Int J Clin Exp Pathol 2016;9(11):11480-11486
www.ijcep.com /ISSN:1936-2625/IJCEP0039542

Original Article

MicroRNA-23b inhibits cell viability and migration but promotes apoptosis in non-small cell lung cancer cells *in vitro*

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Received September 6, 2016; Accepted September 24, 2016; Epub November 1, 2016; Published November 15, 2016

Abstract: Background: Lung cancer is a major public health problem worldwide and currently its effective therapies are still imperfect. MicroRNA-23b (miR-23b) was reported to modulate various cancer pathological conditions. Our study was aimed to reveal the potential effect of miR-23b on non-small cell lung cancer (NSCLC) cells and its possible mechanism. Methods: NSCLC cells line A549 were transfected with miR-23b mimic, miR-23b inhibitor or control, the miR-23b expression was monitored by quantitativepolymerase chain reaction (qPCR). The cell viability, migration and apoptosis of A549 cells were respectively measured by 3-(4, 5-dimethyl-2-thiazolyl)-2, 5-diphenyltetrazolium bromide (MTT), modified two-chamber migration assay, and flow cytometry. Western blot was used to detect the phosphorylation levels of phosphatidylinositol 3-kinase (PI3K), AKT (Protein Kinase B) andglycogen synthase kinase-3 beta (GSK3β) in transfected cells. Results: In A549 cells, miR-23b was effectively overexpressed and suppressed ($P < 0.001$). We observed that miR-23b overexpression significantly suppressed A549 cells viability ($P < 0.05$ or $P < 0.01$) and migration ($P < 0.001$), but enhanced apoptosis ($P < 0.001$). In contrast, miR-23b suppression displayed the contrary results ($P < 0.05$, $P < 0.01$ or $P < 0.001$). In addition, we found that miR-23b overexpression downregulated the phosphorylation levels of PI3K, AKT, and GSK3β in A549 cells, whereas miR-23b suppression could upregulate them. Conclusion: MiR-23b overexpression suppressed A549 cells viability and migration, but enhanced apoptosis through the PI3K/AKT/GSK3β pathway. Overexpression of miR-23b has potential as a novel therapeutic strategy in NSCLC treatment.

Keywords: microRNA-23b, non-small cell lung cancer, cell viability, migration, apoptosis, PI3K/AKT/GSK3β pathway

Introduction

Lung cancer is one of malignant tumors with highest mortality in the human race. Non-small cell lung cancer (NSCLC) is one of the most common types of lung cancer and alsois the leading cause of death among all cancers because of very low survival rate after treatment and the unlikely event of surgical resection. This tumor often presents at the advanced stage, which hinders effective treatment [1, 2]. There were multiple researches have been carried out to prevent, anticipate, and diagnose this cancer [3-5]. However, smaller lesions will inevitably lead to poses diagnostic challenges and more effective therapies needed at earlier stages [6]. Nowadays, a variety of novel molecular methods have been investigated to find effective treatments for NSCLC [7]. Nevertheless, to date only limited response and survival benefit has been shown, in part because of a lack of understanding in the carcinogenesis of NSCLC [8].

MicroRNAs (miRNAs) are small non-coding RNA molecules capable of regulating expression of over 50% of protein-coding genes. It has rapidly emerged as potential biomarkers showing possibility to assist the clinical management of lung cancer [9]. MiRNAs are involved in the pathogenesis of lung diseases such as cystic fibrosis, lung cancer, asthma and *etc* [10]. It has been demonstrated to be relevant to lung cancer development, epithelialmesenchymal transition and response to therapy [11]. A lot of researches certified that miRNAs can affect the migration, invasion and apoptosis of NSCLC cell [12, 13]. A better understanding of the involve-

ment of miRNAs in pathogenesis of this disease could result in the development of new therapeutic and diagnostic tools. Recently, miR-23b has emerged as a promising new cancer biomarker but it's accurately role in NSCLC has not been established yet [14, 15].

