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Downregulation of miR-139 in lung cancer promotes metastasis via ERBB2/Rac1/NF-κB signaling axis

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Abstract: **Background**: Tumor metastasis is a key factor in cancer progression, yet its molecular mechanisms are not fully understood. ERBB2-positive lung cancer exhibits aggressive behavior, and the role of miR-139 in its metastasis requires investigation. **Objectives**: This study aimed to explore the function of miR-139 in ERBB2-positive lung cancer and its underlying molecular mechanism involving the ERBB2/Rac1/NF-κB signaling axis. **Methods**: The study utilized A549 lung cancer cells and tissue samples from 106 lung cancer patients. Methods included RT-PCR, bioinformatics analysis, dual-luciferase reporter assay, Western blot, cell migration/invasion assays, wound healing tests, Rac1 activity assays, and rescue experiments using Rac1-Q61L. **Results**: MiR-139 expression was significantly downregulated in lung cancer tissues, especially in lymph node metastases (P<0.01). MiR-139 directly targeted the 3'UTR of ERBB2 and inhibited its expression (P<0.01). Overexpression of miR-139 reduced Rac1 activity (P<0.01) without affecting RhoA or Cdc42, and decreased NF-κB signaling activity in ERBB2-positive tissues. MiR-139 overexpression significantly suppressed cell migration and invasion (P<0.01), an effect partially reversed by Rac1-Q61L. **Conclusion**: MiR-139 inhibits lung cancer cell migration and invasion by targeting ERBB2, suppressing Rac1 activity, and downregulating NF-κB signaling. Its downregulation promotes metastasis through the ERBB2/Rac1/NF-κB axis.

Keywords: ERBB2; Lung cancer; MiR-139; NF-κB

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INTRODUCTION

Lung cancer remains one of the leading causes of cancer-related mortality worldwide, particularly in Asia, where its incidence continues to rise due to factors such as smoking, environmental pollution, and genetic predisposition (Yang, X *et al*., 2025). At present, signaling pathways in the metastatic cascade have come into focus in related research, which aims to find new treatments for lung cancer. MicroRNAs (miRNAs) are small (Shaheen, N *et al*., 2023), non-coding RNA molecules that play critical roles in post-transcriptional gene regulation, influencing diverse biological processes, including cell proliferation, differentiation, and apoptosis (Dong, L *et al*., 2020). It has been shown in prostate cancer tissue that miR-139 regulates cell cycle. Moreover, miR-139 can alter PIM1-STAT3 signaling in cervical cancer (Chi, X *et al*., 2022). The ERBB2 (HER2) receptor, a member of the epidermal growth factor receptor (EGFR) family (Yoon, J *et al*., 2024, Sanz-Moreno, A *et al*., 2021). is a well-characterized oncogene implicated in the pathogenesis of various solid tumors, including breast and gastric cancers (Zhang, L *et al*., 2023). NF-κB activation is associated with breast cancer (Liang, Y *et al*., 2023, Tan, Y *et al*., 2021). Mice lacking the kBα (IkBα) protein exhibit increased mammary gland epithelial hyperplasia and duct branching, and their development is regulated by NF-kB signaling (Sun, F *et al*., 2021). MMTV-driven c-Rel mice develop various pathological types of delayed breast cancer. IKK inhibition prevents NF-κB activation and decreases xenograft tumor growth (Zhao, C *et al*., 2024). Active NF-κB signal leads to apoptosis and shrinks tumors in ER-negative breast cancer (Wilson, EA *et al*., 2021). Here, we propose for the first time that miR-139 in lung cancer is negatively correlated with ERBB2 level and exerts a role in lung cancer (Fig. 1).

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MATERIALS AND METHODS

Tissue source and cell culture

A549 was purchased from Shanghai Cell Institute and routinely cultured. When reaching a confluence of 80-90%, cells were transfected with Lipofectamine 2000 reagent (Shanghai Thermo Fisher Scientific Co., Ltd.) for 6 hours in serum-free medium. 106 cases of lung cancer (65 males and 41 females with a median age of 64±4.7 years) and 30 cases of adjacent tissues were collected. The patients and their families signed informed consent.

