Heliyon 10 (2024) e38428

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

miR-6884-5p inhibits proliferation and epithelial-mesenchymal transition in non-small cell lung cancer cells

Lianyong Zhang [a], Wei Chi [a], Xue Wang [a], Jingjing Li [a], Fei Li [a], Yuxia Ma [b], Qianyun Zhang [a,*]

[a] Department of Pulmonary and Critical Care Medicine (PCCM) ward II, Cangzhou Central Hospital, No. 16 Xinhua West Road, Cangzhou, 061000, Hebei, China

[b] Department of Geriatrics, Cangzhou Central Hospital, No. 16 Xinhua West Road, Cangzhou, 061000, Hebei, China

ARTICLE INFO

Keywords:
Non-small cell lung cancer
miR-6884-5p
Apoptosis
Epithelial-mesenchymal transition

ABSTRACT

Background: Non-small cell lung cancer (NSCLC) is associated with a high mortality and morbidity rate. MicroRNAs participate in tumorigenesis, progression and metastasis of NSCLC. However, miR-6884-5p has not been previously studied. This study aimed to investigate the role of miR-6884-5p in NSCLC and explore its underlying mechanisms.
Methods: We used miR-6884-5p mimics and inhibitors to assess its effects in NSCLC. miR-6884-5p expression levels in NSCLC cell lines were quantified using qRT-PCR. Cell viability was determined using a cell-counting kit 8 assay. Western blot analysis was employed to measure apoptotic proteins. The impact of miR-6884-5p on cell proliferation was assessed via colony formation assay. Furthermore, Transwell assays were utilized to visualize and quantify the effects of miR-6884-5p on NSCLC migration and invasion.
Results: miR-6884-5p mimic significantly inhibited NSCLC cell proliferation to 71.21 % and 72.26 % of control at 5 days of culture time in H460 and HC9 cells (both $p < 0.01$), respectively, while miR-6884-5p inhibitor significantly promoted cell proliferation to 119.66 % and 126.44 % of control at 5 days of culture time in H460 and HC9 cells (both $p < 0.05$), respectively. In addition, miR-6884-5p promoted apoptosis by reducing the anti-apoptotic protein B-cell lymphoma 2 (BCL2) protein and increasing apoptotic protein BCL2 associated X protein (all $p < 0.01$ at least). Moreover, miR-6884-5p effectively suppressed transforming growth factor β1-induced epithelial-mesenchymal transition, as evidenced by the restored expression of E-cadherin ($p < 0.01$), N-cadherin ($p < 0.01$) and Vimentin ($p < 0.05$), leading to the inhibition of migration and invasion in NSCLC cell lines.
Conclusions: Our findings demonstrate that miR-6884-5p can inhibit NSCLC cell proliferation, migration, and invasion, suggesting its potential as a therapeutic target for NSCLC treatment.

Funding

The study was supported by Key Research Project of Cangzhou City (204106180).

* Corresponding author.
E-mail address: zhangqianyun001@163.com (Q. Zhang).

https://doi.org/10.1016/j.heliyon.2024.e38428
Received 27 June 2023; Received in revised form 17 September 2024; Accepted 24 September 2024
Available online 25 September 2024
2405-8440/© 2024 Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

Non-small cell lung cancer (NSCLC), including a variety of different lung cancers, most notably large cell carcinoma, squamous cell carcinoma and adenocarcinoma, ranks as the second most prevalent cancer in developing countries and is the third leading cause of cancer-related deaths in women, accounting for approximately 500,000 new cases and 300,000 deaths annually [1,2]. The incidence of lung cancer has risen alongside a decrease in the median age at diagnosis. Despite advancements in diagnostic and treatment modalities including various radiotherapies [3,4], the 5-year overall survival rate for NSCLC remains below 30 % [5,6]. Therefore, investigating the molecular mechanisms and identifying new biomarkers of lung cancer is crucial for improving its diagnosis and treatment.

Studies have demonstrated that non-coding RNAs, including microRNAs (miRNAs), play significant roles in the differentiation, proliferation, and apoptosis of tumor cells [7–10]. Several candidate miRNAs in lung cancer have been identified as either oncogenic or tumor-suppressing factors, with abnormal miRNA serving as potential biomarkers for lung cancer diagnosis [11,12]. For example, miR-7 regulates apoptosis and malignant behavior of HeLa and C33A cells in lung cancer by targeting X-linked inhibitor of apoptosis [13]. Additionally, previous study has indicated that upregulation of miR-145 is associated with aggressive progression and poor prognosis in lung cancer [14–18]. Moreover, overexpression of miR-183, hsa-miR-182, hsa-miR-210, hsa-miR-200 and hsa-miR-21 are related to tumor progression, showing their potential as diagnostic biomarkers [19]. In particular, long non-coding RNA RHPN1-AS1 was able to promote ovarian carcinogenesis by sponging miR-6884-5p [20]. Malignant behaviors could be driven by E2F1-activated LINC01224 in esophageal squamous cell carcinoma (ESCC) via targeting miR-6884-5p [21]. A more recent study suggests that miR-6884-5p participated in the regulatory role of circ_0023990 in cell invasion, proliferation and glycolysis of ESCC [22]. These results indicate that miR-6884-5p may play an important role in tumorigenesis. miRNAs play crucial roles in tumorigenesis, progression, and metastasis through various mechanisms. For example, some miRNAs function as oncogenes by downregulating tumor suppressor genes [23]. miRNAs can influence the cell cycle and apoptosis by regulating genes involved in these processes [24]. Moreover, miRNAs can either promote or inhibit epithelial-mesenchymal transition (EMT) [25].

Our previous study reveals that miR-6884-5p expression is downregulated in NSCLC tissues, and its level is inversely correlated with EMT [26]. However, it is a pity that there is no more research of miR-6884-5p on NSCLC and the detailed underlying mechanisms remain largely unknown. Therefore, the present study aims to further substantiate the regulatory role of miR-6884-5p in NSCLC cell proliferation and EMT through additional in *in vitro* experiments.

2. Methods

2.1. Cells

The NSCLC cells lines (A549, H1299, H460, PC9, and H358) and human normal lung epithelial cells (BEAS-2B) were purchased from the American Type Culture Collection (Manassas, VA). These cells were cultured in RPMI 1640 supplemented with 100 units/mL penicillin, 0.1 mg/mL streptomycin, and 10 % fetal bovine serum (Gibco, Grand Island, NY). Cells were incubated in a humidified atmosphere of 5 % CO_2 in air at 37 °C and maintained in logarithmic growth phase.

The miR-6884-5p mimics, inhibitors and corresponding negative controls were synthesized and purchased from RiboBio Company (Guangzhou, China). Cells seeded in 6-well plates were transfected with the constructed plasmids for 48 h by employing Lipofectamine 3000 (Invitrogen, Carlsbad, CA). The transfection time was based on the protocol provided by the manufacturer and current literatures. H460 and PC9 were chosen for most of the experiments due to their lowest expression of miR-6884-5.

2.2. qRT-PCR

Total RNA extraction was carried out using the RNA Easy Fast Tissue/Cell Kit purchased from Tiangen (Beijing, China). The RNA was reverse transcribed to cDNA, and quantified PCR was performed using CFX Connect Real-Time PCR Detection System (Bio-Rad, Hercules, CA). U6 was used as an internal control for normalization. Relative gene expression levels were calculated using $2^{-\Delta\Delta Ct}$ method. qRT-PCR primers (5′-3′): miR-6884-5p: Forward AGAGGCTGAGAAGGTGATGT, Reverse GAACATGTCTGCGTATCTC; U6: Forward CTCGCTTCGGCAGCACA, Reverse AACGCTTCACGAATTTGCGT.

