European Review for Medical and Pharmacological Sciences 2020; 24: 3166-3173

MiR-92b inhibits proliferation and invasion of lung cancer by targeting EZH2

L. CHEN[1], H.-Z. ZHUO[2], J.-Y. WU[3], L.-Y. LIN[1], Z.-L. HUANG[1], J.-X. LU[1], K.-L. CHENG[1]

[1]Department of Cardiothoracic Surgery, Affiliated Hospital of Guangdong Medical University, Zhanjiang, P.R. China
[2]Department of Anaesthesiology, Affiliated Hospital of Guangdong Medical University, Zhanjiang, P.R. China
[3]Clinical Research Center, Affiliated Hospital of Guangdong Medical University, Zhanjiang, P.R. China

Abstract. – OBJECTIVE: To verify that miR-92b inhibits proliferation and invasion of lung cancer by targeting EZH2.

MATERIALS AND METHODS: The expression levels of miR-92b and EZH2 in human bronchial epithelial cell line BEAS-2B and human lung cancer cell line (A549, NCI-H23, NCI-H358, NCI-H1975, PC-9) were detected, and miR-92b mimic, sh-EZH2 expression vector, and plasmid blank vector (blank group) were constructed. Blank group, miR-92b mimic, miR-92b mimic+sh-EZH2 group (combined group) were set up, MTT and transwell were used to detect the proliferation and invasion ability of A549 and NCI-H23 cells, and fluorescein report verified the regulatory relationship of miR-92b to EZH2.

RESULTS: The expression level of miR-92b in A549, NCI-H23, NCI-H358, NCI-H1975, and PC-9 cells was lower than that in BEAS-2B cells ($p<0.05$). The expression level of EZH2 was higher than that of BEAS-2B cells ($p<0.05$). A549 and NCI-H23 cells were selected for transfection. After that, the expression level of miR-92 in miR-92b mimic, combined group A549 and NCI-H23 cells was higher than that in blank group ($p<0.05$), and miR-92b mimic had no difference with joint group ($p>0.05$). The expression level of EZH2 in cells of miR-92b mimic, blank group A549, and NCI-H23 was lower than that of combined group ($p<0.05$), and miR-92b mimic was lower than that of blank group ($p<0.05$). After the overexpression of miR-92b, pmirGLO-EZH2-3'UT Wt luciferase activity decreased significantly ($p<0.05$) but had no effect on pmirGLO-EZH2-3'UTR Mut Luciferase activity ($p>0.05$). Cell proliferation ability and invasion ability of A549 cells and NCI-H23 cells in miR-92b mimic group were lower than those in blank group ($p<0.05$), while those in combined group were higher than those in miR-92b mimic group ($p<0.05$).

CONCLUSIONS: MiR-92b inhibits proliferation and invasion of lung cancer cells through targeted inhibition of EZH2, which is a potential target for future treatment of lung cancer.

Key Words:
MiR-92b, EZH2, Lung cancer, Proliferation, Invasion.

Introduction

Lung cancer is one of the most common cancers, accounting for 17% and 9% of the total number of cancers in men and women respectively, and accounting for 19% of all cancer-related deaths[1,2]. Cancer statistics in 2018 showed that there were 18.1 million new cancer patients and 9.6 million dead patients worldwide, with lung cancer patients ranking first in both new cases (about 11.6%) and deaths (about 18.4%). It has brought serious economic burden to society and individuals[3]. With the aging of the population and growth of smoking behavior trend, the incidence rate of lung cancer shows a further upward trend, increasing by over 5% every year[4,5]. Radiotherapy and chemotherapy is the standard treatment for patients with advanced lung cancer, but in recent years, the efficacy of platinum and other chemotherapy drugs is not satisfactory, the survival time of patients is short, and the 5-year survival rate of those with non-small cell lung cancer is less than 18%[6]. Therefore, it is necessary to find new therapeutic targets.

Gene therapy has always been a hot direction in tumor therapy and research. MiRNAs are a group of non-coding RNA with a length of about 19-24 nucleotides. They are widely expressed

Corresponding Author: Keluo Cheng, MD; e-mail: k4w7f7@163.com

in eukaryote organisms and have pleiotropic effects of gene expression. They regulate post-transcriptional regulation of gene expression through complementary base pairing with specific 3'UTR sequences, which has been greatly utilized in cancer therapy[7,8]. Lately, it has been reported that low expression of miR-92b can maintain the drug resistance of lung cancer cells to cisplatin, and it is a potential biomarker for predicting the clinical efficacy of platinum-based chemotherapy for lung cancer[9], and it can also regulate the proliferation and invasion of lung cancer cells through Phosphatase Gene (PTEN) and Twist[10,11]. Although miRNAs have been found to be deregulated in many types of human cancers, the molecular mechanism by which they regulate various cancer processes is still unclear. Later studies[12] have reported that miR-92b can promote breast cancer cell autophagy and inhibit cells' viability and invasiveness by targeting histone methyltransferase 2 (EZH2), which may be another mechanism by which it acts in lung cancer.