RT-PCR

Trizol was used to extract RNA which was synthesized into cDNA followed by qPCR using SYBR Master Mix with conditions: 95°C 10 min, 40 cycles of 95°C 15s and 60°C 1 min. Gene expression was analyzed using ⊗⊗Ct method with GAPDH or 18S RNA as a control.

Cell migration and invasion assay

The wells of Boyden cells were coated with human type I collagen (10 mg/mL) at 37°C for 1 h for cell migration and coated with Matrigel for cell invasion. It was then separated, followed by centrifugation, and formed a single cell suspension which was added to the membrane at the bottom. After 6 h, the number of migrated and invaded cells was counted after crystal violet staining.

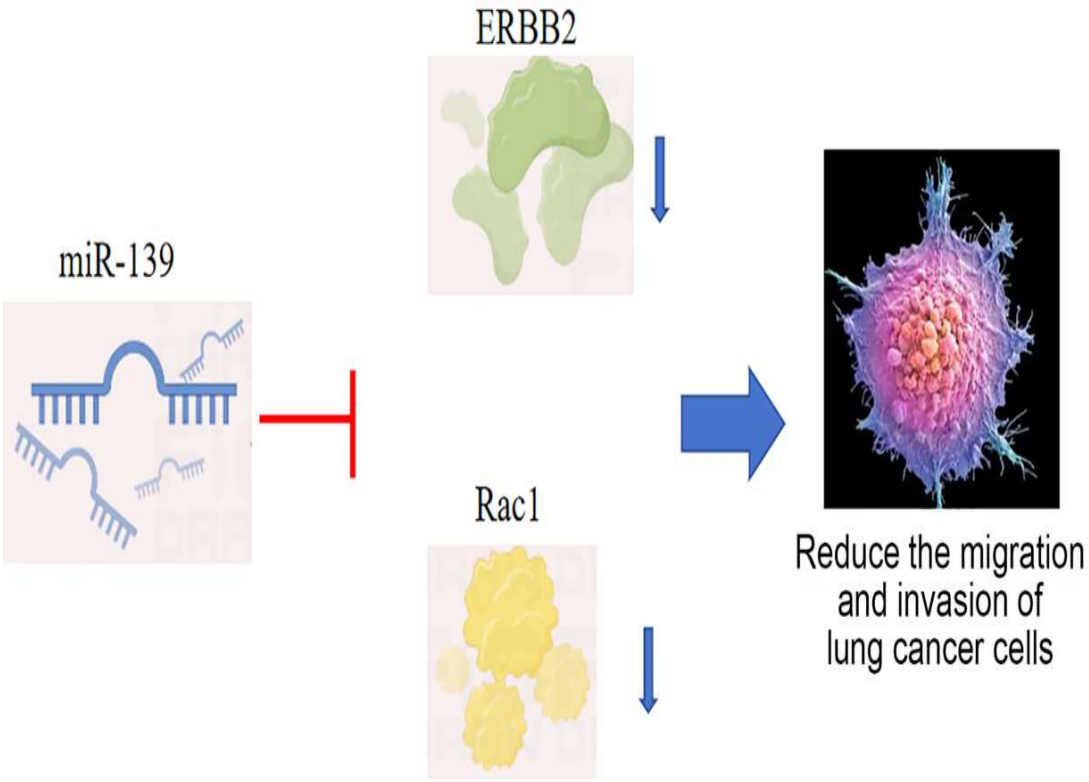

Fig. 1: Mechanism of miR-139 mediating lung cancer

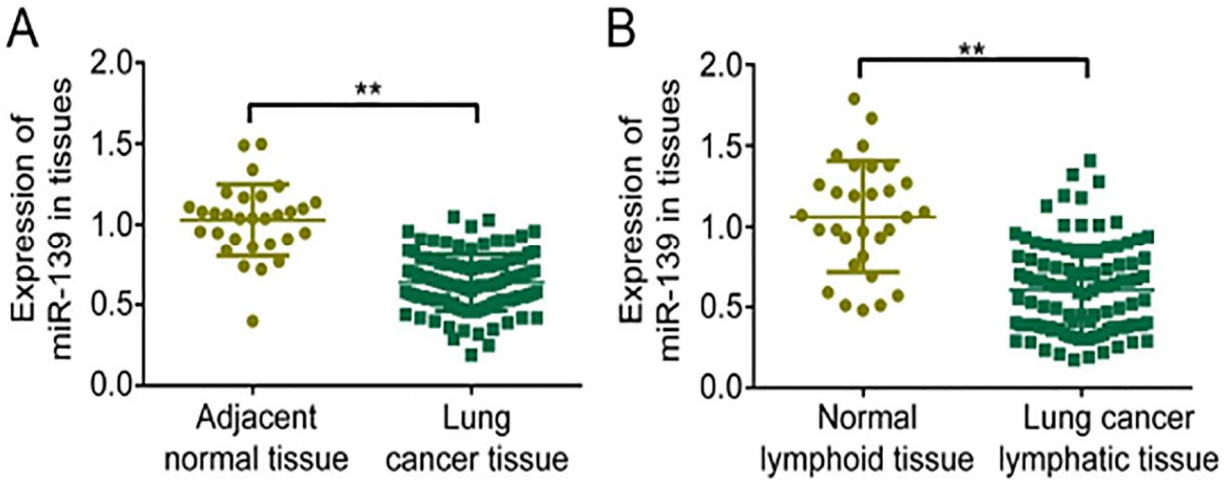

Fig. 2: miR-139 is down-regulated in metastatic lung cancer tissues.
A: RT-PCR detects the expression of miR-139 in the tissue. B: the expression of miR-139 in the adjacent lymph nodes (**p<0.01)

Protein activation test
Cells were transfected with miR-139 mimic or miRNA mimic control as described above followed by measuring RhoA, Cdc42 or Rac1 activation.

Dual luciferase assay
24 hours before transfection, cells were resuspended and distributed evenly in each 6-well plate (1.5×105 cells/well) and treated with 200ng pGL3-ERBB2-3'-UTR plus 80ng pRL-TK along with 60pmol miR-139 mimic or miRNA mimic control, respectively followed by measuring luciferase activity.

Western blot
After transfection, cells were homogenized with lysis buffer to extract protein for Western blot. GAPDH was used as a loading control.