2.3. Cell-counting kit 8 (CCK8) assay

Cell viability was assessed by CCK8 assay (Vazyme Biotec, Nanjing, China). Cells were seeded in 96-well plates and incubated for 1, 2, 3, 4, 5 days following transfection with miR-6884-5p mimics, inhibitor, or negative control.

2.4. Western blot

Total protein was extracted from the cells using radioimmunoprecipitation assay buffer (Beyotime Biotechnology, Shanghai, China), and the protein concentration was determined with the BCA Protein Assay Reagent (Thermo Fisher Scientific, Waltham, MA). Forty micrograms of proteins were loaded onto 10 % sodium dodecyl sulfate-page gels. The separated proteins were transferred to polyvinylidene fluoride membrane and blocked with 5 % non-fat milk for 1 h at room temperature. Primary antibodies used in this study included anti-BAX (BCL2 Associated X) (ab32503, Abcam, Shanghai, China), anti-BCL2 (B-cell lymphoma 2) (ab32124, Abcam),

anti-E-cadherin (ab40772, Abcam), anti-N-cadherin (ab76011, Abcam), anti-Vimentin (ab92547, Abcam) and anti-GAPDH (ab8245, Abcam) antibodies. The bands were quantified using image J software.

2.5. Colony formation assay

For the colony formation assay, cells were plated at a density of 2000 cells per well in 6-well plates and allowed to grow for approximately 10 days. After incubation, colonies were fixed with methanol and stained with 0.5 % crystal violet. Colonies were then counted to assess proliferation and colony formation ability.

2.6. Cell migration and invasion

Cell migration and invasion were determined using Transwell 24-well plates with 8 μm pore size inserts (Corning, NY). After 36 h of stimulation, the cells were fixed with 4 % paraformaldehyde and stained with 1 % crystal violet solution to visualize and quantify migration and invasion capabilities.

2.7. Statistical analysis

Data was exemplified as mean ± standard deviation (SD). Kolmogorov-Smirnov test was used to test the normality of the data before comparisons. Two groups that met normal distribution were conducted using unpaired *t*-test with Welch's correction. More than two groups that met normal distribution were conducted using Welch's ANOVA test followed Dunnett's T3 multiple comparisons test. Data are regarded as statistically relevant if the $P < 0.05$.

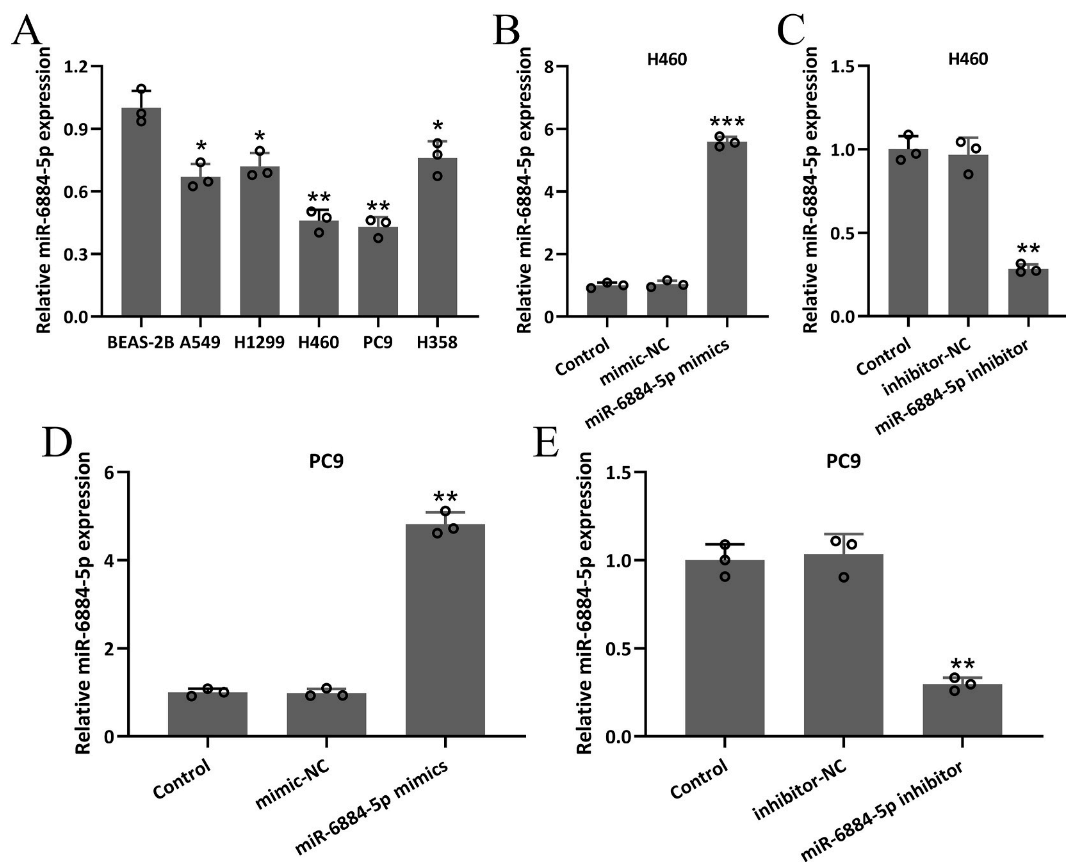

Fig. 1. The expressions of miR-6884-5p in non-small cell lung cancer cells lines. A, qRT-PCR was used to analyze the expressions of miR-6884-5p among different NSCLS cells (U6 was used as a loading control and human normal lung epithelial cells BEAS-2B was used a negative control). The expressions were normalized to BEAS-2B. H460 and PC9 were transfected with miR-6884-5p mimics, inhibitors and corresponding negative controls for 48 h qRT-PCR was used to analyze the expressions of miR-6884-5p (B–E). n = 3. Data were shown as mean ± SD. $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ compared to control. Welch's ANOVA test followed Dunnett's T3 multiple comparisons test.

3. Results

3.1. The expressions of miR-6884-5p in non-small cell lung cancer cells lines

We initially assessed miR-6884-5p expression levels across various cell types: normal cells BEAS-2B, and NSCLS cells, including A2549, H1299, H460, PC9, H358. Compared to BEAS-2B cells, the NSCLC cell lines exhibited notably reduced miR-6884-5p expression, with H460 (decreased by 0.462 ± 0.051, $p = 0.008$) and PC9 (decreased by 0.428 ± 0.047, $p = 0.006$) cells showing particularly significant downregulation (Fig. 1A). Other cell lines (A2549, H1299, H358) also showed a significant decrease in miR-6884-5p expression as well, but the reduction was less pronounced. Subsequently, H460 and PC9 cells were selected for further experiments. Treatment with miR-6884-5p mimics substantially increased miR-6884-5p expression in both H460 (increased by 5.585 ± 0.163, $p < 0.001$ compared to control) and PC9 (increased by 4.797 ± 0.266, $p = 0.003$ compared to control), whereas treatment with miR-6884-5p inhibitors led to a significant decrease in miR-6884-5p levels (decreased by 0.284 ± 0.027 and 0.297 ± 0.037, $p = 0.003$ and 0.002 compared to control) (Fig. 1B-E). These results indicate that miR-6884-5p expression was downregulated in the NSCLC cell lines.

