigate the role and potential mechanism of LncRNA ATB in NSCLC.

Methods: LncRNA ATB expression in NSCLC tissues and cell lines was detected by qRT-PCR. Effects of LncRNA ATB on NSCLC cell proliferation, migration and invasion were assessed by MTS, colony formation and transwell assays. The connection among LncRNA ATB, miR-200b and fibronectin 1 (FN1) was determined by bioformatics prediction and luciferase reporter assay.

Results: In this research, upregulation of LncRNA ATB was discovered in NSCLC tissue samples and cell lines. LncRNA ATB was positively related to advanced tumor phase as well as lymph node metastasis. Cell function assays reflected LncRNA ATB expedited NSCLC cells proliferation, migration and invasion. LncRNA ATB promoted fibronectin 1 (FN1) expression via inhibiting miR-200b. Furthermore, LncRNA ATB depletion suppressed NSCLC cells proliferation, migration and invasion, while miR-200b inhibitor or pcDNA-FN1 rescued these effects.

Conclusion: In summary, our outcomes elucidated that LncRNA ATB/miR-200b axis expedited NSCLC cells proliferation, migration and invasion by up-regulating FN1.

KEYWORDS
FN1, LncRNA ATB, miR-200b, non-small cell lung cancer, proliferation and invasion

1 | INTRODUCTION

With high incidence and mortality rate worldwide, non-small cell lung cancer (NSCLC) occupies around 80% of the whole lung cancer cases.[1,2] Great improvements for NSCLC therapy have been made in recent years, but the long-time survival rate of NSCLC patients is unsatisfactory.[3,4] As a consequence, it is urgent to probe effective targets for NSCLC treatment.

Long noncoding RNAs (lncRNAs) pertain to un-translational transcripts, with over 200 nucleotides.[5] Numerous evidence demonstrated lncRNAs play crucial parts in the pathophysiological processes, containing cell proliferation, migration, along with invasion.[6]

LncRNA ATB is first identified as an oncogenic lncRNA in hepatocellular carcinoma. High expression of LncRNA ATB enhanced invasion of hepatocellular carcinoma cells through combining with the miR-200 family.[7] Moreover, LncRNA ATB could regulate cell malignant behaviors in many cancers.[8–12] However, LncRNA ATB expression in NSCLC and the underlying role remain obscure.

In this research, LncRNA ATB was discovered to be obviously upregulated in NSCLC tissue samples and cell lines. In addition, LncRNA ATB expression was positively related to advanced tumor phase as well as lymph node metastasis. Furthermore, miR-200b could bind to LncRNA ATB. Moreover, LncRNA ATB/miR-200b axis expedited NSCLC cells' proliferation and invasion via up-regulating fibronectin 1 (FN1).

2 | MATERIALS AND METHODS

2.1 | NSCLC samples

A total of 46 NSCLC tumor tissues as well as corresponding normal adjacent normal tissues were obtained from NSCLC patients at the Hospital from February 2016 to February 2018. All patients did not accept radiation treatment or chemotherapy prior to surgery. This study was acquired written informed consent from all patients, as well as approval from the Ethics Committees of the Affiliated Hospital of Medical School of Ningbo University.

2.2 | Cell culture

Cell Bank of the Chinese Academy of Sciences supplied human NSCLC cell lines (A549, H1229, H358 and H522) together with normal human lung epithelial cell line BEAS-2B. Dulbecco's modified essential medium (DMEM, Thermo Fisher) containing with 10% fetal bovine serum (FBS, Hyclone) at 37°C with 5% CO_2 was implemented to culture cells.

2.3 | Quantitative real-time reverse transcription-polymerase chain reaction (qRT-PCR)

Total RNA was extracted from NSCLC tissue samples or cell lines by Trizol reagent (Invitrogen). PrimeScriptTM RT reagent kit (Takara) was implemented to synthesize cDNAs. Then, qPCR was implemented with the SYBR Premix Ex Taq II kit. For microRNA analysis, qRT-PCR was performed using TaqMan microRNA assays (Applied Biosystems). All specific primers were unveiled as follows: LncRNA ATB forward primer; 5'-GGCAGGTAGAAAAGTCGGCT-3', reverse primer; 5'-TGGAAAGAGTGGGAAGGATT-3'; β-actin forward primer; 5'-CTTAGTTGCGTTACACCCTTTCTTG-3', reverse primer; 5'-CTGTCACCTTCACCGTTCCAGTTT-3'. β-actin was an endogenous control.

