Heliyon 10 (2024) e35832

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Overexpression of mir-489–3p inhibits proliferation and migration of non-small cell lung cancer cells by suppressing the HER2/PI3K/AKT/Snail signaling pathway

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ARTICLE INFO

Keywords:
Non-small cell lung cancer
miR-489–3p
HER2
Proliferation
Migration

ABSTRACT

Background: Lung cancer is a highly prevalent malignancy with significant morbidity and mortality rates. MiR-489–3p, a microRNA, has been identified as a regulator of tumor cell proliferation and invasion. Its expression is downregulated in non-small cell lung cancer (NSCLC). Elucidating the molecular mechanisms underlying miR-489–3p′s role in NSCLC pathogenesis is crucial for identifying potential diagnostic and therapeutic targets.

Methods: To investigate the molecular mechanism of miR-489–3p in NSCLC, this study utilized A549, a commonly used NSCLC cell line. MiR-489–3p mimics and inhibitors were transfected into A549 cells. Additionally, co-transfection experiments using wortmannin, an inhibitor of the PI3K/AKT pathway, were performed. Expression of miR-489–3p and related proteins was analyzed by Western blotting and quantitative real-time PCR (qRT-PCR). Cell migration and proliferation were assessed by wound healing and colony formation assays, respectively.

Results: Overexpression of miR-489–3p significantly inhibited the proliferation and migration of A549 cells. This inhibitory effect was further enhanced upon co-transfected with wortmannin. Analysis of human lung specimens showed increased expression of HER2, PI3K, and AKT in lung adenocarcinoma tissues compared to adjacent non-cancerous tissues.

Conclusions: These findings suggest that miR-489–3p overexpression may inhibit NSCLC cell proliferation and migration by suppressing the HER2/PI3K/AKT/Snail signaling pathway. This study elucidates miR-489–3p′s molecular mechanisms in NSCLC and provides experimental basis for identifying early diagnostic markers and novel therapeutic targets.

1. Introduction

Lung cancer is a prevalent malignant tumor with high morbidity and mortality worldwide [1]. Lung adenocarcinoma (LUAD)

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https://doi.org/10.1016/j.heliyon.2024.e35832
Received 19 December 2023; Received in revised form 2 August 2024; Accepted 5 August 2024
Available online 6 August 2024

accounting for approximately 40 % of lung cancer cases and its incidence continues to increase significantly [2,3]. Due to its slow progression and subtle symptoms, LUAD is often diagnosed at advanced stages or with distant metastasis, leading to 5-year survival rates of less than 13 % [4,5]. In recent years, the in-depth study of the molecular mechanism of lung cancer and the clinical application of gene therapy have led to some advances in lung cancer treatment. However, effective early diagnostic methods remain lacking and pathogenesis is incompletely understood. Therefore, elucidating the molecular pathogenesis of lung cancer will facilitate the identification of biomarkers for early diagnosis and effective therapeutic targets.

Non-coding RNA, especially microRNAs (miRNAs), have emerged as critical regulators of cancer pathogenesis. MiRNAs are short, non-coding RNAs of approximately 20–25 nucleotides in length [6]. They are widely involved in regulating key biological processes including cell differentiation, proliferation, metabolism, drug resistance and cell death [7–9]. Aberrant miRNA expression strongly correlates with cancer initiation, progression, diagnosis, treatment response and prognosis [10,11]. Further elucidation of the molecular mechanisms underlying miRNA-mediated regulation will facilitate the development of novel therapeutics and personalized targeted therapy therapies.

Previous studies have shown that miR-489–3p can regulate tumor cell growth and invasion [12–14], and may serve as an independent prognostic risk factor [15]. MiR-489–3p expression has been found to be downregulated in non-small cell lung cancer (NSCLC) [15,16], however its potential molecular mechanisms in the occurrence and development of NSCLC remain unclear. Human epidermal growth factor receptor 2 (HER2) is a transmembrane receptor tyrosine kinase belonging to the epidermal growth factor receptor (EGFR) family. Aberrant HER2 expression is closely associated with tumor development and aggressiveness [17–19]. While absent in normal lung tissues, HER2 is overexpressed in lung cancer and can promote proliferation and survival of NSCLC, breast cancer, and colorectal cancer cells [20–23]. Additionally, HER2 may also regulate cell proliferation and viability by activating downstream phosphatidylinositide3-kinase (PI3K)/protein kinase B (AKT) signaling pathway [24].

In this study, we analyzed HER-2/PI3K/AKT expression in human LUAD tissues by immunohistochemistry. We also revealed the effects of miR-489–3p on A549 cell proliferation and migration in vitro. Our results shed light on the miR-489–3p/HER2/PI3K/AKT/Snail regulatory axis in NSCLC pathogenesis and provide a rationale for targeted therapeutic strategies.