3.2. miR-6884-5p inhibits proliferation of H460 and PC9 cells

To assess the impact of miR-6884-5p on cell proliferation, we carried out CCK8 assays on H460 and PC9 cell lines. Cell viability was measured at 1, 2, 3, 4, and 5 days post-transfection. It was found that miR-6884-5p mimic notably suppressed cell viability in both H460 and HC9 cells (about 71.21 % and 72.26 % of control at 5 days of culture time, $p = 0.007$ and $p = 0.005$) (Fig. 2A and C). Conversely, miR-6884-5p inhibitor significantly promoted cell viability in both cell lines (about 119.66 % and 126.44 % of control at 5 days of culture time, $p = 0.021$ and $p = 0.014$) (Fig. 2B and D). The results demonstrate the regulatory role of miR-6884-5p in NSCLC proliferation.

3.3. miR-6884-5p promotes apoptosis of H460 and PC9 cells

We further evaluated the effects of miR-6884-5p on cell proliferation and apoptosis in H460 and PC9 cells. The protein levels of the

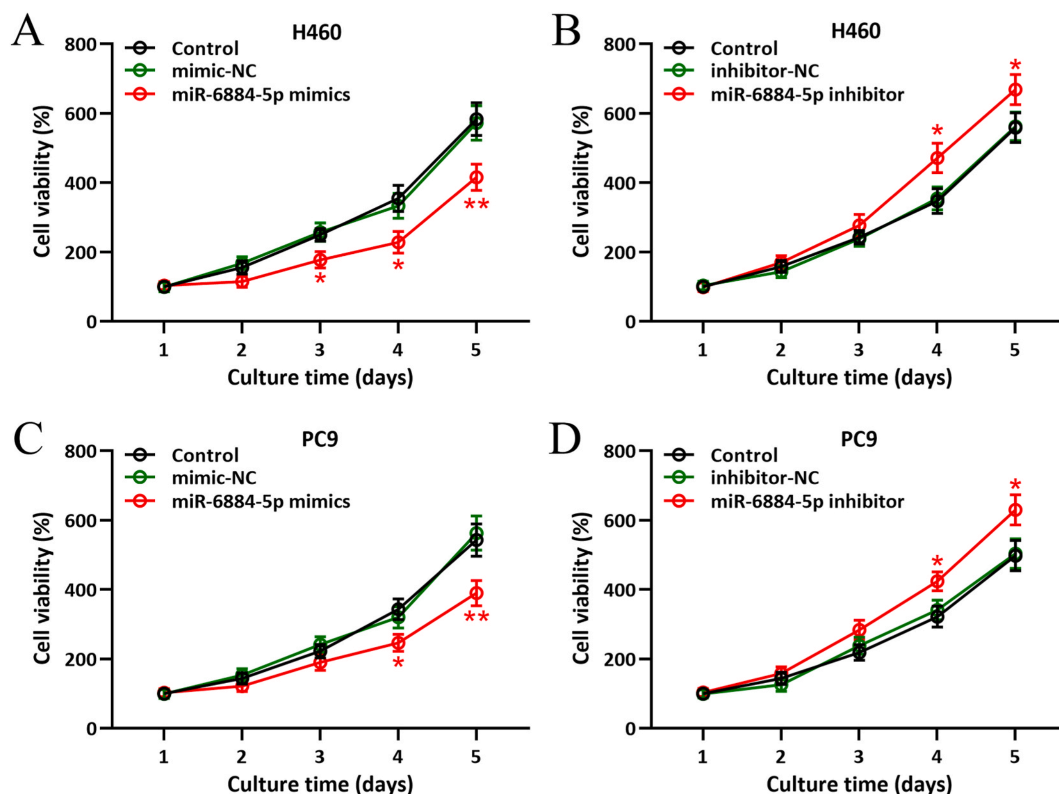

Fig. 2. miR-6884-5p inhibits proliferation of H460 and PC9 cells. H460 and PC9 were transfected with miR-6884-5p mimics, inhibitors and corresponding negative controls. At 1, 2, 3, 4 and 5 days after the transfection, cell viability was measured by CCK8 (A–D). n = 6. Data were shown as mean ± SD. *p < 0.05, **p < 0.01 compared to control. Welch's ANOVA test followed Dunnett's T3 multiple comparisons test.

proapoptotic molecule BAX and the anti-apoptotic molecules BCL2 were determined using Western blot. Compared to control, we observed miR-6884-5p mimic treatment induced a significant increase in BAX levels in H460 (increased by 3.274 ± 0.251, $p = 0.003$) and in PC9 cells (increased by 2.879 ± 0.242, $p = 0.002$), and a decrease in BCL2 levels in H460 (decreased by 0.319 ± 0.038, $p < 0.001$) and in PC9 cells (decreased by 0.437 ± 0.053, $p = 0.004$) (Fig. 3A-F). Furthermore, to further investigate the impact of miR-6884-5p on cell proliferation, we conducted a colony formation assay 10 days post-transfection. The results showed a significant reduction in the number of colonies formed by H460 and PC9 cells in the miR-6884-5p mimics group compared to controls (Fig. 3G-H). In summary, these findings indicate that miR-6884-5p promotes apoptosis and inhibits proliferation of H460 and PC9 cells.