2.4 | Cell transfection

Two human LncRNA ATB siRNAs (si-ATB-1/−2), pcDNA-FN1 overexpression plasmid (pcDNA-FN1), miR-200b mimics/inhibitor were brought from GenePharma Co., Ltd. Cell transfection was carried out by means of Lipofectamine 2000.

2.5 | MTS assay

Briefly, 2000 cells were planted in 96-well plates, followed by the addition of a 100 μl culture medium including 20 μl of MTS reagent (Promega). The absorbance was read at 490 nm by a plate reader.

2.6 | Colony formation assay

NSCLC cells treated with LncRNA ATB siRNAs, miR-200b inhibitor or pcDNA-FN1 were seeded in six-well plates. After 10 days, colonies were fixed and stained. Then, the number of colonies was counted.

2.7 | Transwell assay

For migration assays, 2×10^4 cells in medium without serum were seeded into the upper chamber. For invasion experiments, cells in medium without serum were planted into the upper wells precoated with Matrigel (BD Biosciences). The bottom wells contained growth medium with 10% FBS. Then, the penetrated cells on the filters were fixed, stained and counted.

2.8 | Luciferase assay

LncRNA ATB wild type (LncRNA ATB-WT) with miR-200b binding sites or mutant sites (LncRNA ATB-MUT), as well as the full-length FN1 wild-type 3'-untranslated region (UTR) including miR-200b targeting site (FN1 3'-UTR-WT) or mutant 3'-UTR (FN1 3'-UTR-MUT) were inserted into the psi-CHECK-2 luciferase reporter vector (Promega), respectively. NSCLC cells were co-transfected with luciferase plasmids together with miR-200b mimics or control miRNA. After 48 h, the activities were measured with a Dual-Luciferase Reporter Assay System (Promega).

2.9 | Western blotting assay

Total proteins were extracted from A549 and H522 cells using RIPA buffer containing proteinase inhibitor cocktail (Boster). Then proteins were isolated by SDS-PAGE, and transferred the protein onto polyvinylidene fluoride (PVDF) membranes. The membranes were hatched with the FN1 primary antibodies (CST, USA) at 4°C for an

overnight, followed by adding HRP-linked secondary antibodies, and the protein bands were detected using Super ECL Plus Detection Reagent (Applygen Technologies Inc.).

2.10 | Statistical analysis

All data are expressed as the mean ± standard error (SD). Student's *t*-test was implemented to analyze the differences between two groups. A value of $p < 0.05$ was significant.

3 | RESULTS

3.1 | LncRNA ATB was elevated in NSCLC

qRT-PCR outcomes displayed LncRNA ATB was upregulated in 46 paired NSCLC tissues (Figure 1A). Furthermore, the LncRNA ATB expression was positively related to advanced tumor phase as well as lymph node metastasis (Figure 1B,C). In contrast to the normal lung epithelial cell line, BEAS-2B, LncRNA ATB was high-expressed in four NSCLC cell lines, H1229, H358, A549 and H522 (Figure 1D).

3.2 | LncRNA ATB knockdown inhibited proliferation of NSCLC cells

As shown in Figure 2A, LncRNA ATB was successfully knocked down in NSCLC cells through siRNAs. MTS assay showed that LncRNA ATB depletion reduced the viability of NSCLC cells (Figure 2B). It was also revealed the colony numbers of NSCLC cells were decreased after LncRNA ATB silence (Figure 2C).

3.3 | LncRNA ATB knockdown suppressed NSCLC cells migration and invasion

As indicated in Figure 3A,B, the migratory and invasive potentials underlying NSCLC cells were inhibited due to LncRNA ATB knockdown.