In the present study, we investigated the role of miR-23b in NSCLC cells viability, migration and apoptosis. We observed that overexpression of miR-23b altered A549 cell progresses *in vitro*. Subsequently, we verified the protein levels changes of phosphatidylinositol 3-kinase (PI3K), AKT (Protein Kinase B) and glycogen synthase kinase-3beta (GSK3β) to reveal the possible molecular mechanisms of miR-23b in NSCLC cells. Our finding may offer a partial understanding about miR-23b roles in NSCLC and provide evidences about that miR-23b might be used in NSCLC treatment as novel gene therapeutic target.

Materials and methods

Cell culture and transfection

The NSCLC cells line A549 were obtained from the American Type Culture Collection (ATCC; Rockville, MD, USA), and maintained in Roswell Park Memorial Institute-1640 (RPMI-1640; Hyclone, Logan, UT) medium supplemented with 10% heat-inactivated feral bovine serum (FBS; Hyclone, Logan, UT, USA) and 1% penicillin/streptomycin (GIBCO, Grand Island, NY) in a humidified CO_2 incubator at 37°C [16].

A549 cells were transfected with miR-23b mimic, miR-23b inhibitor, or control (GenePharma, Shanghai, China), respectively. The transfection was performed by using Lipofectamine 3000 (Invitrogen Life Technologies, Carlsbad, CA, USA), according to the manufacturer's instructions. After 48 h of transfection, cells were selected and established a stable cell line as described previously for the further analysis [17].

RNA extraction and quantitative polymerase chain reaction (qPCR)

Total RNA was extracted with Trizol (Invitrogen, USA) and reversely transcribed by MultiScribe RT kit (Applied Biosystems, Foster City, California, USA) according to the manufacturer's instructions. All reverse transcriptase reactions were run in a master cycler gradient (Eppendorf, Germany). Fast START Universal SYBR Green Master (ROX) (Roche, USA) was used for qPCR analysis according to the manufacturer's instructions. The qPCR was performed in triplicate, including no template controls. MiR-23b expressions were normalized to U6 expressions using $2^{-\Delta\Delta CT}$ method [18]. All primers were synthesized by GenePharma (Shanghai, China). The data were analyzed with Real-Time StatMiner (Integromics).

Cell viability assay

MiR-transfected cells were seeded at 2×10^3 cells/well in 96-well plates. After 1-4 days of incubation, 20 μl of 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT; Sigma, St. Louis, MO, USA) were added into each well and incubated for another 4 h at 37°C. Then, 150 μl dimethylsulfoxide (DMSO; Sigma, USA) was added and the plates were shaken for 10 min. The absorbance was measured at 570 nm (OD_{570}) with a microplate reader (Bio-Rad Laboratories, Hercules, CA, US).

Cell migration assay

Cell migration was determined by using a modified two-chamber with 8.0 μm pore membranes (Greiner 662638). MiR-transfected cells were suspended in 200 μl of serum-free culture medium, and were added into the uper compartment of 24-well transwell culture chamber. Add 600 μl complete medium to the lower comparment, then incubated at 37°C for 12 h. After that, cells were fixed with 4% methanol (NIST, USA) for 30 mim, non-transferred cells were removed from the upper surface of the filter carefully with a cotton swab. Traversed cells in the lower were stained with 0.1% crystal violet for 20 min and counted using an vertial microscope (Leica Microsystems, Wetzlar, Germany).

Cell apoptosis assay

Flow cytomety analysis was performed to identify and quantify the apoptotic cells using Annexin V-FITC/PI apoptosis detection kit (Beijin Biosea Biotechnology, Beijing, China) according to manufacturer's recommendations.MiR-transfected cells were collected and suspended in 200 μl of binding buffer containing 10 μl Annexin V-FITC and 5 μl of PI, then incubated at room temperature for 30 min in the dark. Apoptotic cells were measured with flow cytometer (Beckman Coulter, USA) [19].