2. Materials and methods

2.1. Cell culture

The human lung adenocarcinoma cell line A549 was purchased from the Procell Life Science & Technology Co., Ltd (Wuhan, China). A549 cells were cultured in Ham's F–12 K medium (PM150910, Procell, China), supplemented with 10 % fetal bovine serum (FBS, Sijiqing, China), within an incubator maintained at 37 °C with a 5 % CO2 atmosphere.

2.2. Transfection of miRNA mimics and miRNA inhibitor

miR-489–3p mimics (5′-GUGACAUCACAUAUACGGCAGC UGCCGUAUAUGUGAUGUCACUU-3′), miR-NC mimics (5′-UUGUCCGAACGUGUCACGUTT-3′), miR-489–3p inhibitor (5′-GCUGCCGUAUAUGUGAUGUCAC-3′) and miR-NC inhibitor (5′-CAGUACUUUUGUGUAGUACAA-3′) were purchased from Shanghai GenePharma Co., Ltd. A549 cells (0.5×10^6 cells/well) were seeded in 6-well plates. MiRNA mimics or inhibitors were transfected into the cells using Lipofectamine® 3000 (Invitrogen; Thermo Fisher Scientific, Inc., USA) within 24 h, when the cells were approximately 70%confluent. According to the manufacturer's instructions, miRNA (150 pmol) was mixed with Lipofectamine 3000 (5 μL) and P3000 (5 μL) in serum-free medium. The mixture was allowed to stand at room temperature for 10–15 min, during which the culture medium in the wells was replaced with serum-free medium. Subsequently, the mixture was added to each well and incubated at 37 °C. After 6–9 h of transfection, the culture medium was replaced with fresh medium containing 10 % FBS. The cells were then cultured for an additional 48 h at 37 °C before proceeding with the subsequent experiments.

2.3. Cell viability assay

A549 cells were seeded at a density of 2×10^3 cells per well in 96-well plates and then incubated in 10 % FBS medium for 24 h. Cells were collected at 0, 12,24,48, and 72 h post-transfection. After transfection, 20 μL of MTT solution(5 mg/ml) (M8180, Solarbio, China) was added to the appropriate test wells. Following continuous incubation for 4 h, 150 μL of DMSO solution was added to each well and shaken for 30 min at 37 °C. The absorbance was then measured at a wavelength of 490 nm.

2.4. RNA isolation and quantitative RT-PCR (qRT-qPCR)

Total RNA was isolated from A549 cells using TRIzol® reagent (Invitrogen; Thermo Fisher Scientific, Inc., USA). The concentration of total RNA was determined using NanoDrop 2000 (Thermo Fisher Scientific, Inc., USA). To detect the expression levels of miR-489–3p, RNA was reverse transcribed using the Hairpin-it™ miRNAs and U6 One-step qRT-PCR Probe Kit (GenePharma Co., China) according to the manufacturer's protocol. U6 served as an internal control for miR-489–3p. Relative mRNA expression levels of Snail were normalized to GAPDH. The primer sequences were as follows: miR-489–3p forward, 5′-ACACTCCAGCTGGGGTGACATCACATATAC-3′ and reverse, 5′-CTCAACTGGTGTCGTGGAGTCGG-3'. U6 forward, 5′-ACACTCCAGCTGGGCGCAAATTCGTGAAGC-3′ and reverse, 5′-CTCAACTGGTGTCGTGGAGTCGG-3'. Snail forward, 5′-TGGTTCTTCTGCGCTACTGC-3′, reverse, 5′-

GCTGGAAGGTAAACTCTGGATT-3'. GAPDH forward, 5′-AATGGGCAGCCGTTAGGAAA-3′, reverse, 5′-GCCCAATACGACCAAATCAGAG-3'. The relative expression levels were calculated using equation $2^{-\Delta\Delta Ct}$.

2.5. Protein isolation and western blotting

A549 cells were lysed using a RIPA lysis buffer (Sigma-Aldrich; Merck KGaA). Proteins were separated by SDS-PAGE. After electrophoresis(80 V/20min, 120 V/60min), the proteins were semi-dry electrophoretic transferred(25 V/35min) to PVDF membranes, blocked with 5 % skim milk at room temperature for 2 h. The membranes were then incubated with a primary antibody against HER-2 (HUABIO, ER0106, China), Snail (Affinity, AF6032, USA) and β-actin (BOSTER, BM0627, China) at 4 °C overnight. After three washes in TBST, they were incubated with the secondary antibody at room temperature for 1 h. Following three more washes, the membranes were detected by an ECL chemiluminescence Substrate kit (Biosharp, Beijing, China). The intensity of the bands was analyzed using ImageJ.

2.6. Wound healing assay

The wound healing assay was used to assess cell migratory capacity. Transfected A549 cells (0.5×10^6/well) were seeded in a 6-well plate for the assay. Once the cell monolayer covered the surface, wounds were created by scratching with a 10 μL pipette tip. Wound images were captured at 0, 12, 24 and 36 h, and wound healing percentage was calculated by ImageJ.