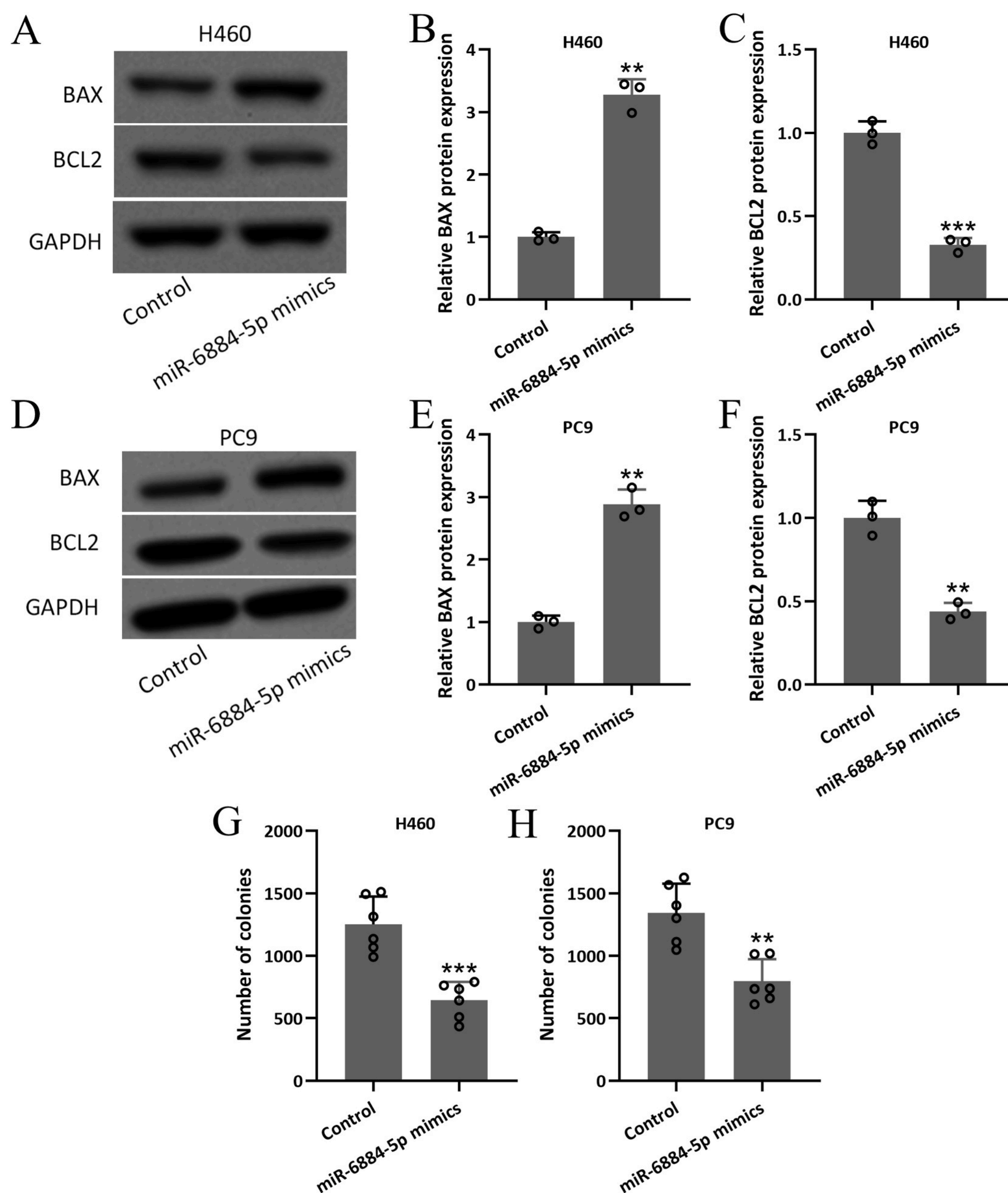

Fig. 3. miR-6884-5p promotes apoptosis and inhibits proliferation of H460 and PC9 cells. H460 and PC9 were transfected with miR-6884-5p mimics for 72 h. The protein level of proapoptotic molecules BAX, and anti-apoptotic molecules BCL2 were examined by Western blot (A and D). The uncropped gels were provided in the supplementary materials. GAPDH was used as a loading control and the expressions were normalized to control (B, C and E, F). n = 3. The colony formation assay was conducted 10 days after transfection and the number of colonies were calculated (G and H). n = 6. Data were shown as mean ± SD. **p < 0.01, ***p < 0.001 compared to control. Unpaired *t*-test with Welch's correction.

3.4. miR-6884-5p inhibits transforming growth factor (TGF)-β1-induced migration and invasion of H460 cells

We then investigated whether miR-6884-5p could inhibit cell migration and invasion in H460 cells. H460 cells were transfected with miR-6884-5p mimics for 48 h, followed by stimulation with TGF-β1 (10 ng/ml) for 36 h. The Transwell assays were performed to assess cell migration and invasion. Our results showed that the number of migrated cells (Fig. 4A, TGF-β1: 470.03 ± 62.09, TGF-β1+miR-mimics: 309.31 ± 46.75, $p = 0.001$) as well as invasive cells (Fig. 4B, TGF-β1: 389.68 ± 48.94, TGF-β1+miR-mimics: 223.72 ± 35.53, $p < 0.001$) were significantly reduced upon treatment with miR-6994-5p mimics when compared to TGF-β1 treatment group. These findings collectively suggest that miR-6884-5p can effectively inhibit TGF-β1-induced migration and invasion in H460 cells.

3.5. miR-6884-5p inhibits TGF-β1-induced EMT of H460 cells

We then evaluated whether miR-6884 could inhibit TGF-β1-induced EMT in H460 cells. The protein levels of EMT-related markers, including E-cadherin, N-cadherin and Vimentin, were examined using Western blot analysis (Fig. 5A). Our findings indicated that E-cadherin expression was significantly reduced in the TGF-β1 treatment group, but this reduction was attenuated by miR-6884-5p mimics treatment (Fig. 5B, TGF-β1: 0.342 ± 0.047, TGF-β1+miR-mimics: 1.032 ± 0.121, $p = 0.005$). Conversely, the protein levels of N-cadherin and Vimentin were markedly increased in response to TGF-β1 stimulation, and miR-6884-5p effectively suppressed the elevation of N-cadherin (TGF-β1: 6.784 ± 0.593, TGF-β1+miR-mimics: 2.634 ± 0.315, $p = 0.003$) and Vimentin protein levels (TGF-β1: 4.372 ± 0.317, TGF-β1+miR-mimics: 3.172 ± 0.263, $p = 0.001$) (Fig. 5C and D). Overall, these data suggest that miR-6884-5p inhibits TGF-β1-induced EMT.

4. Discussion

In recent years, significant progress has been made in the diagnosis and treatment of NSCLC, utilizing therapies such as chemotherapy, radiotherapy, immunotherapy, and targeted therapies like tyrosine kinase inhibitors. However, despite these advances, the 5-year overall survival rate for NSCLC patients remains disappointingly low [27,28]. Recurrence and tumor metastasis continue to pose significant challenges in clinical cancer treatment [29]. Therefore, there is critical need to develop novel therapeutic approaches targeting tumor invasiveness and motility to overcome current limitations in lung cancer treatment [30,31]. In this study, we investigated the anti-tumor effects of miR-6884-5p on two NSCLC cell lines *in vitro*. Our findings demonstrate that miR-6884-5p exerts cytotoxic effects on NSCLC cells, inducing apoptosis by modulating the BCL2/BAX ratio. Additionally, miR-6884-5p inhibits cell migration and invasion, and mitigates the dysregulation of key oncogenic factors during EMT in NSCLC cells.

Aberrant expression of miRNAs is closely linked to tumorigenesis, influencing processes such as malignant transformation, apoptosis, invasion and metastasis of cancer [32–36]. miRNAs are intricately involved in cancer regulation by targeting specific genes and influencing multiple signaling pathways [37–40]. They play pivotal roles in metastasis and EMT in NSCLC. For example, miR-107 has been shown to inhibit hepatocellular carcinoma proliferation and metastasis by modulating the Wnt signaling pathway [41]. Similar regulatory roles have been observed in other cancer types, where Zhang et al. also showed such regulatory events in pre-adipocytes [42]. Li et al. found that the miR-6884-3p/CCNB1 axis is involved in the tumorigenic behavior of hepatocellular carcinoma cells mediated by RP11-295G20.2 [43].