3.4 | LncRNA ATB bound to miR-200b in NSCLC cells

Previous literatures have proved that LncRNA ATB could compete with miR-200b in tumors.[7,9] Therefore, we assumed that LncRNA ATB might participate in NSCLC by regulating the miR-200b expression. As illustrated in Figure 4A, LncRNA ATB possesses a potential binding site of miR-200b. MiR-200b was low-expressed in NSCLC cells upon LncRNA ATB silence (Figure 4B). The results of luciferase assay indicated that the luciferase intensity of LncRNA ATB-WT was significantly reduced in NSCLC cells due to miR-200b elevation, but that of LncRNA ATB-MUT was not affected by miR-200b increase (Figure 4C). Additionally, LncRNA ATB was reduced in NSCLC cells after miR-200b elevation, whereas significantly increased upon miR-200b inhibition (Figure 4D).

3.5 | MiR-200b targeted FN1 in NSCLC cells

Previous studies showed that miR-200c could regulate gastric cancer progression by binding to FN1.[13] Here, we hypothesized that LncRNA ATB/miR-200b axis might promote NSCLC progression by regulating FN1. As uncovered in Figure 5A, FN1 3'-UTR possesses a potential binding site of miR-200b. Luciferase assay revealed that

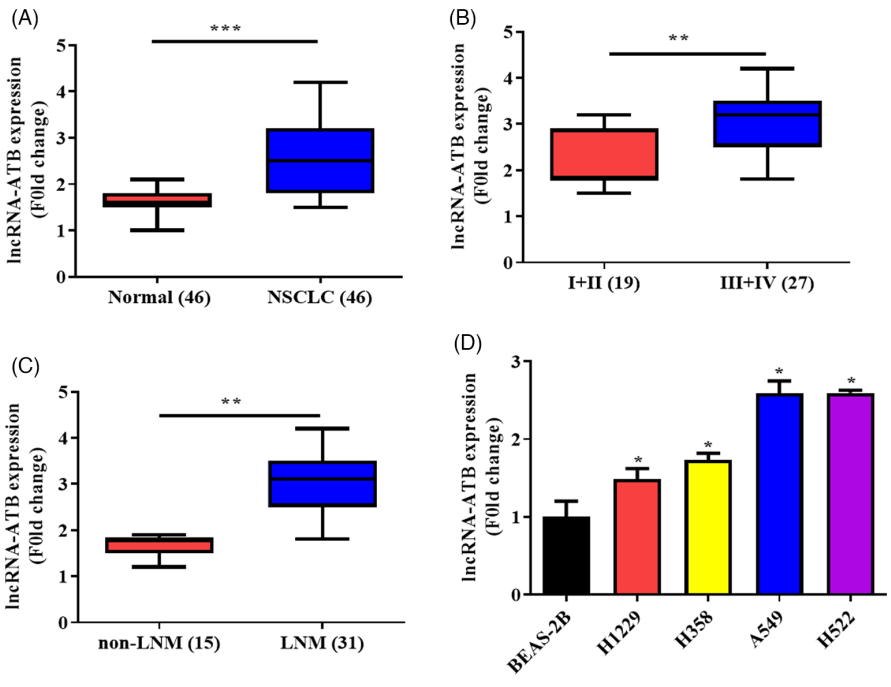

FIGURE 1 Up-regulation of LncRNA ATB in NSCLC. (A) LncRNA ATB expression underlying NSCLC tissues and adjacent normal tissues ($n = 46$); (B) Relation of LncRNA ATB expression and tumor stage; (C) Relation of LncRNA ATB expression as well as lymph node metastasis; (D) LncRNA ATB expression underlying human noncancerous cell lines and the cancer cell lines (H1299, H358, A549 and H522) from qRT-PCR. $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$