2.7. Colony formation assay

Transfected A549 cells were cultured in a 6-well plate (1000/well). The plate was inoculated at 37 °C with 5 % CO2 for 14 days. After 14 days, cell colonies were stained with crystal violet solution and counted using ImageJ.

2.8. Statistical analysis

All statistical data were analyzed using GraphPad Prism 8.0.2. Results were presented as mean ± SD (standard deviation). Student's t-test was used for comparing differences between two groups, while one-way or two-way analysis of variance (ANOVA) was used for three groups or more. Differences were considered statistically significant at $p < 0.05$.

3. Results

3.1. The expression of miR-489–3p in LUAD cells

The expression levels of miR-489–3p were measured in human bronchial epithelial cell Beas-2b, NSCLC cell lines A549, and

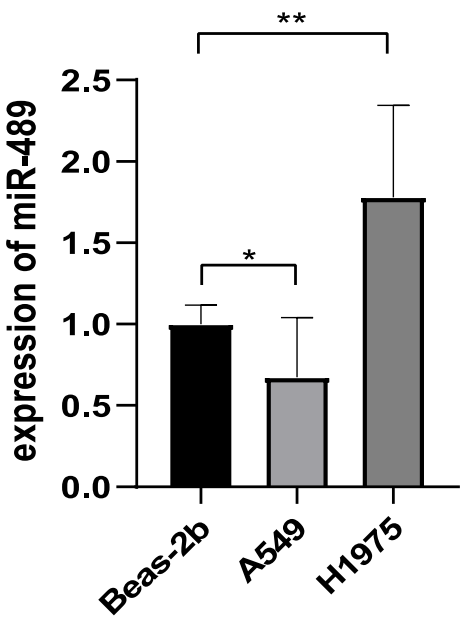

Fig. 1. The Expressions of miR-489 in lung adenocarcinoma. Expressions of miR-489–3p in human bronchial epithelial cell Beas-2b, NSCLC cell lines A549 and H1975 by qRT-PCR. (n = 3, *$P < 0.05$ **$P < 0.01$).