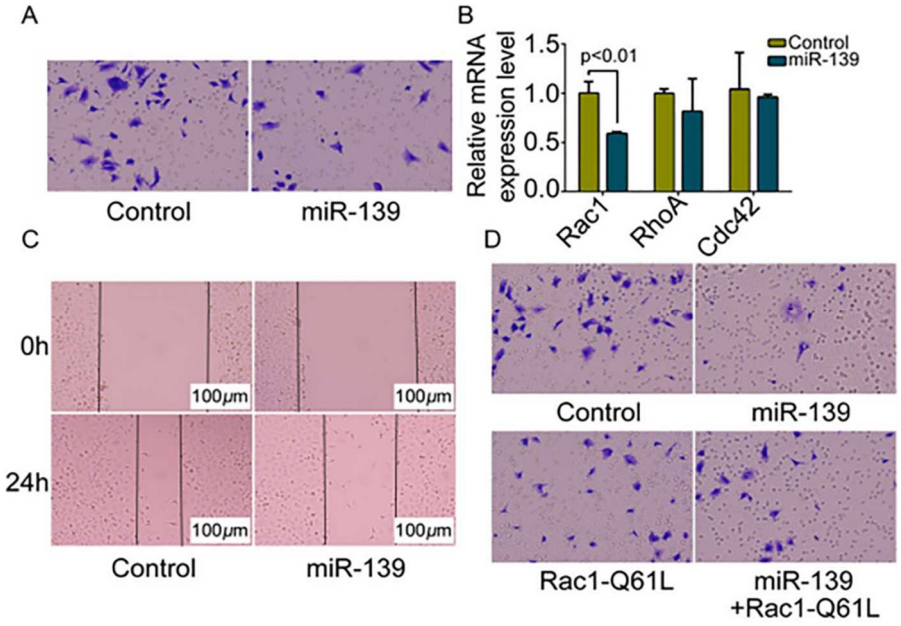

Fig. 3: miR-139 inhibits lung cancer cell migration, invasion and Rac1 activation.
(A. Invasion assay (×100 magnification) The miR-139 group exhibited significantly fewer invading cells compared to controls, indicating miR-139's potent inhibitory effect on lung cancer cell invasiveness. B. RT-PCR analysis of Rho GTPase expression: miR-139 specifically downregulated Rac1 mRNA expression ($p < 0.01$), while RhoA and Cdc42 levels remained unchanged, suggesting selective targeting of Rac1. C. Wound healing assay: Scratch wound closure was markedly slower in miR-139-treated cells at 24h versus controls (scale bar = 100 μm), confirming impaired migration. D. Rescue experiment with constitutively active Rac1 (Q61L): Overexpression of Rac1-Q61L partially reversed miR-139-mediated invasion suppression, demonstrating Rac1's pivotal role in this regulatory mechanism.) Note: Data are mean ± SEM; **$p<0.01$ vs. control by Student's t-test (n=3 independent experiments).

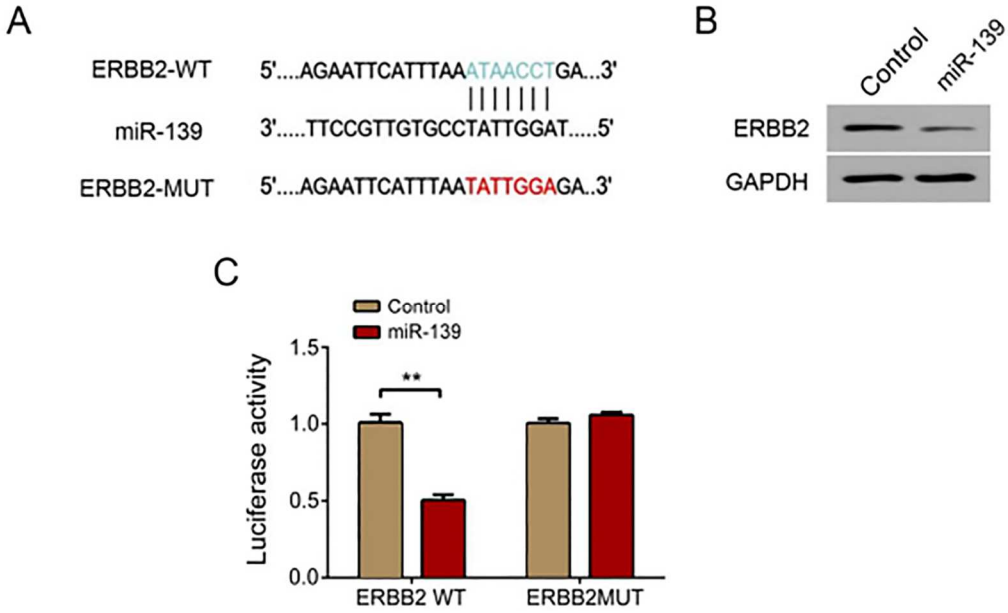

Fig. 4: ERBB2 is a target of miR-139.
(A. Bioinformatics prediction of miR-139-ERBB2 interaction: TargetScan analysis revealed perfect complementarity between miR-139's seed region and the wild-type (WT) ERBB2 3'UTR, while this binding was disrupted in the mutant (MUT) sequence (Fig. 3A), suggesting a direct regulatory relationship. B. ERBB2 protein suppression by miR-139: Western blot analysis demonstrated significant downregulation of ERBB2 protein in miR-139-treated cells compared to controls (Fig. 3B). GAPDH served as the loading control, confirming equivalent protein input across samples. C. Functional validation through luciferase assay: The luciferase reporter assay demonstrated a 70% reduction in activity for ERBB2-WT + miR-139 ($p<0.01$) but no significant change for ERBB2-MUT, confirming miR-139's specific suppression of ERBB2 through 3'UTR binding.)