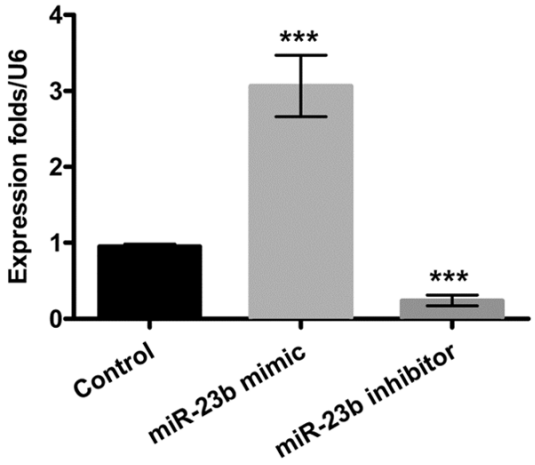

Figure 1. Effects of transfection on miR-23b expression in A549 cells. The miR-23b expression was analyzed by qPCR, using U6 RNA as an internal control. Data are represented as mean ± SD. MiR-23b, microRNA-23b; qPCR, quantitative polymerase chain reaction; SD, standard deviation. ***, $P < 0.001$.

Western blot analysis

Cell lysates were extracted by lysis buffer (Beyotime Biotechnology, Shanghai, China) supplemented with protease inhibitor (Roche, Guangzhou, China) from miR-transfected cells. Proteins were resolved over 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto polyvinylidene fluoride (PVDF) membranes. Membranes were blocked for 1 h using 5% non-fat dry milk and probed with the respective primary antibodies (all 1:1,000). Then the blots were incubated for 1 h with horseradish peroxidase (HRP)-conjugated secondary antibodies (Santa Cruz biotech) and detected using enhanced chemiluminescence (ECL) Western Blotting Substrate (Thermo Scientific) and autoradiograph. The protein loading was normalized using antibody to glyceraldehyde-3-phosohate dehydrogenase (GAPDH). The data were analyzed using Image Lab (Bio-Rad) software. The following antibodies were used in this study: PI3K (ab86714), phosphorylated PI3K (p-PI3K, ab182651), AKT (ab8805), phosphorylated AKT (p-AKT, ab38449), GSK3β (ab32391), phosphorylated GSK3β (p-GSK3β, ab75745) and GAPDH (ab9485) (Abcam, Cambridge, MA, USA).

Statistical analysis

Results were expressed as the mean ± standard deviation (SD), and statistical analyses were performed usinga one-way analysis of variance (ANOVA) or two-tailed paired-sample *t* test in GraphPad Prism 5 (GraphPad, La Jolla, CA, USA). A P value of < 0.05 was considered statistically significant.

Results

MiR-23b overexpression reduced cell viability and migration of A549 cells

In order to investigate the role of miR-23b in NSCLC, A549 cells were respectively transfected with miR-23b mimic, miR-23B inhibitor, or control. We observed that miR-23b was effectively overexpressed or suppressed ($P < 0.001$) in A549 cells after transfection (**Figure 1**). Then, cell viability was examined by MTT assay after cultured for 1-4 days. The results shown in **Figure 2A** indicated that miR23b overexpression caused a significant decrease in cell viability at 2 ($P < 0.05$), 3 ($P < 0.01$), and 4 days ($P < 0.01$) as compared to control group. MiR-23b suppression could significantly promote cell viability at the same time point ($P < 0.05$ or $P < 0.01$). The results from migration assay (**Figure 2B**) showed that cell migration was significantly decreased by miR-23b overexpression ($P < 0.001$), while was increased by miR-23b suppression ($P < 0.001$) compared with the control group. Collectively, these results indicated that miR-23b might affect cell viability and migration in NSCLC cells.

Overexpression of miR-23b promoted A549 cells apoptosis

The results of apoptotic cells classified by flow cytometry were shown in **Figure 3A** and **3B**. We observed that the apoptotic cell rate was significantly enhanced ($P < 0.001$) in miR-23b mimic transfected cells, while miR-23b suppression group showed the opposite result ($P < 0.01$). Hence, the miR-23b might be involved in NSCLC cells apoptosis.