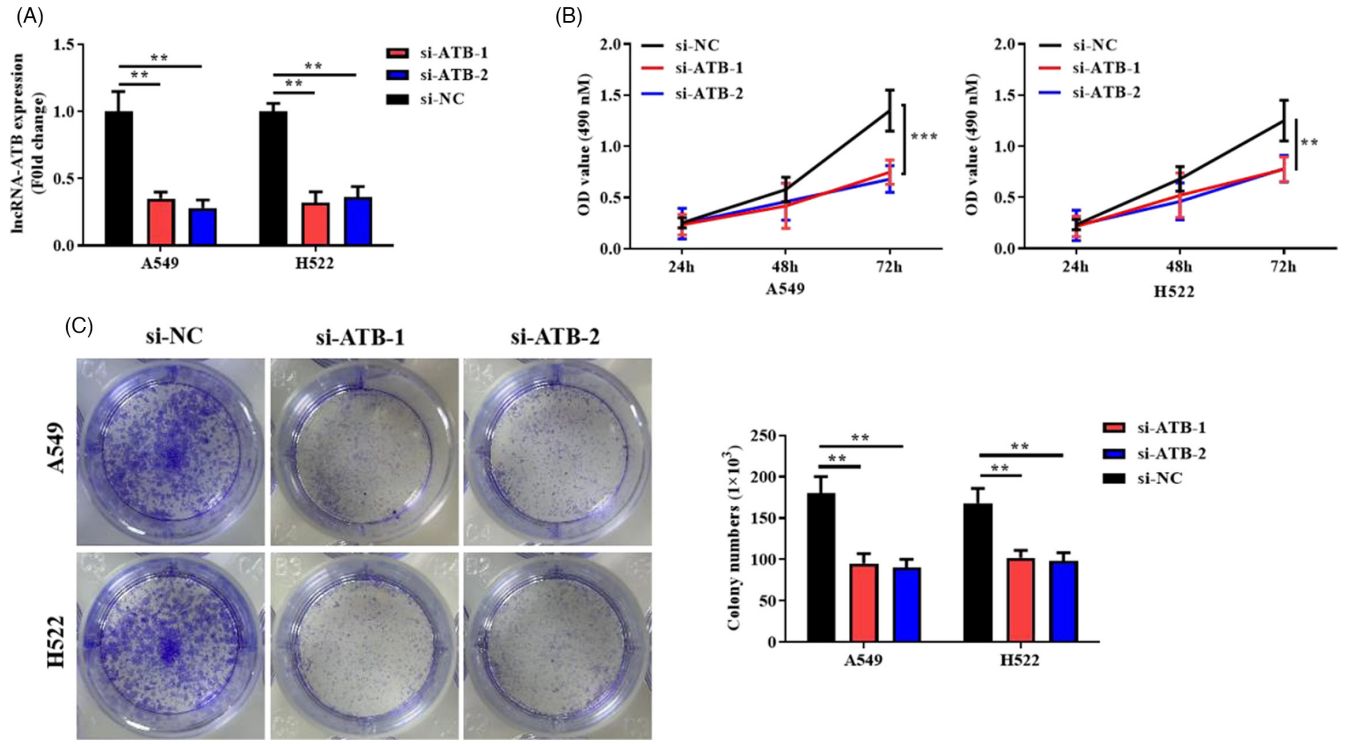

FIGURE 2 LncRNA ATB depletion impaired NSCLC cells proliferation. (A) LncRNA ATB expression underlying NSCLC cells after LncRNA ATB silence from qRT-PCR; (B) MTS assay of NSCLC cells after LncRNA ATB silence; (C) Colony formation assay of NSCLC cells after LncRNA ATB silence. $^{**}p < 0.01$