Hence, this study explores the changes of proliferation and invasion ability of lung cancer cells by regulating miR-92b and EZH2, in order to find new targets for clinical treatment of lung cancer.

Materials and Methods

Objects of Research

The human bronchial epithelial cell line BEAS-2B and human lung cancer cell line (A549, NCI-H23, NCI-H358, NCI-H1975, PC-9) were all purchased from Beina Biology (Beijing, China), and their serial number was respectively BNCC338205, BNCC337696, BNCC341422, BNCC341638, BNCC100301, BNCC340767. BEAS-2B cell culture medium was BEBM culture medium (Thermo Fisher Scientific, Waltham, MA, USA, Art. No. 12677019) + 10% fetal calf serum (FCS; Thermo Fisher Scientific, Shanghai, China, Art. No. 16250086), and human lung cancer cell line culture medium was Roswell Park Memorial Institute-1640 (RPMI-1640) culture medium (Thermo Fisher Scientific, Art. No. 61870044) + 10% fetal calf serum. Then, cells were cultured in a constant temperature incubator with 37°C, 5% CO_2.

Construction and Transfection of Expression Vectors

The miR-92b mimic, sh-EZH2 expression vector, and plasmid blank vector (blank group) were constructed by Shanghai GenePharma Biology Company, China. Trypsin was used to digest human lung cancer cell lines 24 h before transfection. Transfection of the expression vector was carried out when the cells were fused to about 80%. Blank group, miR-92b mimic, miR-92b mimic+ sh-EZH2 group (joint group) were set up. Please refer to the kit instructions for specific procedures. The cells were cultured in a 37°C 5% CO_2 incubator for 48 h and the culture medium was changed every 6 h. QRT-PCR was used to detect transfection results. Lipofectamine™2000 transfection kit was purchased from Shanghai Yanjin Biotechnology Co., Ltd., China.

QRT-PCR

The concentration of cell suspension was adjusted to $1*10^7$, and TRIzol lysate (Guangzhou Lanji Biotechnology Co., Ltd., China) was used to lyse cells to extract total RNA. The extraction steps were carried out according to the kit instructions. The concentration and purity of the extracted RNA were analyzed by micro ultraviolet spectrophotometer DanoProp1000 (Thmorgan Biotechnology Co., Ltd.). The A260/A280 value was between 1.8 and 2.1, which was considered to meet the experimental requirements. The integrity of RNA was analyzed by 3% agarose gel electrophoresis (gel electrophoresis kit was purchased from Shanghai Jingke Chemical Technology Co., Ltd.,). QRT-PCR reaction was carried out after RNA extraction. The reverse transcription reaction system was as follows: 5 * TransScript All in one Superfix for PCR 4 μL, total RNA 2 μg, ribonuclease-free distilled water added to 20 μL, 25°C for 10 min, 42°C for 30 min, deactivation of reverse transcriptase at 85°C for 5 s; the reaction is over; the PCR amplification system was as below: cDNA template 2 μL, 2 * TransTaq HiFi PCR SuperMix II 25 μl, upstream primer and downstream primer 1 μL each, double distilled water added to 50 μL, pre-denaturation at 95°C for 3 min, 94°C for 2 min, 94°C for 30 s, 55°C for 30 s, 72°C for 1-2 kb/min, with 42 cycles; extension at 72°C for 5 min after the cycle was completed, U6 was used as the reaction internal reference, and the results were analyzed by $2^{-\Delta\ CT}$ method. TransScript® All-in-One First-Strand cDNA Synthesis SuperMix for PCR was purchased from TransGen Biotech (Beijing, China), the primer sequence was designed and synthesized by Hepeng (Shanghai, China) Biotechnology Co., Ltd (Table I).

Table I. Primer sequences.