MiR-23b overexpression influenced on A549 cells by regulating PI3K, AKT and GSK3β

Activated PI3K, AKT and GSK3β are associated with differentiation and proliferation of cells in various types of cancers including lung cancer [20-23]. To define the molecular mechanism of the miR-23b effect on NSCLC cells, we detected the protein levels of PI3K, AKT and GSK3β in miR-transfected cells by western blot analysis.

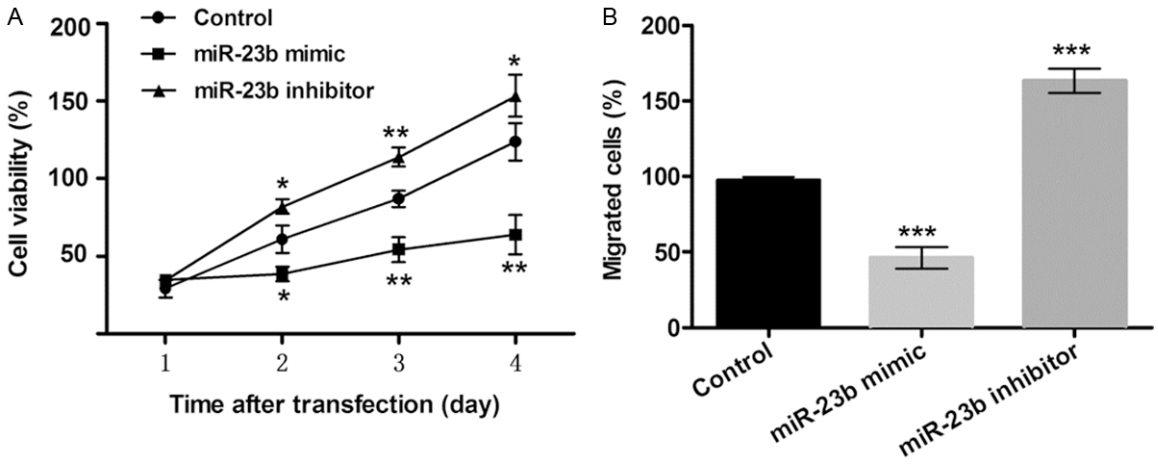

Figure 2. Overexpression of miR-23b inhibited cell viability and migration. MiR-23b mimic, miR-23b inhibitor or control was transfected into A549 cells respectively. A. After the transfected cells were cultured for 1-4 days, cell viability was determined by MTT assay. B. The migration of transfected cells were measured by Transwell assay. Data were represented as mean ± SD. MiR-23b, microRNA-23b; MTT, 3-(4, 5-dimethyl-2-thiazolyl)-2, 5-diphenyltetrazolium bromide; SD, standard deviation. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