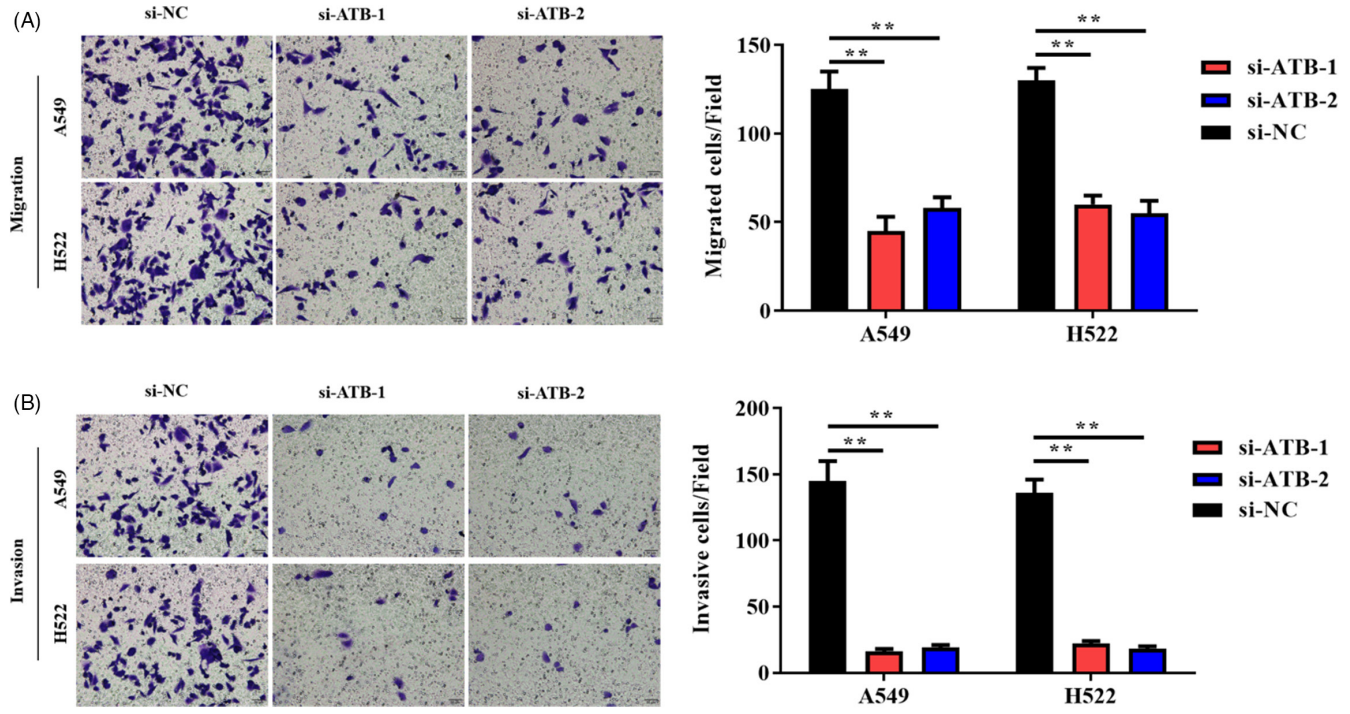

FIGURE 3 LncRNA ATB reduction repressed NSCLC cells migration and invasion. (A–B) Migration and invasion assays underlying NSCLC cells upon LncRNA ATB silence. $^{**}p < 0.01$ and $^{***}p < 0.001$

the luciferase intensity was impaired in NSCLC cells cotransfected with FN1 3'-UTR-WT together with miR-200b mimics. However, the luciferase intensity was not changed in NSCLC cells cotransfected with FN1 3'-UTR-MUT along with miR-200b mimics (Figure 5B,C). Moreover, FN1 protein level was significantly decreased in NSCLC cells upon miR-200b increase (Figure 5D).