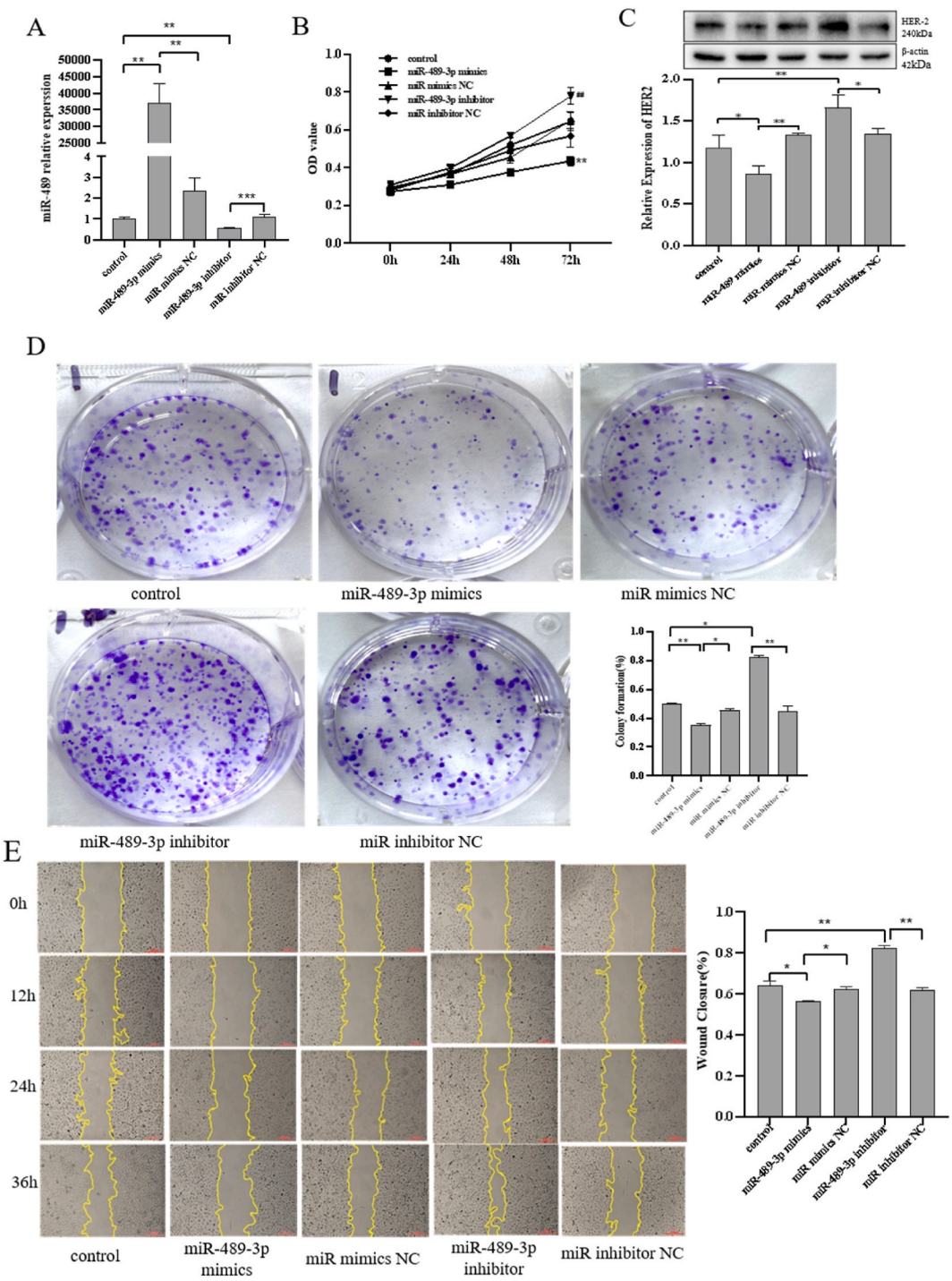

Fig. 2. The effect of miR-489 on the proliferation and migration of lung adenocarcinoma cells. Compared with miR mimics NC and miR inhibitor NC group, (A) miR-489–3p levels were significantly increased observed in the miR-489–3p mimics group, miR-489–3p inhibitor group was significantly decreased. (B) miR-489–3p mimics decreased the cell viability, while miR-489–3p inhibitor increased the cell viability (n = 5, $^{**}P < 0.01$ *vs* miR mimics NC, $^{\#\#}P < 0.01$ *vs* miR inhibitor NC). (C) miR-489–3p mimics decreased HER2 at protein expression levels, while miR-489–3p inhibitor increased the HER2 expression levels. (D) miR-489–3p mimics inhibited cell proliferation, while miR-489–3p inhibitor promoted cell proliferation. (E) miR-489–3p mimics inhibited the cell migratory ability of A549 cells, miR-489–3p inhibitor promoted the cell migratory ability. (n = 3, $^{*}P < 0.05$ $^{**}P < 0.01$).

NCI–H1299 using qRT-PCR. The results demonstrated that, in comparison to BEAS-2b, A549 cells exhibited lower levels of miR-489–3p cells, whereas H1975 displayed higher levels of miR-489–3p (Fig. 1).

3.2. The effect of miR-489–3p on the proliferation and migration of LUAD cells

To investigate the role of miR-489–3p in NSCLC, miR-489–3p mimics and miR-489–3p inhibitors were transfected into A549 cells. RT-qPCR was performed to detect the miR-489–3p expression levels in A549. In comparison to the miRNA mimics NC group, the miR-489–3p levels were significantly increased observed in the miR-489–3p mimics group, while compared to the miRNA inhibitor NC group, miR-489–3p levels were significantly decreased in the miR-489–3p inhibitor group (Fig. 2A). Subsequently, we performed an MTT assay to determine the effect of miR-489–3p on the cell viability of A549 cells. Relative to the miR mimics NC and miRNA inhibitor NC groups, miR-489–3p mimics decreased the cell viability, whereas miR-489–3p inhibitor increased cell viability (Fig. 2B). To validate the regulatory effect of miR-489–3p on HER2 expression, the HER2 expression levels were analyzed. Compared to the miRNA mimics NC and miRNA inhibitor NC groups, miR-489–3p mimics significantly decreased HER2 protein expression levels in A549 cells, while miR-489–3p inhibitor increased HER2 expression levels (Fig. 2C). Furthermore, results from the cell colony formation assay showed that miR-489–3p mimics inhibited cell proliferation, whereas miR-489–3p inhibitor promoted cell proliferation (Fig. 2D). In addition, the wound healing assay corroborated these findings, showing that miR-489–3p mimics inhibited the migratory ability of A549 cells, while miR-489–3p inhibitor promoted cell migration (Fig. 2E). miR-489–3p is associated with the expression of HER2, and miR-489–3p may affect LUAD cell proliferation and migration through HER2.

3.3. Expression of HER-2/PI3K/AKT expression in LUAD tissues

We conducted survival analysis on 180 samples, including 88 cases of adjacent non-cancerous tissue and 92 cases of LUAD patients, and observed a significant difference in survival rates among clinical stages 1, 2, 3 and 4, with a p-value of 0.007 (Fig. 3A). Subsequently, we performed HE staining on LUAD tissues. In comparison with adjacent tissues, we clearly observed the proliferation and canceration of basal cells beneath the epithelium of bronchioles in LUAD (Fig. 3B). By immunohistochemistry staining, we found enhanced expression of HER2/PI3K/AKT in LUAD tissues when compared to adjacent tissues (Fig. 3C).