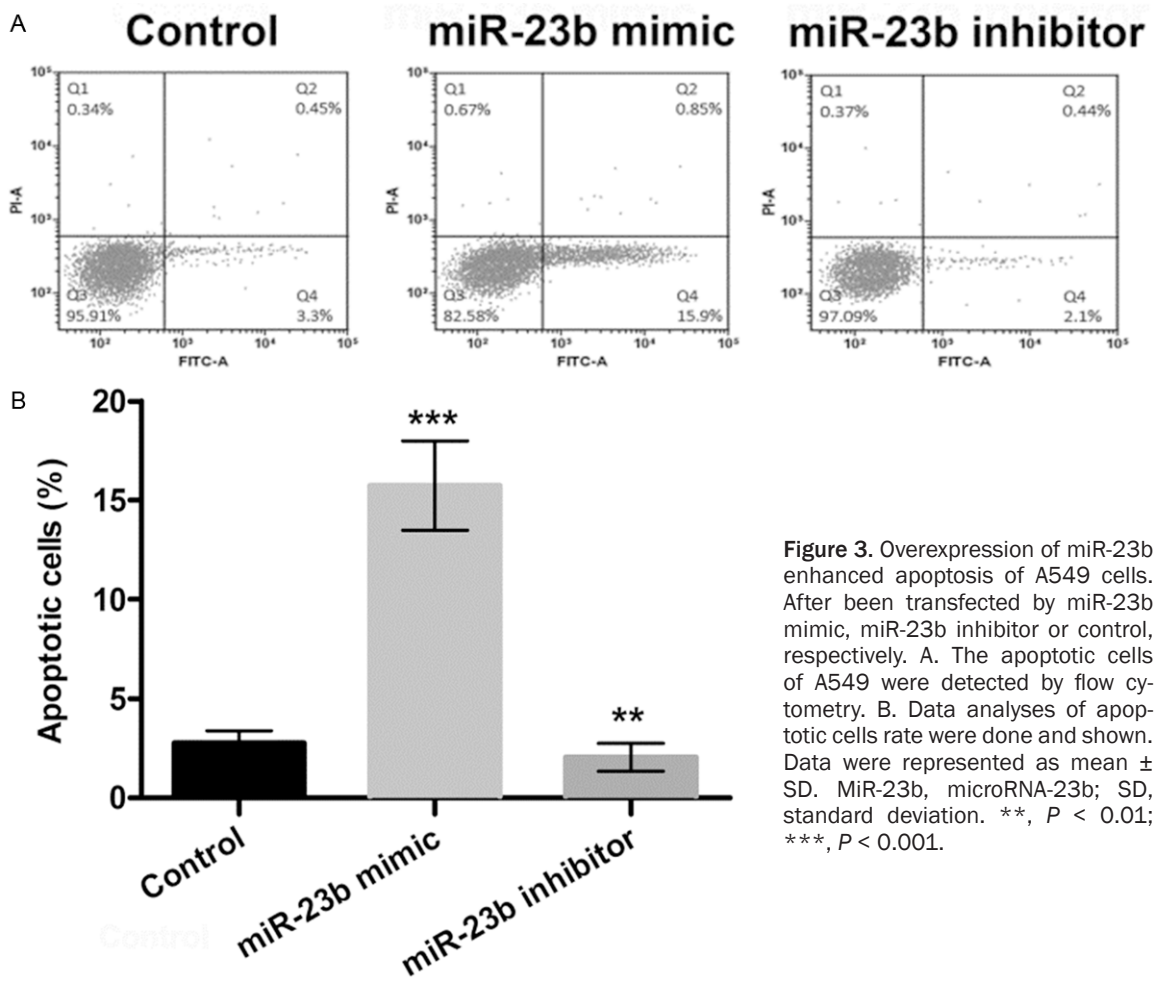

Figure 3. Overexpression of miR-23b enhanced apoptosis of A549 cells. After been transfected by miR-23b mimic, miR-23b inhibitor or control, respectively. A. The apoptotic cells of A549 were detected by flow cytometry. B. Data analyses of apoptotic cells rate were done and shown. Data were represented as mean ± SD. MiR-23b, microRNA-23b; SD, standard deviation. **, $P < 0.01$; ***, $P < 0.001$.

As shown in **Figure 4A** and **4B**, the phosphorylation level of these three proteins were significantly downregulated by miR-23b overexpression ($P < 0.05$ or $P < 0.01$) when compared with