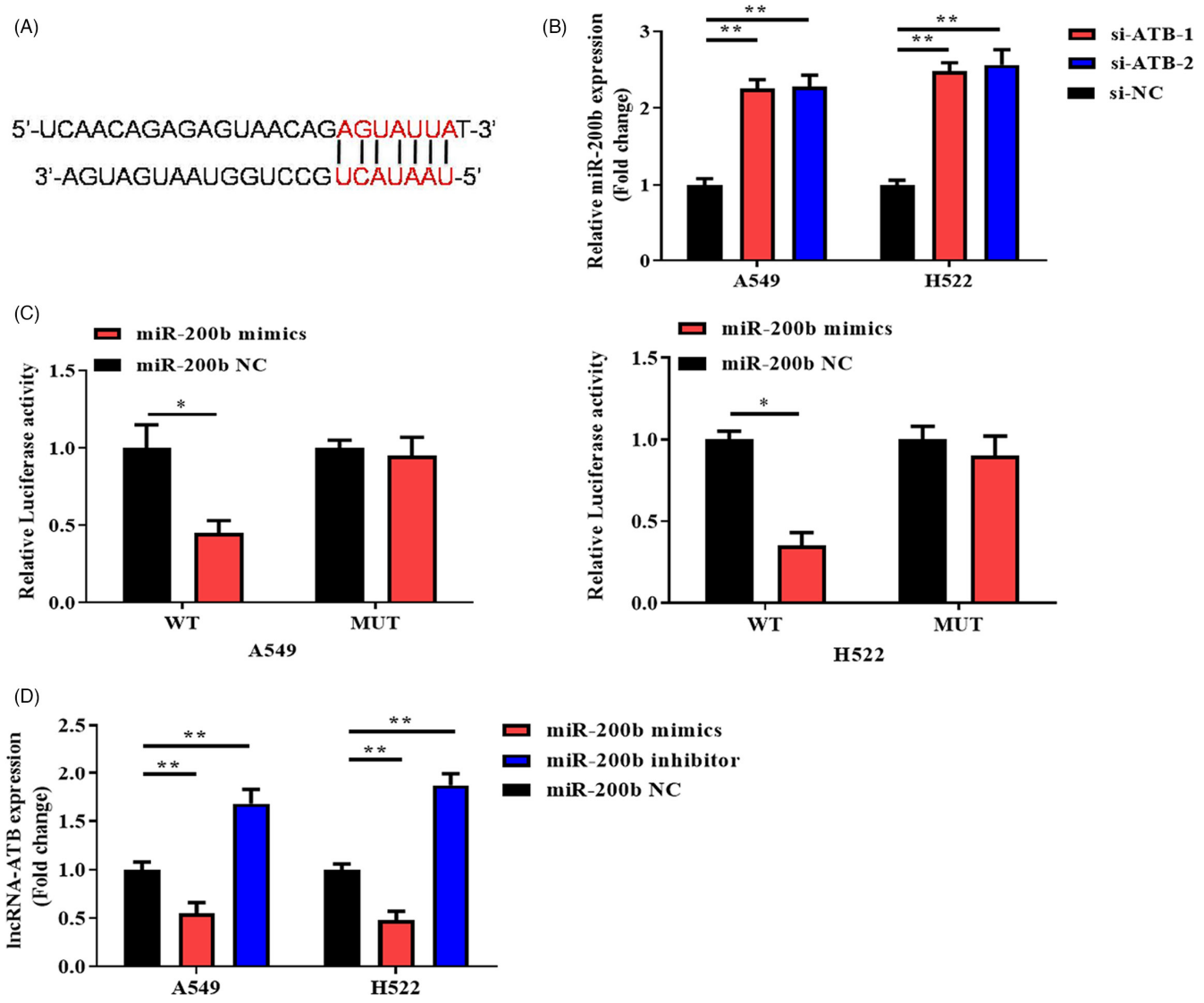

FIGURE 4 MiR-200b bound to LncRNA ATB. (A) MiR-200b binding sites on the LncRNA ATB transcript; (B) MiR-200b expression in NSCLC cells after LncRNA ATB silence by qRT-PCR; (C) Luciferase activity of LncRNA ATB-WT/MUT in NSCLC cells after miR-200b elevation; (D) LncRNA ATB expression in NSCLC cells upon transfecting with miR-200b mimics or inhibitor by qRT-PCR. $^{*}p < 0.05$ and $^{***}p < 0.01$

3.6 | LncRNA ATB/miR-200b axis facilitated NSCLC cells proliferation and invasion by regulating FN1

Next, whether FN1 was involved in the impacts of LncRNA ATB/miR-200b axis on NSCLC cells proliferation and invasion was further explored. LncRNA ATB knockdown significantly decreased FN1 protein level in NSCLC cells, while miR-200b inhibitor or pcDNA-FN1 offset the effects of LncRNA ATB knockdown on FN1 expression (Figure 6A). MST assay displayed LncRNA ATB decrease suppressed the proliferation of NSCLC cells, while miR-200b inhibition or FN1 amplification rescued the effects (Figure 6B). Furthermore, miR-200b inhibition or FN1 amplification counteracted the repressive influence underlying LncRNA ATB abrogation on cell migration and invasion (Figure 6C,D).