	Forward primer	Reverse primer
miR-92b	5′-TATTGCACTCGTCCCGGCCTCC-3′	5′-CAGTGCGTGTCGTGGAGT-3′
EZH2 mRNA	5′-CGCTTTTCTGTAGGCGATGT-3′	5′-TGGGTGTTGCATGAAAAGAA-3′
U6	5’-GCGCGTCGTGAAGCGTTC-3’	5’-GTGCAGGGTCCGAGGT-3’

Western Blot

Repeated freeze-thaw method was used to extract protein from cells. The bicinchoninic acid assay (BCA) method was used to detect the protein concentration. Then, it was adjusted to 4 μg/μL. Altogether 12% polyacrylamide gel electrophoresis (PAGE) was used to separate protein. The initial voltage was 90 V, and then it was increased to 120 V to move the sample to the appropriate position of the separation gel. After electrophoresis was completed and membrane was transferred, 100 V constant voltage was applied for 100 min and 37°C was closed for 60 min. Next, the transfer membrane was placed in 5% skim milk for sealing, and then immune reaction was carried out; the membrane was incubated with primary antibody (1:1000) at 4°C overnight, phosphate-buffered saline (PBS) was washed three times with 5 min each time on the next day, and then enhanced chemiluminescence (ECL) reagent was incubated with secondary antibody (1:1000) for 1 h at room temperature; next, ECL luminescent reagent was developed and fixed, the strip scanned by the film was statistically analyzed by Quantity One software, and the relative protein expression level = strip gray value/internal reference gray value. BCA protein kit, ECL luminescent kit, and trypsin were all purchased from Thermo Scientific™ (Waltham, MA, USA), and their article numbers were 23250, 35055 and 90058, respectively. Rabbit anti-EZH2 monoclonal antibody and goat anti-rabbit IgG secondary antibody were all purchased from Abcam (Cambridge, MA, USA), and their article numbers were ab84989 and ab6721, respectively.

Dual-Luciferase Report

Human embryonic kidney cell 293T was purchased from Beina Biotechnology, serial number BNCC100530. After cell culture to logarithmic growth phase, plasmid and miR-92b mimics were transfected. PGL3-wt-EZH2, PGL3-mt-EZH2, miR-92b mimics were designed by Thermo Fisher Scientific (China), and the fluorescence intensity was measured 48 h after transfection by a Dual-Luciferase assay system (American Beckman CytoFLEX S flow cytometer, Brea, CA, USA).

Cell Proliferation Experiment In Vitro

Lung cancer cells were prepared into $4*10^6$ cells/mL of single cell suspension. The cells were inoculated with 200 μL cell suspension and cultured on 96-well cell culture plates. After incubation for 12 h, 24 h, 48 h, and 72 h respectively, 20 μL MTT (5 mg/mL) solution was added to remove the supernatant containing impurities. Dimethylsulfoxide (DMSO) was added to the supernatant, and it was then placed on a horizontal vibration table for 10 min. The absorbance at 570 nm was measured by an enzyme reader (Shanghai Flash Spectrum Biotechnology Co., Ltd., China), and MTT test kit was purchased from Beijing Equation Jiahong Technology Co., Ltd., China.

Transwell Invasion Experiment In Vitro

The prepared cell suspension (5×105/mL) was inoculated with 100 μL in transwell chamber, and the number of cells passing through was detected 24 h later. Three parallel experiments were conducted simultaneously. Transwell chamber was purchased from Shanghai Shengbo Bio-pharmaceutical Technology Co., Ltd., China.

Statistical Analysis

SPSS 19.0 (IBM, Armonk, NY, USA) was applied. The measurement data were expressed by mean±SD. Student's *t*-test was used for comparison between the two groups, one-way analysis of variance (ANOVA) was used for comparison among multiple groups, LSD test was used for comparison in back testing, repeated measures analysis of variance was used for comparison at different time points in the group, and LSD test was used for comparison in back testing. $p<0.05$ had statistical significance.

Results

Analysis of the Expression Level of MiR-92b in Each Cell Line

The expression level of miR-92b in A549, NCI-H23, NCI-H358, NCI-H1975, and PC-9 cells was lower than that in BEAS-2B cells ($p<0.05$; Figure 1).

Analysis of the Expression Level of EZH2 in Each Cell Line

The expression level of EZH2 in A549, NCI-H23, NCI-H358, NCI-H1975, and PC-9 cells was higher than that in BEAS-2B cells ($p<0.05$; Figure 2).

Analysis of Transfection Results

A549 and NCI-H23 cells were selected for transfection. After that, the expression level of miR-92 in miR-92b mimic, joint group A549 and NCI-H23 cells was higher than that in blank group ($p<0.05$), and miR-92b mimic had no difference with combined group ($p>0.05$). The expression level of EZH2 in cells of miR-92b mimic, blank group A549 and NCI-H23 was lower than that of combined group ($p<0.05$), and miR-92b mimic was lower than that of blank group ($p<0.05$; Figure 3).