However, research specifically linking miR-6884-5p to known signaling pathways in NSCLC is still relatively limited. Based on

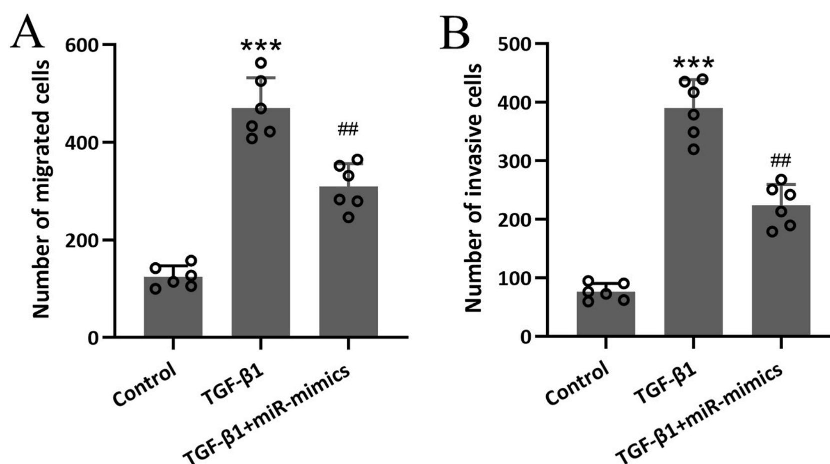

Fig. 4. miR-6884-5p inhibits TGF-β1-induced migration and invasion of H460 cells. H460 cells were transfected with miR-6884-5p mimics for 48 h and then stimulated with TGF-β1 (10 ng/mL) for 36 h. The cell migration and invasion were determined using Transwell 24-well plates. The number of migrated and invasive cells were compared (A, B). n = 6. Data were shown as mean ± SD. ***p < 0.001 compared to control, ##p < 0.01 compared to TGF-β1. Welch's ANOVA test followed Dunnett's T3 multiple comparisons test.

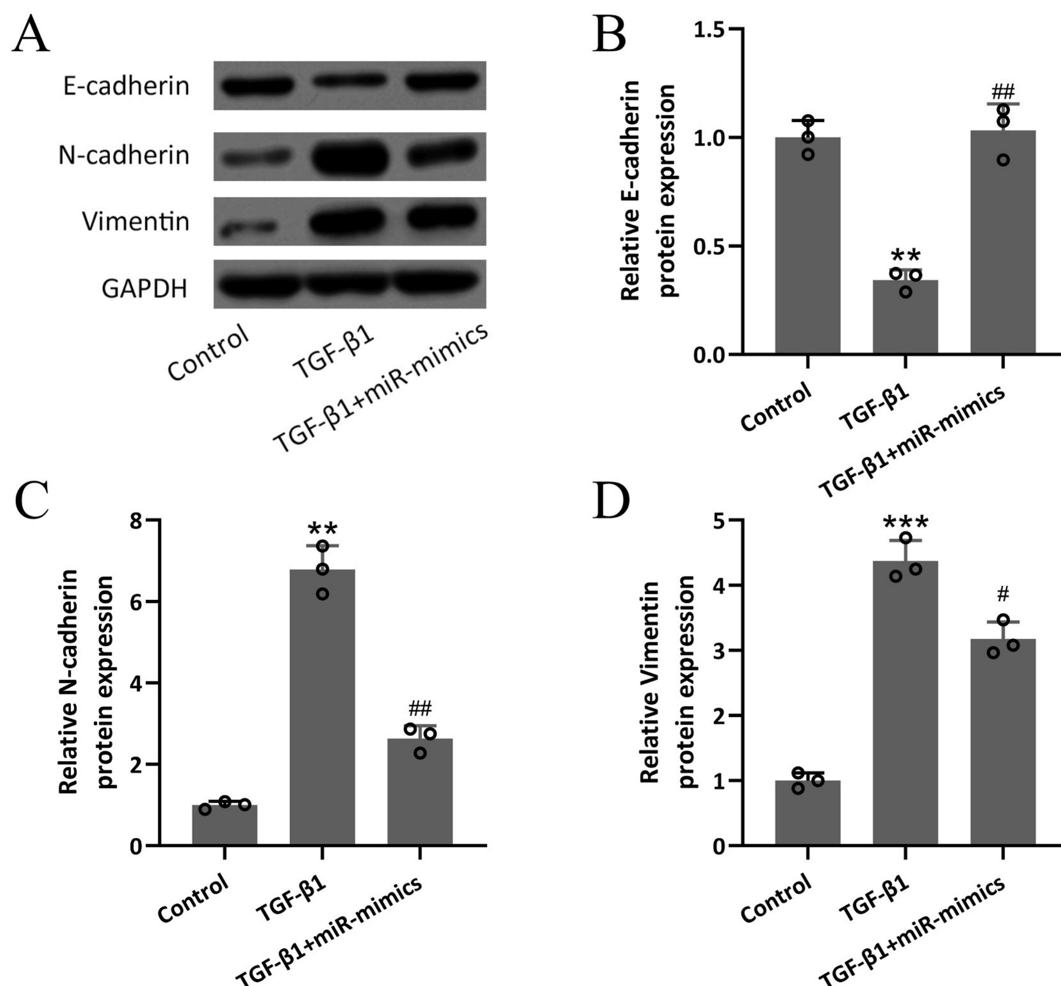

Fig. 5. miR-6884-5p inhibits TGF-β1-induced EMT of H460 cells. H460 cells were transfected with miR-6884-5p mimics for 48 h and then stimulated with TGF-β1 (10 ng/mL) for 36 h. The protein level of EMT-related proteins including E-cadherin, N-cadherin and Vimentin were detected by Western blot (A). The uncropped gel was provided in the supplementary materials. GAPDH was used as a loading control and the expressions were normalized to control (B–D). n = 3. Data were shown as mean ± SD. **p < 0.01, ***p < 0.001 compared to control, #p < 0.05, ##p < 0.01 compared to TGF-β1. Welch's ANOVA test followed Dunnett's T3 multiple comparisons test.

general miRNA mechanisms and potential interactions, miR-6884-5p might influence key pathways involved in NSCLC. For example, miR-6884-5p may target genes involved in PI3K/AKT/mTOR, potentially acting as a tumor suppressor by downregulating components of this pathway, leading to reduced cell proliferation and tumor growth. Components of PI3K/Akt/mTOR signaling pathway have emerged as promising targets for microRNA-based therapies [44]. It was revealed that miR-4524b-5p attenuated the proliferation and radioresistance of glioblastoma via PI3K/AKT/mTOR signaling [45]. MAPK/ERK pathway, also involved in cell proliferation and survival, could be another important target for miR-6884-5p. A very recent study suggests that miR-889-3p could promote the development of retinoblastoma through MAPK/ERK signaling pathway [46].

Metastasis represents a significant cause of cancer-related mortality, and EMT is pivotal in facilitating this process [47]. EMT involves a series of stages including local invasion, internal invasion, external invasion and distant proliferation, making it a complex and currently uncontrollable process. A hallmark of EMT is the downregulation of E-cadherin coupled with increased expression of N-cadherin and vimentin [48–51]. Our study observed that miR-6884-5p introduction resulted in decreased E-cadherin expression and increased levels of N-cadherin and vimentin in NSCLC cell lines. These findings suggest that miR-6884-5p induces EMT in NSCLC cells. Previous research has also demonstrated that downregulation of miR-6884-5p in esophageal squamous cell carcinoma promotes cell proliferation, invasion, and migration, while its upregulation inhibits these processes. Our results align with these findings, indicating that miR-6884-5p inhibits cell proliferation and EMT processes in NSCLC. Mechanically, our previous study suggests that miR-6884-5p directly binds to the 3′UTR of S100A16, leading to the inhibition of S100A16 expression, which then contributes to its inhibitory effect on EMT process in NSCLC [26].Certainly, there are several limitations in our current study that warrant further investigation. Firstly, while we have demonstrated the anti-cancer effects of miR-6884-5p *in vitro*, validating these findings with *in vivo* data is crucial to confirm their relevance in a physiological context. Secondly, elucidating the specific mechanisms and signaling pathways through

which miR-6884-5p exerts its inhibitory effects on NSCLC requires further detailed study. Understanding these pathways could provide insights into potential therapeutic targets. Thirdly, assessing the expression of miR-6884-5p in clinical specimens from NSCLC patients is essential to establish its clinical relevance and potential as a biomarker. Correlating miR-6884-5p expression levels with clinical outcomes such as patient survival and response to treatment would provide valuable insights into its role in NSCLC progression and prognosis. Addressing these limitations will be crucial for advancing our understanding of miR-6884-5p′s role in NSCLC and its potential as a therapeutic target or diagnostic marker.