4 | DISCUSSION

LncRNAs regulate various cellular processes of NSCLC.[14] For instance, lncRNA AWPPH promoted proliferation and inhibits apoptosis of NSCLC cells.[15] SIK1-LNC repressed the proliferative of NSCLC cells.[16] Therefore, lncRNAs could be potential in diagnosis and treatment of NSCLC cancer.

LncRNA ATB, first identified as an oncogenic lncRNA in hepatocellular carcinoma cells.[7] Lei et al proposed that LncRNA ATB promoted gastric cancer cell proliferation through miR-141-3p/TGFβ2 axis.[11] Another literature pointed LncRNA ATB promoted lung cancer development.[17,18] Whereas, the expression and potential of lncRNA ATB in NSCLC are uncharted. In this work, we discovered lncRNA ATB was high-expressed in NSCLC tissues and was positively related to advanced tumor phase as well as lymph node metastasis.

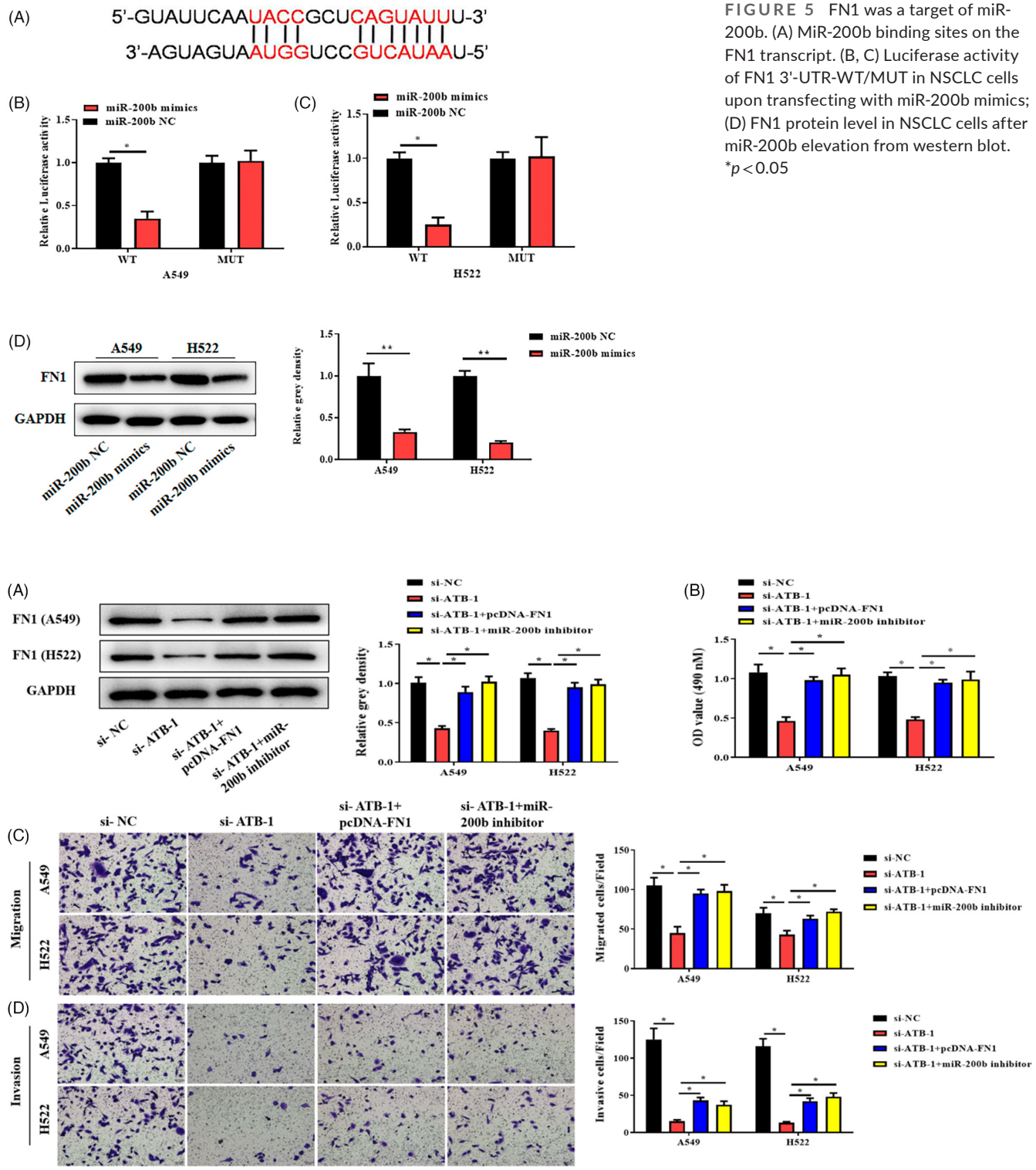

FIGURE 5 FN1 was a target of miR-200b. (A) MiR-200b binding sites on the FN1 transcript. (B, C) Luciferase activity of FN1 3'-UTR-WT/MUT in NSCLC cells upon transfecting with miR-200b mimics; (D) FN1 protein level in NSCLC cells after miR-200b elevation from western blot. $^{*}p < 0.05$

FIGURE 6 LncRNA ATB/miR-200b axis promoted NSCLC cells proliferation and invasion by regulating FN1. (A) FN1 protein level was analyzed in NSCLC cells after co-transfection with LncRNA ATB siRNA-1 and miR-200b inhibitor or pcDNA-FN1 by western blot. (B) MTS assay of NSCLC cells after co-transfection with LncRNA ATB siRNA-1 and miR-200b inhibitor or pcDNA-FN1; (C–D) Migration and invasion assays underlying NSCLC cells upon co-transfection with LncRNA ATB siRNA-1 and miR-200b inhibitor or pcDNA-FN1. $^{*}p < 0.05$

Moreover, LncRNA ATB abrogation hindered NSCLC cells proliferation, migration and invasion.

For an another class of noncoding RNAs, microRNAs (miRNAs) also control physiological processes of cancers.[19] Previous studies indicated that lncRNAs might work as miRNA sponges in tumor progression.[20] Therefore, we hypothesized that LncRNA ATB might participate in NSCLC through competing with miRNAs. A previous report unveiled that miR-200b/c repressed NSCLC cells proliferation