Fluorescein Report

After the overexpression of miR-92b, pmirGLO-EZH2-3'UT Wt Luciferase activity decreased significantly ($p<0.05$) but had no effect on pmirGLO-EZH2-3'UTR Mut Luciferase activity ($p>0.05$; Figure 4).

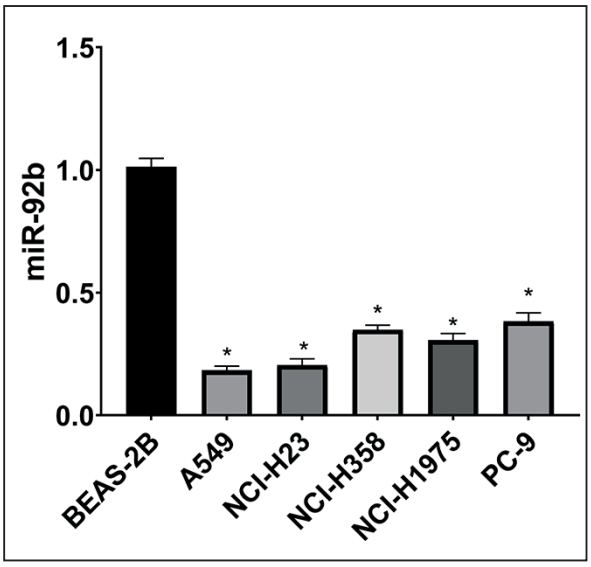

Figure 1. Analysis of the expression level of miR-92b in each cell line *indicates the comparison with BEAS-2B cells ($p<0.05$).

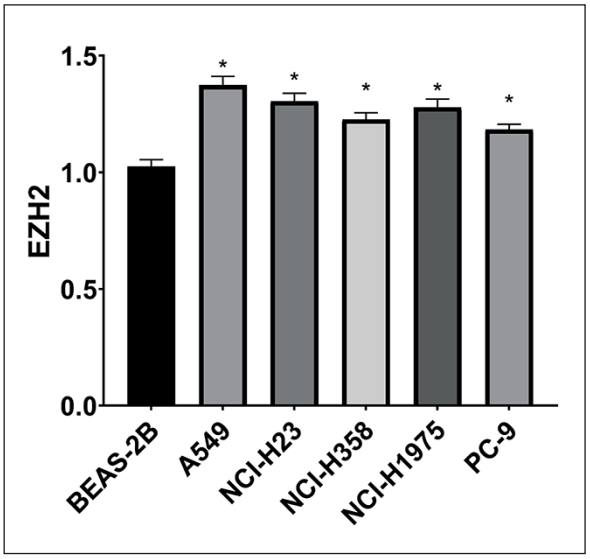

Figure 2. Analysis of the expression level of EZH2 in each cell line *indicates the comparison with BEAS-2B cells ($p<0.05$).

Results of Proliferation Test In Vitro

The proliferation ability of A549 and NCI-H23 cells in miR-92b mimic group was lower than those in blank group ($p<0.05$), while those in combined group were higher than those in miR-92b mimic group ($p<0.05$; Figure 5).

Experimental Results of Invasion In Vitro

The invasion ability of A549 and NCI-H23 cells in miR-92b mimic group was lower than that in blank group ($p<0.05$), while that in combined group was lower than that in miR-92b mimic group ($p<0.05$; Figure 6).

Discussion

Lung cancer is the major cause of cancer-related deaths worldwide, with non-small cell lung cancer (NSCLC) accounting for more than 80% of all lung cancer cases. Although some progress has been made in early detection and treatment, the prognosis of lung cancer patients is still very poor[13]. Where possible, surgical resection is still the only treatment for patients with early non-small cell lung cancer. However, nearly 50% of surgical patients have relapse and poor prognosis,