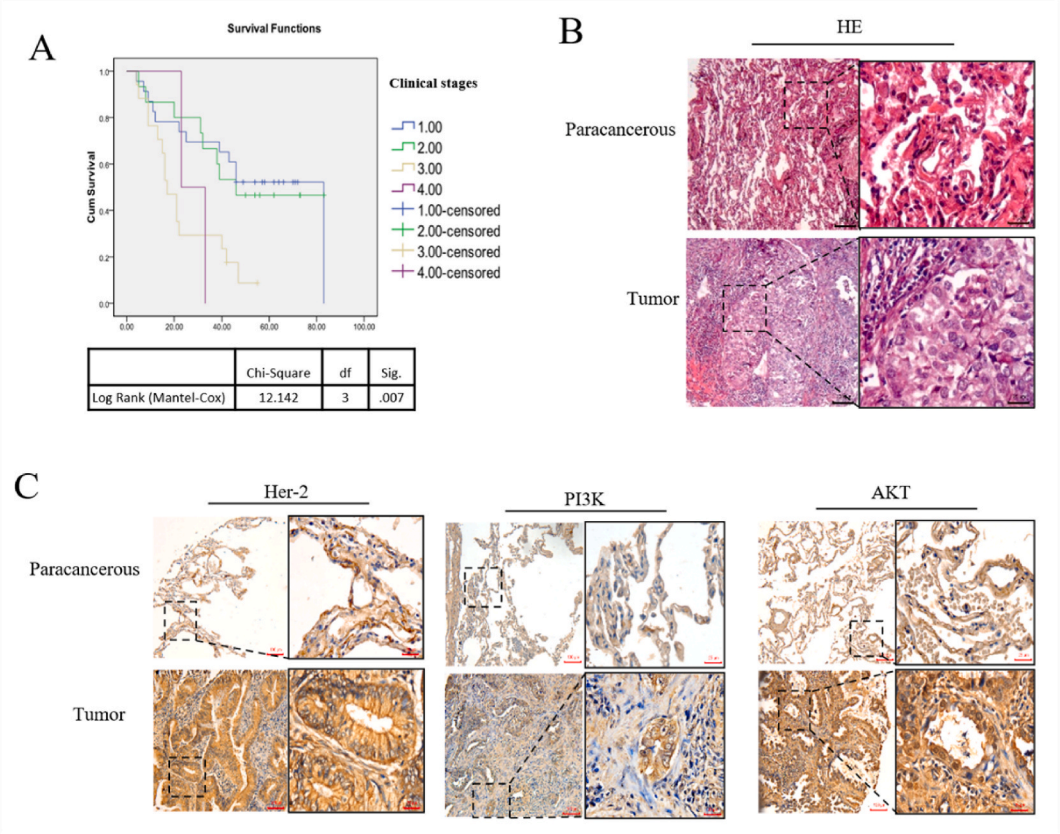

Fig. 3. HER-2/PI3K/AKT expression in lung adenocarcinoma tissues. (A) The survival rate of patients with clinical stage 1, 2, 3 and 4, $P = 0.007$. (B) Lung adenocarcinoma tissue and paracancerous tissue were stained for HE. (C) HER2/PI3K/AKT were stained for immunohistochemistry in lung adenocarcinoma tissue and paracancerous tissue (Fig. 3C).

3.4. Inhibition of PI3K may affect on LUAD cells and snail expression

To confirm the regulatory role of PI3K in LUAD, we overexpressed miR-489–3p and subsequently applied the PI3K inhibitor wortmannin (1 μM,MCE, HY-10197) to A549 cells. We then analyzed the expression levels of Snail. We performed an MTT assay to assess the effect of wortmannin on the cell viability of A549 cells. Compared to the control group, both the miR-489–3p mimics group and the wortmannin group exhibited decreased cell viability. Co-transfection of miR-489–3p mimics and wortmannin intensified this effect (Fig. 4A). Furthermore, we observed that miR-489–3p mimics group and the wortmannin group, in comparison to the control group, exhibited decreased Snail levels at both the mRNA and protein expression levels in A549 cells. Co-transfection of miR-489–3p