Although our results demonstrate the potential of miR-6884-5p based therapy for NSCLC treatment, how to deliver miR-6884-5p specifically to lung cancer cells without affecting normal tissues is still a major challenge. Additionally, ensuring that miR-6884-5p reach the target cells in sufficient quantities is difficult due to the potential degradation by nucleases in the bloodstream and clearance by the immune system. To date, various delivery systems, such as nanoparticles, liposomes, and viral vectors, have been developed, each comes with its own set of issues, including toxicity, immune reactions, and the risk of unintended gene modifications. This requires more safe and efficient delivery strategies.

5. Conclusion

In summary, the current study identified the anti-tumor effects of miR-6884-5p *in vitro*. Specifically, miR-6884-5p can inhibit NSCLC cell proliferation and promote cell apoptosis by regulating BAX/BCL2 ratio. Furthermore, miR-6884-5p can inhibit migration, invasion, and EMT process by suppressing TGF-β1 in NSCLC. The current results suggest the regulatory role of miRNAs in NSCLC, and reveal the potential of miR-6884-5p as a therapeutic target for the treatment of NSCLC. Nevertheless, the observed findings could be validated in clinical samples and NSCLC animal models in the future to fully unravel the importance of miR-6881-5p in NSCLC. Specifically, the levels of miR-6884-5p in patient blood samples should be investigated as a non-invasive biomarker for early detection of NSCLC. Similar to other microRNAs, miR-6884-5p holds promise as a therapeutic target in NSCLC due to its potential role in tumor suppression, and as a diagnostic/prognostic biomarker. However, further research is needed to fully understand its mechanisms and to develop effective strategies for clinical application. Besides, synthetic miR-6884-5p mimics and efficient delivery systems that can be delivered to NSCLC tissues should be developed in clinic.

Data availability statement

Data could be obtained upon request to the corresponding author.

CRediT authorship contribution statement

Lianyong Zhang: Writing – review & editing, Writing – original draft, Validation, Data curation. **Wei Chi:** Writing – review & editing, Writing – original draft, Data curation. **Xue Wang:** Writing – review & editing, Writing – original draft, Data curation. **Jingjing Li:** Writing – review & editing, Writing – original draft, Data curation. **Fei Li:** Writing – review & editing, Writing – original draft, Data curation. **Yuxia Ma:** Writing – original draft, Data curation. **Qianyun Zhang:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Funding acquisition, Data curation, Conceptualization.

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.

Abbreviations

(NSCLC) Non-small cell lung cancer
(EMT) epithelial-mesenchymal transition
(CCK8) Cell-counting kit 8
(SD) standard deviation
(BCL2) B-cell lymphoma 2
(BAX) BCL2 Associated X
(TGF) transforming growth factor

Appendix ASupplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e38428.

References

[1] N. Duma, R. Santana-Davila, J.R. Molina, Non-small cell lung cancer: epidemiology, screening, diagnosis, and treatment, Mayo Clin. Proc. 94 (8) (2019) 1623–1640, https://doi.org/10.1016/j.mayocp.2019.01.013.

[2] M. Yuan, L.L. Huang, J.H. Chen, J. Wu, Q. Xu, The emerging treatment landscape of targeted therapy in non-small-cell lung cancer, Signal Transduct Target Ther 4 (2019) 61, https://doi.org/10.1038/s41392-019-0099-9.

[3] Q. Bi, X. Lian, J. Shen, F. Zhang, T. Xu, Exploration of radiotherapy strategy for brain metastasis patients with driver gene positivity in lung cancer, J. Cancer 15 (7) (2024) 1994–2002, https://doi.org/10.7150/jca.91875.

[4] Q. Bi, J. Shen, P. Li, Y. Zeng, X. Lian, F. Zhang, Efficacy of whole-brain radiotherapy plus simultaneous integrated boost (SIB-WBRT) for lung cancer brain metastases, J. Cancer 15 (14) (2024) 4636–4642, https://doi.org/10.7150/jca.95804.

[5] M. Cao, H. Li, D. Sun, W. Chen, Cancer burden of major cancers in China: a need for sustainable actions, Cancer Commun. 40 (5) (2020) 205–210, https://doi.org/10.1002/cac2.12025.

[6] E.C. Ko, D. Raben, S.C. Formenti, The integration of radiotherapy with immunotherapy for the treatment of non-small cell lung cancer, Clin. Cancer Res. 24 (23) (2018) 5792–5806, https://doi.org/10.1158/1078-0432.CCR-17-3620.

[7] E. Hermyt, N. Zmarzly, B. Grabarek, C. Kruszniewska-Rajs, J. Gola, A. Jeda-Golonka, K. Szczepanek, U. Mazurek, A. Witek, Interplay between miRNAs and genes associated with cell proliferation in endometrial cancer, Int. J. Mol. Sci. 20 (23) (2019), https://doi.org/10.3390/ijms20236011.

[8] N.M. White, T.F. Chow, S. Mejia-Guerrero, M. Diamandis, Y. Rofael, H. Faragalla, M. Mankaruous, M. Gabril, A. Girgis, G.M. Yousef, Three dysregulated miRNAs control kallikrein 10 expression and cell proliferation in ovarian cancer, Br. J. Cancer 102 (8) (2010) 1244–1253, https://doi.org/10.1038/sj.bjc.6605634.

[9] J. Zhang, Q. Luo, X. Li, J. Guo, Q. Zhu, X. Lu, L. Wei, Z. Xiang, M. Peng, C. Ou, Y. Zou, Novel role of immune-related non-coding RNAs as potential biomarkers regulating tumour immunoresponse via MICA/NKG2D pathway, Biomark. Res. 11 (1) (2023) 86, https://doi.org/10.1186/s40364-023-00530-4.

[10] A. Zaravinos, J. Radojicic, G.I. Lambrou, D. Volanis, D. Delakas, E.N. Stathopoulos, D.A. Spandidos, Expression of miRNAs involved in angiogenesis, tumor cell proliferation, tumor suppressor inhibition, epithelial-mesenchymal transition and activation of metastasis in bladder cancer, J. Urol. 188 (2) (2012) 615–623, https://doi.org/10.1016/j.juro.2012.03.122.

[11] L. Wang, Z. Hu, Q. Guo, L. Yang, Y. Pang, W. Wang, MiR-23b functions as an oncogenic miRNA by downregulating Mcl-1S in lung cancer cell line A549, J. Biochem. Mol. Toxicol. 34 (7) (2020) e22494, https://doi.org/10.1002/jbt.22494.