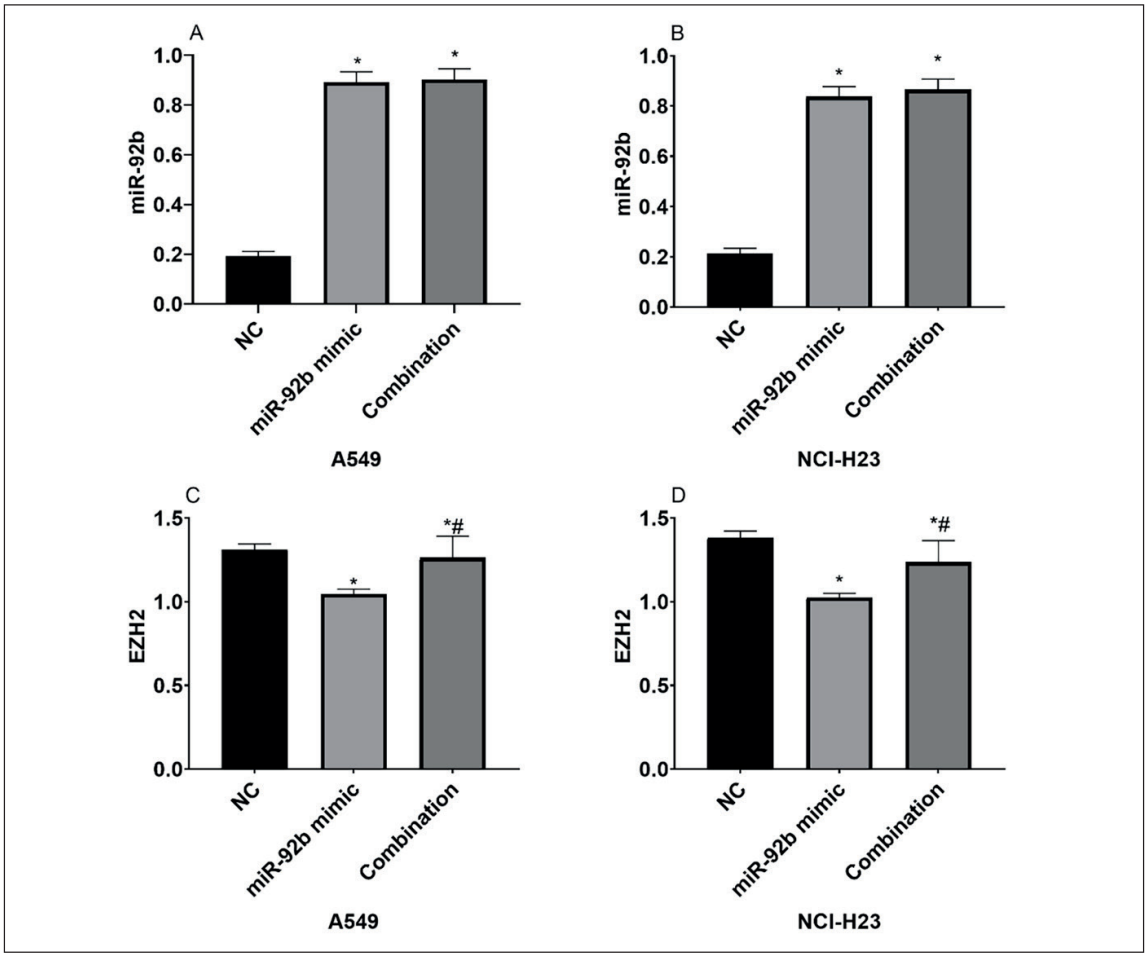

Figure 3. Transfection results. **A,** Expression level of miR-92b in transfected A549 cells. **B,** Expression level of miR-92b in transfected NCI-H23 cells. **C,** Expression level of EZH2 in transfected A549 cells. **D,** Expression level of EZH2 in transfected NCI-H23 cells *indicates the comparison with NC group (p<0.05) and comparison with miR-92b mimic group (p<0.05).

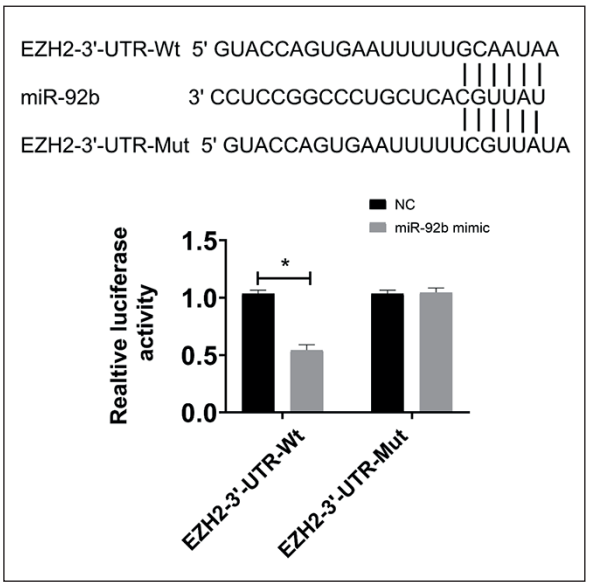

Figure 4. Fluorescein report *indicates p<0.05.

and chemotherapy for advanced patients is histologically dependent[14,15]. At present, there are very few molecular markers which can be used in clinical application and do not depend on the histological differences of non-small cell lung cancer. It is of great significance to study the molecular markers related to non-small cell lung cancer for their treatment and prognosis evaluation.