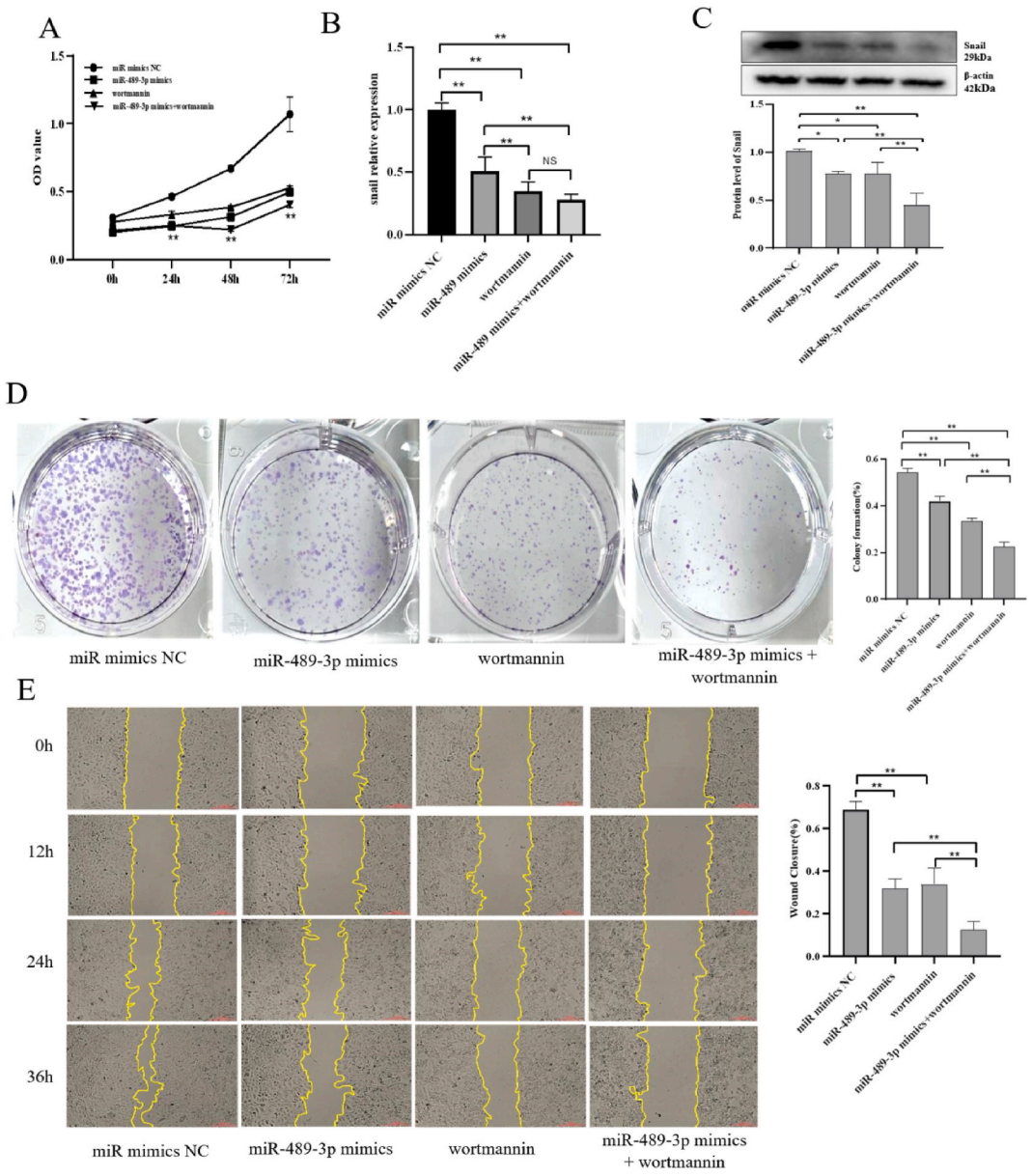

Fig. 4. Inhibit the effects of PI3K on lung adenocarcinoma cells and the expression of snail. (A) MTT assay detected the effects of wortmannin on the cell viability of A549, compared with control group, miR-489–3p mimics group and wortmannin group decreased the cell viability, co-transfected miR-489–3p mimics and wortmannin aggravate this phenomenon. (B– C) Compared with control group, miR-489–3p mimics group and wortmannin group decreased Snail at mRNA and protein expression levels in A549 cells, co-transfected miR-489–3p mimics and wortmannin decreased Snail levels more significantly. (D) miR-489–3p mimics and wortmannin decreased the cell proliferation ability, after co-transfection miR-489–3p mimics and wortmannin, the cell proliferation ability decreased more significantly. (E) miR-489–3p mimics and wortmannin inhibited the cell migratory ability of A549 cells, and co-transfected miR-489–3p mimics and wortmannin aggravate the decline of cell migration ability. (n = 3, $*P < 0.05$ $**P < 0.01$).

mimics and wortmannin led to a more significant reduction in Snail levels (Fig. 4B and C). In the cell colony formation assay, we found that we found that the miR-489–3p mimics group and the wortmannin group showed reduced cell proliferation ability compared to the control group. Co-transfection of miR-489–3p mimics and wortmannin resulted in a more substantial decrease in cell proliferation ability (Fig. 4D). The wound healing assay further confirmed these findings. Both the miR-489–3p mimics and wortmannin inhibited the migratory ability of A549 cells when compared to the control group. Co-transfection of miR-489–3p mimics and wortmannin exacerbated the decline in cell migration ability (Fig. 4E). miR-489–3p may affect LUAD cell proliferation and migration through PI3K/AKT.