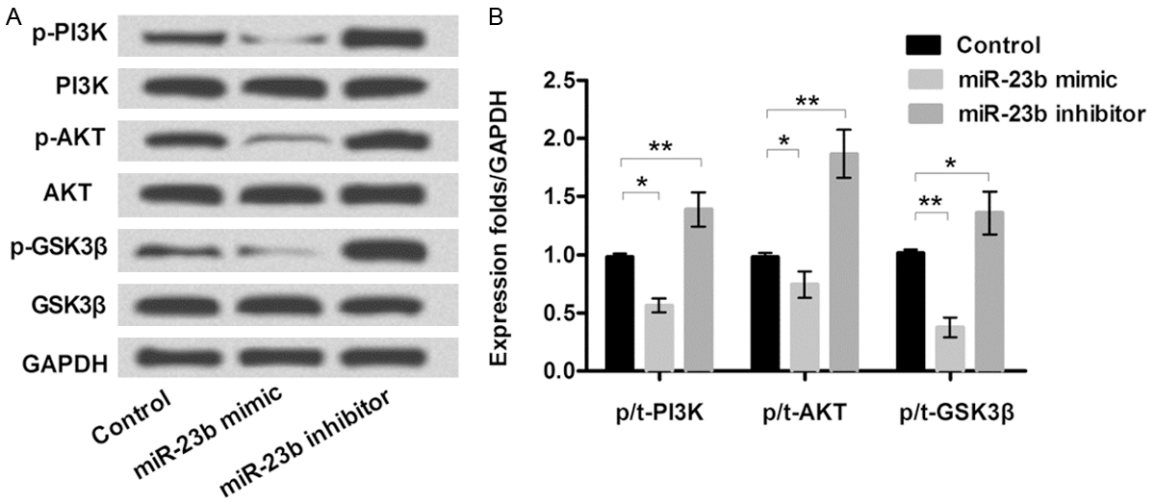

Figure 4. MiR-23b downregulated the expression of p-PI3K p-AKT, and p-GSK3β in A549 cells. Cell lysates from miR-23b mimic and miR-23b inhibitor transfected cells were analyzed for expression of p/t-PI3K, p/t-AKT, and p/t-GSK3β using specific antibodies. These blots were reprobed with an antibody to GAPDH for comparison of protein loading in each lane. A. The protein level of p/t-PI3K, p/t-AKT, and p/t-GSK3β and GAPDH expression in miR-23b treated A549 cells were detected by western blot. B. Densitometric analyses of these proteins were done by using Image Lab software and shown. Data were represented as mean ± SD. MiR-23b, microRNA-23b; PI3K, phosphatidylinositol-3-kinase; p-PI3K, phosphorylated-PI3K, p-AKT, phosphorylated-AKT; GSK3β, Glycogen synthesis kinase 3 beta; p-GSK3β, phosphorylated- GSK3β; GAPDH, glyceraldehyde-3-phosohate dehydrogenase; SD, standard deviation. *, $P < 0.05$; **, $P < 0.01$.

the control group, while the miR-23b suppression significantly upregulated the expression of them ($P < 0.01$ or $P < 0.05$). Therefore, we inferred miR-23b suppressed the PI3K/AKT/GSK3β signaling pathway to affect A549 cells.

Discussion

Researchers have reported that altered miRNAs expression contributed in carcinogenesis and was implicated in cancer cell proliferation and apoptosis [24]. MiRNAs might function as regulatory molecules and acted as tumor suppressors or oncogenes [25]. Nowadays, plenty of miRNAs have been found to affect NSCLC cell invasion, induct apoptosis and *etc*. by its own post-transcriptional regulation, self-regulation of expression, epigenetic regulation and anti-tumor properties [26, 27]. Nora *et al.* have reported that miR-15a/miR-16 were implicated in cell cycle control and likely contribute to the tumorigenesis of NSCLC [28]. In the current study we presented *in vitro* data suggesting a possible role for miR-23b in NSCLC cells viability and migration inhibition and apoptosis induction. More importantly, the protein expression levels of p-PI3K, p-AKT and p-GSK3β in A549 cells were all downregulated by transfected with miR-23b mimic.

MiR-23b which belongs to the miR-23b-27b-24-1 cluster (9q22.32), has been described as a pleiotropic modulator in different organs especially with regard to cancer development [29]. MiR-23b has been verified play important roles in cancer progression, and have dual role in carcinogenesis, both as a tumor suppressor and as an oncogene [30]. For examples, miR-23b promoted tumorigenesis of breast cancer cells [31], inhibited carcinogenesis in prostate cancer cells [32] andinhibited migration and metastasis in colorectal cancer cells [33]. It also has been verified miR-23b suppressed lymph node metastasis in breast cancer [34], blocked gastric cancer progression [33] and *etc*. Previous studies indicated that miR-23b was significant amplification in NSCLC, and it was a potential oncogene for the genesis and progression of NSCLC [35]. But the exactly effects of miR-23b on NSCLC cells activity has not been deeply explored. In our studies, we measured biological functions of miR-23b on A549 cells *in vitro*. Our results indicated thatmiR-23b overexpression could significantly inhibit cell viability and migration, while induce apoptosis in A549 cells. Therefore, miR-23b might exert tumor suppressor function in NSCLC cells by regulating cell viability, migration and apoptosis.