The analysis results of this study show that compared with normal human bronchial epithelial cells, miR-92b has low expression in lung cancer cell lines and EZH2 has high expression, suggesting that miR-92b and EZH2 may be related to the occurrence and development of lung cancer. Zhang et al[16] reported that miR-138 was able to target EZH2 to inhibit proliferation of non-small cell lung cancer cells. EZH2 is a catalytic subunit of multi-comb inhibition complex 2, mediates histone methyltransferase activity,

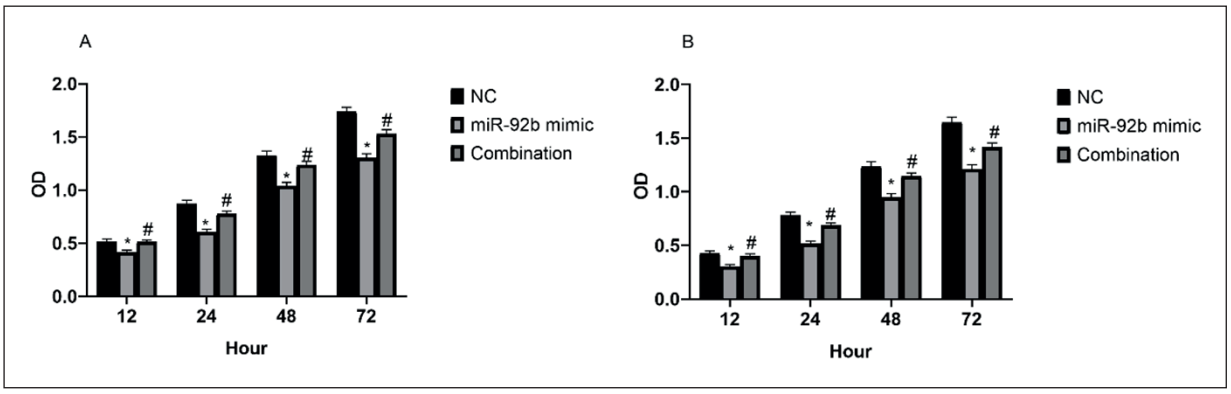

Figure 5. Proliferation experiment *in vitro*. **A,** Analysis of the proliferation ability of A549 cells. **B,** Analysis of the proliferation ability of NCI-H23 cells *indicates the comparison with the NC group at the same time point ($p<0.05$); # indicates the comparison with the miR-92b mimic group at the same time point ($p<0.05$).

and acts as a transcription inhibitor involved in gene silencing[17]. Many studies[18,19] have reported that EZH2 expression is elevated in lung cancer and is tied to poor prognosis of patients. There are few reports about miR-92b and lung cancer. Zhao et al[9] have verified that miR-92b is related to cisplatin resistance in lung cancer patients.

We further analyzed the effect of miR-92b on the biological behavior of lung cancer cells and its mechanism of action. In 6 lung cancer cell lines, we selected A549 and NCI-H23 cell lines for analysis. We transfected miR-92b mimetics. Compared with blank group, we found that the expression level of miR-92b in A549 and NCI-H23 cells increased significantly, while the expression of EZH2 decreased. The proliferation and invasion of A549 and NCI-H23 cells can be restored to a certain extent by overexpressing miR-92b and EZH2 at the same time, which indicates that miR-92b may inhibit the proliferation and invasion of lung cancer cells by regulating EZH2. To further verify the relationship between miR-92b and EZH2, we first predicted the target gene downstream of miR-92b through