4. Discussion

NSCLC, one of the most prevalent lung cancer subtypes, presents a significant global health threat. Despite extensive research, the precise etiology of lung cancer remains unclear. The 5-year survival rate for patients with advanced lung adenocarcinoma is only about 10 % [25]. Consequently, there has been a surge of interest in recent years in studying immunotherapy, targeted therapy, and other approaches to treating lung cancer. The primary aim is to discover safe and effective treatment methods, understand the mechanisms underlying the development of lung cancer, and identify suitable treatment targets. In this study, we investigated the expression levels of miR-489–3p in lung cancer cells and made a noteworthy discovery. We found that miR-489–3p is expressed at low levels in lung cancer cells. Moreover, when we artificially increased the expression of miR-489–3p, it had a profound inhibitory effect on the proliferation and migration of A549 cells, which are a specific type of lung cancer cells.

MiR-489–3p has been implicated in the occurrence and development of various tumors, primarily demonstrating tumor-inhibitory effects in different cancer types. Studies have shown its involvement in melanoma [26], breast cancer [27], ovarian cancer [28], prostate cancer [29], and pancreatic cancer [30], where it has been shown to suppress tumor growth and progression. In the case of NSCLC, miR-489–3p may serve as a significant prognostic marker [15]. Low expression of miR-489–3p in NSCLC tissue has been associated with poor prognosis in patients [31]. In our study, we compared the expression levels of miR-489–3p in A549 cells with those in human bronchial epithelial cells. The results showed that miR-489–3p was expressed at lower levels in A549 cells. To further investigate its role in lung cancer, we conducted experiments using miR-489–3p inhibitors and mimics in A549 cells.

We found that overexpression of miR-489–3p significantly reduced the viability of A549 cells and decreased the rate of cell colony formation. Additionally, the wound healing assay revealed that the healing rate was markedly slowed following the overexpression of miR-489–3p. Conversely, inhibiting miR-489–3p produced opposite effects. These alterations may be mediated through impacts on cell cycle regulation, proliferation, apoptosis pathways, and metabolic processes. miR-489–3p could directly or indirectly modulate key genes associated with cell proliferation, such as cyclins and cyclin-dependent kinases (CDKs), and apoptosis-related genes, such as those in the Bcl-2 family, thereby affecting cell viability. It might also regulate processes of proliferation and migration by modulating epithelial-mesenchymal transition (EMT) associated transcription factors (e.g., Snail and Slug) or by affecting EMT marker genes (e.g., E-cadherin). Among these pathways, the PI3K/AKT signaling pathway is likely to be a major signaling pathway regulating cell proliferation and survival. miR-489–3p could regulate cell viability by influencing key components of this pathway. Therefore, we have conducted further studies on the HER2/PI3K/AKT/Snail signaling pathway. Further research is warranted to gain a comprehensive understanding of its molecular mechanisms and interactions with target genes. This knowledge could pave the way for novel therapeutic strategies in the treatment of NSCLC and potentially other cancers.

HER2 is known to be highly expressed in various tumors, including breast, ovary, stomach, and lung cancer [32,33]. Numerous studies have confirmed that HER2 overexpression can promote tumor cell proliferation and inhibit apoptosis in NSCLC [22,23]. In our investigation using human tissue specimens, we observed an increase in the expression of HER2, PI3K, and AKT in lung adenocarcinoma tissue compared to adjacent tissues. Based on these findings, we formulated a hypothesis that miR-489–3p might regulate the proliferation and migration of A549 cells through the HER2/PI3K/AKT/Snail pathway. Our data suggest that there is a relationship between the expression of HER2 and miR-489–3p in A549 cells. This indicates that miR-489–3p may play a role in modulating the activity of the HER2/PI3K/AKT/Snail pathway, which could be crucial for A549 cell functions, including proliferation and migration. Further research is needed to fully elucidate the intricate molecular mechanisms involved in this pathway and to determine how miR-489–3p precisely influences HER2 and downstream signaling components.

As of now, the precise mechanism of how miR-489–3p functions in the progression of NSCLC remains unclear. However, it is known that the PI3K/AKT signaling pathway plays a critical role in regulating NSCLC tumorigenesis and progression [34]. A pertinent has demonstrated that GINS2 promotes EMT in non-small cell lung cancer by modulating the PI3K/AKT signaling pathway [35]. Given the significance of the PI3K/AKT pathway in NSCLC, inhibitors targeting this pathway have shown potential anti-tumor activity. Wortmannin, for instance, is a PI3K inhibitor known to effectively block the PI3K signaling pathway. In our research, we employed wortmannin to inhibit the PI3K pathway, resulting in reduced proliferation and migration capabilities. Furthermore, co-transfection of miR-489–3p mimics with wortmannin in our study led to a more pronounced decrease in cell proliferation and migration compared to wortmannin alone. Additionally, the expression of Snail, a downstream component of the PI3K/AKT pathway was also reduced.