[12] Z. Wu, Q. Yuan, C. Yang, X. Zhang, P. Qi, H. Huang, Z. Ma, Downregulation of oncogenic gene TGFbetaR2 by miRNA-107 suppresses non-small cell lung cancer, Pathol. Res. Pract. 216 (1) (2020) 152690, https://doi.org/10.1016/j.prp.2019.152690.

[13] A.P. Singh, Y.H. Hung, M.T. Shanahan, M. Kanke, A. Bonfini, M.K. Dame, M. Biraud, B.C.E. Peck, O.O. Oyesola, J.M. Freund, R.L. Cubitt, E.G. Curry, L. M. Gonzalez, G.A. Bewick, E.D. Tait-Wojno, N.A. Kurpios, S. Ding, J.R. Spence, C.M. Dekaney, N. Buchon, P. Sethupathy, Enteroendocrine progenitor cell-enriched miR-7 regulates intestinal epithelial proliferation in an xiap-dependent manner, Cell. Mol. Gastroenterol. Hepatol. 9 (3) (2020) 447–464, https://doi.org/10.1016/j.jcmgh.2019.11.001.

[14] Y. Chang, W. Yan, C. Sun, Q. Liu, J. Wang, M. Wang, miR-145-5p inhibits epithelial-mesenchymal transition via the JNK signaling pathway by targeting MAP3K1 in non-small cell lung cancer cells, Oncol, Lett 14 (6) (2017) 6923–6928, https://doi.org/10.3892/ol.2017.7092.

[15] W.C. Cho, A.S. Chow, J.S. Au, Restoration of tumour suppressor hsa-miR-145 inhibits cancer cell growth in lung adenocarcinoma patients with epidermal growth factor receptor mutation, Eur. J. Cancer 45 (12) (2009) 2197–2206, https://doi.org/10.1016/j.ejca.2009.04.039.

[16] J. Hu, M. Qiu, F. Jiang, S. Zhang, X. Yang, J. Wang, L. Xu, R. Yin, MiR-145 regulates cancer stem-like properties and epithelial-to-mesenchymal transition in lung adenocarcinoma-initiating cells, Tumour Biol 35 (9) (2014) 8953–8961, https://doi.org/10.1007/s13277-014-2158-8.

[17] J. Li, Y. Song, B. Yu, Y. Yu, TNFAIP2 promotes non-small cell lung cancer cells and targeted by miR-145-5p, DNA Cell Biol. 39 (7) (2020) 1256–1263, https://doi.org/10.1089/dna.2020.5415.

[18] H. Shen, J. Shen, L. Wang, Z. Shi, M. Wang, B.H. Jiang, Y. Shu, Low miR-145 expression level is associated with poor pathological differentiation and poor prognosis in non-small cell lung cancer, Biomed. Pharmacother. 69 (2015) 301–305, https://doi.org/10.1016/j.biopha.2014.12.019.

[19] X. Du, J. Zhang, J. Wang, X. Lin, F. Ding, Role of miRNA in lung cancer-potential biomarkers and therapies, Curr. Pharm. Des. 23 (39) (2018) 5997–6010, https://doi.org/10.2174/1381612823666170714150118.

[20] S. Cui, F. Li, RHPN1-AS1 promotes ovarian carcinogenesis by sponging miR-6884-5p thus releasing TOP2A mRNA, Oncol. Rep. 46 (4) (2021), https://doi.org/10.3892/or.2021.8172.

[21] G. Qiang, Q. Yu, K. Su, Y. Guo, D. Liu, C. Liang, E2F1-activated LINC01224 drives esophageal squamous cell carcinoma cell malignant behaviors via targeting miR-6884-5p/DVL3 axis and activating Wnt/beta-catenin signaling pathway, Pathol. Res. Pract. 235 (2022) 153873, https://doi.org/10.1016/j.prp.2022.153873.

[22] H. Tian, G. Liang, Q. Qin, C. Yu, J. He, Circ_0023990 Promotes the Proliferation, Invasion, and Glycolysis of Esophageal Squamous Cell Carcinoma Cells via Targeting miR-6884-5p/PAK1 Axis, Biochem. Genet, 2024, https://doi.org/10.1007/s10528-024-10674-z.

[23] H. Xu, G. Lu, S. Zhou, F. Fang, MicroRNA-19a-3p acts as an oncogene in gastric cancer and exerts the effect by targeting SMOC2, Appl. Biochem. Biotechnol. 194 (9) (2022) 3833–3842, https://doi.org/10.1007/s12010-022-03944-2.

[24] Y. Chen, F. Liu, X. Chen, W. Li, K. Li, H. Cai, S. Wang, H. Wang, K. Xu, C. Zhang, S. Ye, Y. Shen, T. Mou, S. Cai, J. Zhou, J. Yu, microRNA-622 upregulates cell cycle process by targeting FOLR2 to promote CRC proliferation, BMC Cancer 24 (1) (2024) 26, https://doi.org/10.1186/s12885-023-11766-6.

[25] W. Hu, Y. Wang, Q. Zhang, Q. Luo, N. Huang, R. Chen, X. Tang, X. Li, H. Luo, MicroRNA-199a-3p suppresses the invasion and metastasis of nasopharyngeal carcinoma through SCD1/PTEN/AKT signaling pathway, Cell. Signal. 110 (2023) 110833, https://doi.org/10.1016/j.cellsig.2023.110833.

[26] L. Zhang, W. Chi, X. Wang, J. Li, F. Li, Y. Ma, Q. Zhang, The role of miR-6884-5p in epithelial-mesenchymal transition in non-small cell lung cancer, Aging (Albany N. Y.) 16 (2) (2024) 1968–1979, https://doi.org/10.18632/aging.205474.

[27] S. Cedres, A. Prat, P. Martinez, E. Pallisa, G. Sala, J. Andreu, J.M. del Campo, I. Quispe, J. Baselga, E. Felip, Clinical surrogate markers of survival in advanced non-small cell lung cancer (NSCLC) patients treated with second-third line erlotinib, Lung Cancer 66 (2) (2009) 257–261, https://doi.org/10.1016/j.lungcan.2009.01.014.

[28] N. Ghaderi, J.H. Jung, D.J. Odde, J. Peacock, Clinically validated model predicts the effect of intratumoral heterogeneity on overall survival for non-small cell lung cancer (NSCLC) patients, Comput Methods Programs Biomed 212 (2021) 106455, https://doi.org/10.1016/j.cmpb.2021.106455.

[29] F. Ren, T. Zhao, B. Liu, L. Pan, Neutrophil-lymphocyte ratio (NLR) predicted prognosis for advanced non-small-cell lung cancer (NSCLC) patients who received immune checkpoint blockade (ICB), OncoTargets Ther. 12 (2019) 4235–4244, https://doi.org/10.2147/OTT.S199176.

[30] J.R. Brahmer, R. Govindan, R.A. Anders, S.J. Antonia, S. Sagorsky, M.J. Davies, S.M. Dubinett, A. Ferris, L. Gandhi, E.B. Garon, M.D. Hellmann, F.R. Hirsch, S. Malik, J.W. Neal, V.A. Papadimitrakopoulou, D.L. Rimm, L.H. Schwartz, B. Sepesi, B.Y. Yeap, N.A. Rizvi, R.S. Herbst, The Society for Immunotherapy of Cancer consensus statement on immunotherapy for the treatment of non-small cell lung cancer (NSCLC), J Immunother Cancer 6 (1) (2018) 75, https://doi.org/10.1186/s40425-018-0382-2.