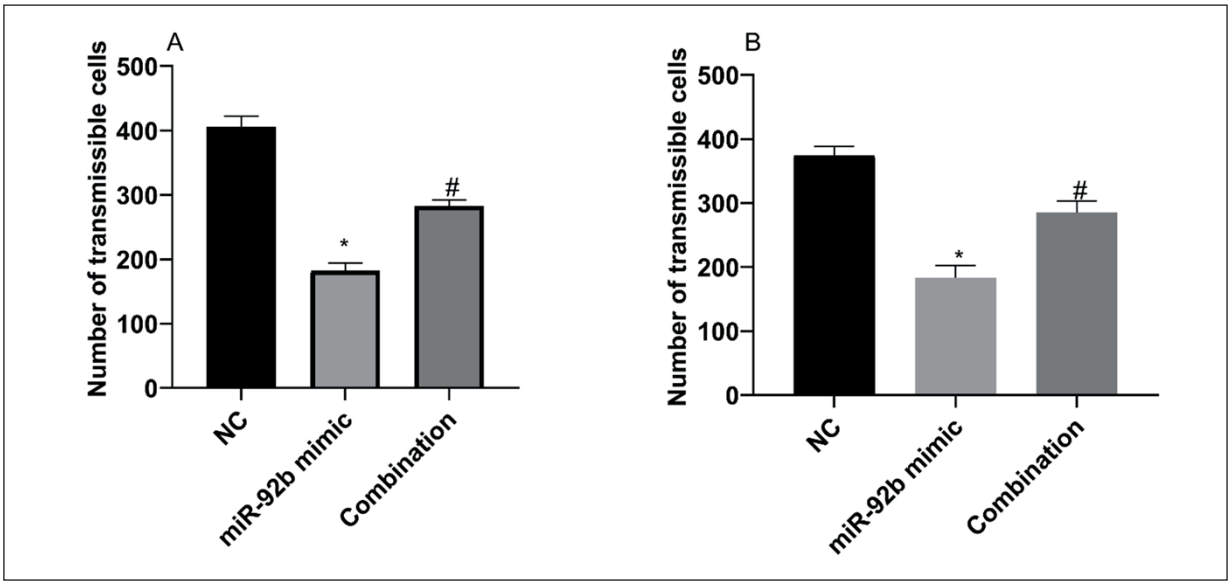

Figure 6. Invasion experiment *in vitro*. **A,** Analysis of the invasion ability of A549 cell. **B,** Analysis of the invasion ability of NCI-H23 cells *indicates the comparison with the NC group ($p<0.05$) and comparison with the miR-92b mimic group ($p<0.05$).

Targetscan7.2 and found that there was a targeted binding site between EZH2 and miR-92b. What's more, the Dual-Luciferase report analysis results also showed that miR-92b and EZH2 also had a targeted regulatory relationship.

Another mode of action of miR-92b in tumors has been found in some reports. Huang et al[20] reported that miR-92b was able to target inhibit DAB2 interacting protein (DAB2IP) to promote epithelial-mesenchymal transformation of bladder cancer cells, thus promoting migration and invasion of bladder cancer cells. Long et al[21] reported that the decrease of miR-92b level was related to poor prognosis of pancreatic cancer patients; overexpression of miR-92b was able to inhibit the proliferation and invasion of pancreatic cancer cells by inhibiting the expression of γ-aminobutyric acid receptor α3 subunit (GABRA3), thus leading to inactivation of important oncogenic pathways such as AKT/mTOR and JNK pathways. These studies reported that miR-92b had two different modes of action in cancer promotion and cancer suppression, which suggested that miR-92b might have a complex mechanism of action in lung cancer, requiring further research.

There are also some deficiencies in this report. It only explores the effect of miR-92b on lung cancer cells from *in vitro* cell experiments. Although we have carried out experiments on multiple cell lines, at present, *in vitro* experiments cannot simulate the complex tumor microenvironment of the body. The role of miR-92b in lung cancer needs further proof of animal experiments and clinical experiments. In addition, this study also has some limitations of experimental conditions and fails to verify the effect of miR-92b on apoptosis, autophagy, epithelial mesenchymal transition, and other phenotypes of lung cancer cells, which will be further analyzed in our future research.

Conclusions

In brief, miR-92b inhibits proliferation and invasion of lung cancer cells through targeted inhibition of EZH2, which is a potential target for future treatment of lung cancer.

Conflict of Interest

The Authors declare that they have no conflict of interests.