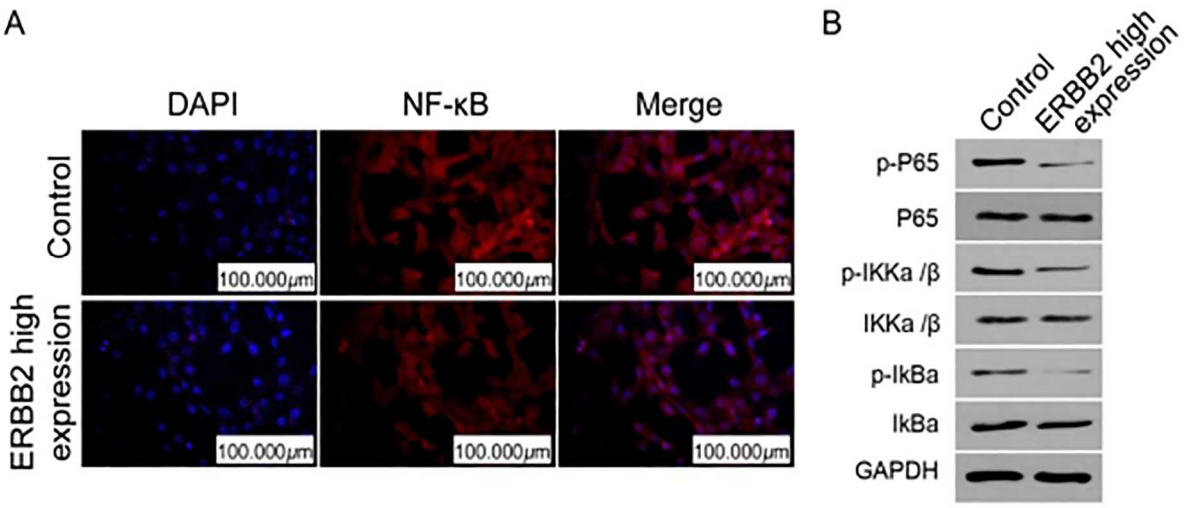

Fig. 5: NF-κB is down-regulated in ERBB2-positive lung cancer tissues.
A shows the fluorescence signal detection, and B shows the protein expression detection.

Statistical analysis

All values were displayed as mean ± standard deviation (SEM) and assessed by student's t test. $P<0.05$ means a significance.

RESULTS

miR-139 is down-regulated in metastatic lung cancer tissues

miR-139 was significantly decreased in lung cancer tissues (Fig. 2A) and lymph node metastatic tissues (Fig. 2B).

miR-139 inhibits cell invasion and Rac1 activation

miR-139 mimics significantly reduced cell migration and invasion (Fig. 3A) .Quantitative analysis showed miR-139 reduced Rac1-GTP levels by 65±8% ($P<0.01$), while RhoA and Cdc42 activities remained unchanged (RhoA: 102±5%; Cdc42: 98±3%, $p>0.05$) (Fig. 3B). We next determined whether Rac1 activation is required for cell migration and invasion and found that overexpression of miR-139-mediated inhibition of cell migration was rescued by constitutively activating Rac1-Q61L (Fig. 3C). miR-139 can significantly inhibit the invasion of lung cancer cells (as shown in Fig. 3D).

ERBB2 acts miR-139 target

Software predicts ERBB2 to be miR-139 target (Fig. 4A). Dual luciferase assay showed decreased relative luciferase activity after transfection of pGL3-ERBB2-3'-UTR and miR-139 mimics (Fig. 4B). In addition, ERBB2 in miR-139 transfected cells was significantly reduced (Fig. 4C).

NF-κB is reduced in lung cancer tissues

Compared with ERBB2-negative lung cancer, the signal expression of most ERBB2-positive lung cancer samples was suppressed (Fig. 5A) along with downregulated p-IκBα and p-P65 protein (Fig. 5B).