[31] C. Rolfo, P.C. Mack, G.V. Scagliotti, P. Baas, F. Barlesi, T.G. Bivona, R.S. Herbst, T.S. Mok, N. Peled, R. Pirker, L.E. Raez, M. Reck, J.W. Riess, L.V. Sequist, F. A. Shepherd, L.M. Sholl, D.S.W. Tan, H.A. Wakelee, I.I. Wistuba, M.W. Wynes, D.P. Carbone, F.R. Hirsch, D.R. Gandara, Liquid biopsy for advanced non-small cell lung cancer (NSCLC): a statement paper from the iaslc, J. Thorac. Oncol. 13 (9) (2018) 1248–1268, https://doi.org/10.1016/j.jtho.2018.05.030.

[32] M.S. Fawzy, E.A. Toraih, A. Ibrahiem, H. Abdeldayem, A.O. Mohamed, M.M. Abdel-Daim, Evaluation of miRNA-196a2 and apoptosis-related target genes: ANXA1, DFFA and PDCD4 expression in gastrointestinal cancer patients: a pilot study, PLoS One 12 (11) (2017) e0187310, https://doi.org/10.1371/journal.pone.0187310.

[33] J. Zhang, S. Li, Y. Li, H. Liu, Y. Zhang, Q. Zhang, miRNA-218 regulates the proliferation and apoptosis of cervical cancer cells via targeting Gli 3, Exp. Ther. Med. 16 (3) (2018) 2433–2441, https://doi.org/10.3892/etm.2018.6491.

[34] M. Zhang, F. Wang, X. Zhang, miRNA-627 inhibits cell proliferation and cell migration, promotes cell apoptosis in prostate cancer cells through upregulating MAP3K1, PTPRK and SRA1, Int. J. Clin. Exp. Pathol. 11 (1) (2018) 255–261.

[35] Q.Y. Fang, Q.F. Deng, J. Luo, C.C. Zhou, MiRNA-20a-5p accelerates the proliferation and invasion of non-small cell lung cancer by targeting and downregulating KLF9, Eur. Rev. Med. Pharmacol. Sci. 24 (5) (2020) 2548–2556, https://doi.org/10.26355/eurrev_202003_20522.
[36] T. Tan, X.H. Xu, X.H. Lu, X.W. Wang, MiRNA-200a-3p suppresses the proliferation, migration and invasion of non-small cell lung cancer through targeting IRS2, Eur. Rev. Med. Pharmacol. Sci. 24 (2) (2020) 712–720, https://doi.org/10.26355/eurrev_202001_20050.
[37] T. Ghasemi, M. Khalaj-Kondori, M.A. Hosseinpour Feizi, P. Asadi, lncRNA-miRNA-mRNA interaction network for colorectal cancer; an in silico analysis, Comput. Biol. Chem. 89 (2020) 107370, https://doi.org/10.1016/j.compbiolchem.2020.107370.
[38] S.J. Kim, J.W. Ha, B.T. Zhang, Constructing higher-order miRNA-mRNA interaction networks in prostate cancer via hypergraph-based learning, BMC Syst. Biol. 7 (2013) 47, https://doi.org/10.1186/1752-0509-7-47.
[39] Z. Li, Z. Peng, S. Gu, J. Zheng, D. Feng, Q. Qin, J. He, Global analysis of miRNA-mRNA interaction network in breast cancer with brain metastasis, Anticancer Res. 37 (8) (2017) 4455–4468, https://doi.org/10.21873/anticanres.11841.
[40] R. Ma, C. Wang, J. Wang, D. Wang, J. Xu, miRNA-mRNA interaction network in non-small cell lung cancer, Interdiscip Sci 8 (3) (2016) 209–219, https://doi.org/10.1007/s12539-015-0117-8.
[41] M. Tantawy, J.M. Collins, D. Wang, Genome-wide microRNA profiles identify miR-107 as a top miRNA associating with expression of the CYP3As and other drug metabolizing cytochrome P450 enzymes in the liver, Front. Pharmacol. 13 (2022) 943538, https://doi.org/10.3389/fphar.2022.943538.
[42] T. Zhang, L. Chen, H. Ding, P. Wu, G. Zhang, Z. Pan, K. Xie, G. Dai, J. Wang, Construction of miRNA-mRNA network in the differentiation of chicken preadipocytes, Br. Poult. Sci. 63 (3) (2022) 298–306, https://doi.org/10.1080/00071668.2021.2000585.
[43] J. Li, T. Xia, J. Cao, D. He, Z. Chen, B. Liang, J. Song, RP11-295G20.2 facilitates hepatocellular carcinoma progression via the miR-6884-3p/CCNB1 pathway, Aging (Albany N. Y.) 12 (14) (2020) 14918–14932, https://doi.org/10.18632/aging.103552.
[44] P. Singh, MicroRNA based combinatorial therapy against TKIs resistant CML by inactivating the PI3K/Akt/mTOR pathway: a review, Med. Oncol. 40 (10) (2023) 300, https://doi.org/10.1007/s12032-023-02161-z.
[45] H. Yu, X. Li, Y. Li, T. Wang, M. Wang, P. Mao, MiR-4524b-5p-targeting ALDH1A3 attenuates the proliferation and radioresistance of glioblastoma via PI3K/AKT/mTOR signaling, CNS Neurosci, Ther 30 (2) (2024) e14396, https://doi.org/10.1111/cns.14396.
[46] Y. Gao, P. Du, miR-889-3p targeting BMPR2 promotes the development of retinoblastoma via JNK/MAPK/ERK signaling, Sci. Rep. 14 (1) (2024) 7277, https://doi.org/10.1038/s41598-023-49994-2.
[47] S. Tanabe, E.M.T. Mechanism, Lung cancer metastasis, and microRNA, Front. Mol. Biosci. 8 (2021) 731788, https://doi.org/10.3389/fmolb.2021.731788.
[48] L. Chen, T. Munoz-Antonia, W.D. Cress, Trim 28 contributes to EMT via regulation of E-cadherin and N-cadherin in lung cancer cell lines, PLoS One 9 (7) (2014) e101040, https://doi.org/10.1371/journal.pone.0101040.
[49] S. Liao, C. Yu, H. Liu, C. Zhang, Y. Li, X. Zhong, Long non-coding RNA H19 promotes the proliferation and invasion of lung cancer cells and regulates the expression of E-cadherin, N-cadherin, and vimentin, OncoTargets Ther. 12 (2019) 4099–4107, https://doi.org/10.2147/OTT.S185156.
[50] S. Liu, Y. Miao, C. Fan, Y. Liu, J. Yu, Y. Zhang, S. Dai, E. Wang, Clinicopathologic correlations of liver kinase B1, E-cadherin, and N-cadherin expression in non-small cell lung cancer, Appl. Immunohistochem. Mol. Morphol. 21 (4) (2013) 334–340, https://doi.org/10.1097/PAI.0b013e31826b128b.
[51] N. Zheng, Z. Huo, B. Zhang, M. Meng, Z. Cao, Z. Wang, Q. Zhou, Thrombomodulin reduces tumorigenic and metastatic potential of lung cancer cells by up-regulation of E-cadherin and down-regulation of N-cadherin expression, Biochem. Biophys. Res. Commun. 476 (4) (2016) 252–259, https://doi.org/10.1016/j.bbrc.2016.05.105.