and invasion via controlling RhoE expression.[21] In this research, we assumed LncRNA ATB might participate in NSCLC by interacting with miR-200b. MiR-200b inhibitor rescued the anti-tumor potential of LncRNA ATB knockdown.

FN1 involves in various biological processes of tumors.[22] For example, FN1 facilitated A431 tumor cells the migration.[23] In a Mouse Glioma Model, knockdown of FN1 delayed the growth of glioma cells.[24] Xiu-Xia Zhang et al pointed out that high expression of FN1 is correlated with poor prognosis and immune infiltrates in breast cancer.[25] FN1 overexpression could promote gastric cancer progression.[26] Herein, miR-200b was found to directly target FN1 in NSCLC cells. Importantly, pcDNA-FN1 rescued the inhibitory influence of LncRNA ATB depletion on NSCLC progression.

5 | CONCLUSION

In sum, our study interestingly demonstrated that LncRNA ATB was high-expressed in NSCLC tissue samples along with cell lines, and was positively related to advanced tumor phase as well as lymph node metastasis. Furthermore, LncRNA ATB facilitated NSCLC cells proliferation, migration together with invasion via regulating miR-200b/FN1 axis. Our study might provide potential targets for NSCLC treatments.

AUTHOR CONTRIBUTIONS

Shifang Sun and Decai Zhu conceived the experiments and wrote the paper. Yifan Zou, Ningjie Xu, Jiarong Lv and Bin Hu performed the experiments. Yifeng Mai and Shanshan Rong gave experimental guidance in the lab. Liren Ding and Kaiyue Wang designed the experiments and analyzed the data. All authors approved the final version of the article.

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CONFLICT OF INTEREST

All authors declare that there is no conflict of interest.

DATA AVAILABILITY STATEMENT

Data generated in this study are available from the corresponding author under reasonable requests.

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