DISCUSSION

Lung cancer is highly malignant, and its metastatic mechanism is not fully understood. (Wilson, EA *et al*., 2021). We found reduced miR-139 level in lung cancer samples, Our study reveals that **miR-139 is significantly downregulated in lung cancer tissues**, particularly in lymph node metastases (Fig. 2A-B), corroborating its role as a tumor suppressor, as previously reported in prostate cancer and TNBC (Nam, RK *et al*., 2021). Here, we provide mechanistic evidence that miR-139 suppresses metastasis by targeting the ERBB2/Rac1/NF-κB axis, offering new insights into lung cancer progression. This is the first study to prove that miR-139 can regulate the growth of lung cancer tissues. Meanwhile, ERBB2 and miR-139 showed negative feedback regulation. miR-139 in most metastatic lymph node tissues in ERBB2-positive lung cancer was further reduced.

miR-139 overexpression can inhibit cyclin D1 level and cell proliferation (Bao, B *et al*., 2022). Low miR-139 level can up-regulate microchromosome maintenance protein 2 (MCM2) (Tanigawa, K *et al*., 2023). We demonstrate for the first time that **miR-139 directly targets ERBB2** (HER2), a key oncogene in solid tumors (Yoon, J *et al*., 2024, Sanz-Moreno, A *et al*., 2021), via binding to its 3′-UTR (Fig. 4A-B). ERBB2 overexpression is linked to poor prognosis, and our data show that miR-139 restoration reduces ERBB2 protein levels (Fig. 4C), thereby attenuating downstream signaling. Intriguingly, miR-139 levels were further suppressed in ERBB2-positive metastatic lymph nodes, suggesting a negative feedback loop that exacerbates invasion. In this article, our experiments verified the inhibitory effect of miR-139 on ERBB2-positive lung cancer cells via inhibiting ERBB2 and Rac1 activation. In high ERBB2 cells, TGF-beta recruits actin and actinomycin to HER2, which then

activates Rac1 and increases cell invasion (Kato, C *et al*., 2024). Studies have reported that without activation of Rac1, ERBB2-induced migration is unlikely to occur (Conlon, NT *et al*., 2021). P130Cas/PI3K/Akt/Rac1 signaling regulate ERBB2-dependent invasion of breast epithelial cells in 3D culture (Pan, L *et al*., 2024). Consistently, we found ERBB2/Rac1 signaling regulates lung cancer cells.

Notably, miR-139 selectively suppressed Rac1 but not RhoA or Cdc42. This specificity may arise from miR-139's indirect regulation of Rac1 through ERBB2, as ERBB2-driven TGF-β signaling is known to activate Rac1 but not other Rho GTPases in lung adenocarcinoma (Kato, C *et al*., 2024). Conversely, RhoA and Cdc42 are primarily regulated by alternative pathways (e.g., LPA for RhoA) (Tran, KC *et al*., 2021), which may explain their insensitivity to miR-139. In addition, anti-HER2 drugs can block the NF-κB activity in SKBR3 cells, reflecting the effect of specific IKK inhibitors (De La, *et al*., 2024; Cruz P *et al*., 2024, Wu, Q *et al*., 2022). Our data showed that NF-κB activity (p-P65) was significantly reduced in ERBB2-positive tumors with low miR-139. This contrasts with breast cancer models where HER2 activates NF-κB (Zhang, L *et al*., 2023), suggesting tissue-specific signaling crosstalk. Mechanistically, NF-κB inhibition may suppress metastasis by downregulating MMP-9 (a known NF-κB target (Mirzaei, S *et al*., 2022) and inducing apoptosis via Bcl-2 suppression (Han, D *et al*., 2022), as observed in our invasion assays. While our study establishes miR-139's role in ERBB2/Rac1/NF-κB signaling, further work is needed to Validate these findings in in vivo models of lung cancer metastasis.

CONCLUSION

miR-139 may at least partially regulate NF-κB activity by targeting ERBB2/Rac1 signaling pathway during human lung cancer cell migration and invasion.

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Authors' contributions
Jiao He: Conceptualization, Methodology, Investigation, Formal analysis, Writing - Original Draft.
Yaolan Zhen: Validation, Investigation, Data Curation, Visualization.
Lei Liu: Supervision, Project administration, Writing - Review & Editing.
All authors have read and approved the final manuscript.

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Data availability statement
The data that support the findings of this study are available from the corresponding author upon reasonable request.

Ethical approval
The use of patient-derived lung cancer cells in this study has been approved by the Ethics Review Committee, with approval number GLY2024028.

Conflicts of interest
There are no conflicts to declare.

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