References

1) Cheng TY, Cramb SM, Baade PD, Youlden DR, Nwogu C, Reid ME. The international epidemiology of lung cancer: latest trends, disparities, and tumor characteristics. J Thorac Oncol 2016; 11: 1653-1671.
2) Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. CA Cancer J Clin 2015; 65: 87-108.
3) Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2018; 68: 394-424.
4) Didkowska J, Wojciechowska U, MaĐczuk M, Łobaszewski J. Lung cancer epidemiology: contemporary and future challenges worldwide. Ann Transl Med 2016; 4: 150.
5) Markou A, Sourvinou I, Vorkas PA, Yousef GM, Lianidou E. Clinical evaluation of microRNA expression profiling in non small cell lung cancer. Lung Cancer 2013; 81: 388-396.
6) Soria JC, Ohe Y, Vansteenkiste J, Reungwetwattana T, Chewaskulyong B, Lee KH, Dechaphunkul A, Imamura F, Nogami N, Kurata T, Okamoto I, Zhou C, Cho BC, Cheng Y, Cho EK, Voon PJ, Planchard D, Su WC, Gray JE, Lee SM, Hodge R, Marotti M, Rukazenkov Y, Ramalingam SS; for the FLAURA Investigators. Osimertinib in untreated EGFR-mutated advanced non-small-cell lung cancer. N Engl J Med 2018; 378: 113-125.
7) Berindan-Neagoe I, Calin GA. Molecular pathways: microRNAs, cancer cells, and microenvironment. Clin Cancer Res 2014; 20: 6247-6253.
8) Marta GN, Garicochea B, Carvalho AL, Real JM, Kowalski LP. MicroRNAs, cancer and ionizing radiation: where are we? Rev Assoc Med Bras 2015; 61: 275-281.
9) Zhao J, Fu W, Liao H, Dai L, Jiang Z, Pan Y, Huang H, Mo Y, Li S, Yang G, Yin J. The regulatory and predictive functions of miR-17 and miR-92 families on cisplatin resistance of non-small cell lung cancer. BMC Cancer 2015; 15: 731.
10) Li Y, Li L, Guan Y, Liu X, Meng Q, Guo Q. MiR-92b regulates the cell growth, cisplatin chemosensitivity of A549 non small cell lung cancer cell line and target PTEN. Biochem Biophys Res Commun 2013; 440: 604-610.
11) Liu X, Tian XD, Liu Y, Zhang T, Chen L. Regulation of Twist in the metastasis of non-small cell lung cancer by miR-92b. Eur Rev Med Pharmacol Sci 2016; 20: 4003-4010.
12) Liu F, Sang M, Meng L, Gu L, Liu S, Li J, Geng C. MiR 92b promotes autophagy and suppresses viability and invasion in breast cancer by targeting EZH2. Int J Oncol 2018, 53: 1505-1515.
13) Jemal A, Siegel R, Xu J, Ward E. Cancer statistics, 2010. CA Cancer J Clin 2010; 60: 277-300.
14) Herbst RS, Baas P, Kim DW, Felip E, Pérez-Gracia JL, Han JY, Molina J, Kim JH, Arvis CD, Ahn MJ, Majem

M, Fidler MJ, de Castro G Jr, Garrido M, Lubiniecki GM, Shentu Y, Im E, Dolled-Filhart M, Garon EB. Pembrolizumab versus docetaxel for previously treated, PD-L1-positive, advanced non-small-cell lung cancer (KEYNOTE-010): a randomised controlled trial. Lancet 2016; 387: 1540-1550.

15) Brahmer J, Reckamp KL, Baas P, Crinò L, Eberhardt WE, Poddubskaya E, Antonia S, Pluzanski A, Vokes EE, Holgado E, Waterhouse D, Ready N, Gainor J, Arén Frontera O, Havel L, Steins M, Garassino MC, Aerts JG, Domine M, Paz-Ares L, Reck M, Baudelet C, Harbison CT, Lestini B, Spigel DR. Nivolumab versus docetaxel in advanced squamous-cell non–small-cell lung cancer. N Engl J Med 2015; 373: 123-135.

16) Zhang H, Zhang H, Zhao M, Lv Z, Zhang X, Qin X, Wang H, Wang S, Su J, Lv X, Liu H, Du W, Zhou W, Chen X, Fei K. MiR-138 inhibits tumor growth through repression of EZH2 in non-small cell lung cancer. Cell Physiol Biochem 2013; 31: 56-65.

17) Kikuchi J, Takashina T, Kinoshita I, Kikuchi E, Shimizu Y, Sakakibara-Konishi J, Oizumi S, Marquez VE, Nishimura M, Dosaka-Akita H. Epigenetic therapy with 3-deazaneplanocin A, an inhibitor of the histone methyltransferase EZH2, inhibits growth of non-small cell lung cancer cells. Lung Cancer 2012; 78: 138-143.

18) Cao W, Ribeiro Rde O, Liu D, Saintigny P, Xia R, Xue Y, Lin R, Mao L, Ren H. EZH2 promotes malignant behaviors via cell cycle dysregulation and its mRNA level associates with prognosis of patient with non-small cell lung cancer. PLoS One 2012; 7: e52984.

19) Geng J, Li X, Zhou Z, Wu CL, Dai M, Bai X. EZH2 promotes tumor progression via regulating VEGF-A/AKT signaling in non-small cell lung cancer. Cancer Lett 2015; 359: 275-287.

20) Huang J, Wang B, Hui K, Zeng J, Fan J, Wang X, Hsieh JT, He D, Wu K. MiR-92b targets DAB2IP to promote EMT in bladder cancer migration and invasion. Oncol Rep 2016; 36: 1693-1701.

21) Long M, Zhan M, Xu S, Yang R, Chen W, Zhang S, Shi Y, He Q, Mohan M, Liu Q, Wang J. MiR-92b-3p acts as a tumor suppressor by targeting Gabra3 in pancreatic cancer. Mol Cancer 2017; 16: 167.