Our study demonstrates that overexpression of miR-489–3p inhibits the proliferation and migration of LUAD cells by targeting the HER2/PI3K/AKT/Snail signaling pathway. This finding has significant implications for understanding the role of the PI3K/AKT pathway in LUAD progression, as it is often dysregulated in cancer, driving uncontrolled growth and resistance to apoptosis. By suppressing this pathway, miR-489–3p may act as a tumor suppressor in LUAD, suggesting that the PI3K/AKT pathway is not only a key driver of cancer progression but also a potential therapeutic target. The downstream mechanism Snail, a transcription factor involved in EMT, which is critical for cancer metastasis. Snail promotes EMT by repressing epithelial markers and inducing mesenchymal genes, enhancing cell migration and invasion. The suppression of Snail by miR-489–3p indicates that the microRNA may

inhibit EMT, preventing LUAD cells from acquiring a more invasive and metastatic phenotype. For instance, Snail regulates genes involved in cell adhesion, migration, and invasion, and identifying these genes could reveal new therapeutic targets. Of course, further characterization of the interaction between miR-489–3p and the PI3K/AKT/Snail pathway is also necessary, including determining the direct targets of miR-489–3p and the mechanisms by which it inhibits Snail expression. In summary, our study suggests that miR-489–3p and the PI3K/AKT/Snail pathway are key factors in LUAD progression. Elucidating their interactions and downstream effects could lead to the development of new diagnostic and therapeutic strategies for this devastating disease.

These results suggest that miR-489–3p may influence A549 cells by regulating the PI3K/AKT signaling pathway. Co-treatment with miR-489–3p mimics and wortmannin appears to have a synergistic effect in inhibiting cell proliferation and migration, possibly through Snail pathway suppression. However, further investigations are needed to fully understand the intricate molecular mechanisms of miR-489–3p in NSCLC progression and its interaction with the PI3K/AKT pathway. Our study has revealed the potential tumor-suppressing effects of miR-489–3p in NSCLC, opening up new possibilities for the development of treatment strategies and presents a novel approach to miRNA therapy. miR-489–3p could serve as a new biomarker for the early diagnosis and prognostic assessment of lung cancer. The expression level of miR-489–3p may become an important indicator for evaluating the condition of NSCLC patients and predicting treatment outcomes. In future research, it would be valuable to further explore the mechanisms of action of miR-489–3p in NSCLC and its potential role in other types of cancer. As our understanding of miR-489–3p deepens, we have reason to believe that it will play an increasingly important role in the diagnosis, treatment, and prognostic assessment of NSCLC.

In this study, we acknowledge several limitations and shortcomings for improvement. T Although we analyzed the expression of miR-489–3p in multiple cell lines and clinical samples, the scarcity of fresh clinical specimens restricted our analysis to only three representative cell types, this limitation may have influenced our assessment of miR-489–3p expression patterns in NSCLC. To address this, we intend to investigate miR-489–3p expression in a more diverse array of clinical samples in subsequent experiments. Moreover, we are committed to conducting comprehensive research on the mechanisms by which miR-489–3p functions and to exploring potential therapeutic strategies.

5. Conclusion

In conclusion, our study indicates that the overexpression of miR-489–3p may inhibit the malignant progression of A549 cells by modulating the HER2/PI3K/AKT/Snail pathway. These findings suggest that miR-489–3p may have a tumor-suppressive role in NSCLC and could potentially serve as a promising therapeutic target for this disease. Nevertheless, it is imperative to emphasize that these findings require further experimental validation and verification. Subsequent experiments should be dedicated to thoroughly investigating the mechanism by which miR-489–3p influences NSCLC. Further studies could delve into the precise molecular interactions and downstream effects of miR-489–3p in NSCLC cells, elucidating its involvement in critical cellular processes such as proliferation, migration, and apoptosis. Moreover, comprehensive research may illuminate potential interactions between miR-489–3p and other signaling pathways, providing a more holistic understanding of its role in NSCLC pathogenesis.

Ethics statement

This study was approved by Ethics Committee of Shanghai Xinchao Biotechnology Co., Ltd. (ID: SHYJS-CP-2206001).

Funding

This work is supported by the Horizontal Cooperation project with The First Affiliated Hospital of Shaoyang University (2020HX03) and the Natural Sciences Foundation of Hunan Province (2022JJ50192).

Data availability

Data will be made available on request.

CRediT authorship contribution statement

Di Cheng: Project administration, Data curation. **Zhong Liu:** Project administration, Data curation. **Renren Sun:** Software, Methodology, Investigation. **Yun Jiang:** Software, Methodology. **Zhaoming Zeng:** Software, Formal analysis. **Rui Zhao:** Writing – original draft, Conceptualization. **Zhongcheng Mo:** Writing – review & editing, Supervision.

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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e35832.

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