In order to reveal the underlying molecular mechanisms by which miR-23b affected NSCLC cells activity, the protein expression levels of p-PI3K, p-AKT, and p-GSK3β were assessed. We found that expression levels of these three proteins were decreased significantly in miR-23b mimic transfected cells. Among these kinases, PI3K and AKT were the important kinases in the PI3K/ATK signaling pathway.The PI3K/AKT signaling pathway plays an essential role in maintaining tumor cell proliferation and apoptosis [36]. GSK3β is one of the downstream negatively regulated targets of PI3K/AKT pathway, which couldinduce apoptosis via an apoptosis protein of B cell lymphoma/lewkmia-2 (Bcl-2) family myeloidcelleukemia-1 (Mcl-1) phosphorylation and also participate in dicers cellular processes including proliferation, differentiation, motility and survival [37, 38]. Thus our study indicated that miR-23b might affect the cell progresses in A549 cells via regulating the PI3K/AKT/GSK3β pathway.

In conclusion, we found that miR-23b was the major mediator of cell viability, migration and apoptosis *in vitro*, and this might be related with PI3K/AKT/GSK3β signaling pathway. Further exploration need to be focused on the precise role of miR-23b in the other types of NSCLC cells.

Disclosure of conflict of interest

None.

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References

[1] Jemal A, Siegel R, Xu J, Ward E. Cancer statistics CA: Cancer J Clin 2010; 60: 277-300.

[2] Jack RH, Davies EA and Moller H. Lung cancer incidence and survival in different ethnic groups in South East England. Br J Cancer 2011; 105: 1049-1053.

[3] Daskalos A, Nikolaidis G, Xinarianos G, Savvari P, Cassidy A, Zakopoulou R, Kotsinas A, Gorgoulis V, Field JK and Liloglou T. Hypomethylation of retrotransposable elements correlates with genomic instability in non-small cell lung cancer. Int J Cancer 2009; 124: 81-87.

[4] Tomida S, Koshikawa K, Yatabe Y, Harano T, Ogura N, Mitsudomi T, Some M, Yanagisawa K, Takahashi T, Osada H and Takahashi T. Gene expression-based, individualized outcome prediction for surgically treated lung cancer patients. Oncogene 2004; 23: 5360-5370.

[5] Yanagisawa K, Yu S, Xu BJ, Massion PP, Larsen PH, White BC, Roberts JR, Edgerton M, Gonzalez A and Nadaf S. Proteomic patterns of tumour subsets in non-small-cell lung cancer. Lancet 2003; 362: 433-439.

[6] Sidransky D. Emerging molecular markers of cancer. Nat Rev Cancer 2002; 2: 210-219.

[7] Chen G, Gharib TG, Wang H, Huang CC, Kuick R, Thomas DG, Shedden KA, Misek DE, Taylor JM, Giordano TJ, Kardia SL, Iannettoni MD, Yee J, Hogg PJ, Orringer MB, Hanash SM and Beer DG. Protein profiles associated with survival in lung adenocarcinoma. Proc Natl Acad Sci U S A 2003; 100: 13537-13542.

[8] Pallis AG, Gridelli C, Wedding U, Faivre-Finn C, Veronesi G, Jaklitsch M, Luciani A and O'Brien M. Management of elderly patients with NSCLC; updated expert's opinion paper: EORTC Elderly Task Force, Lung Cancer Group and International Society for Geriatric Oncology. Ann Oncol 2014; 25: 1270-1283.

[9] Liloglou T, Bediaga NG, Brown BRB, Field JK and Davies MPA. Epigenetic biomarkers in lung cancer. Cancer Lett 2014; 342: 200-212.

[10] Ebrahimi A and Sadroddiny E. MicroRNAs in lung diseases: Recent findings and their pathophysiological implications. Pulm Pharmacol Ther 2015; 34: 55-63.

[11] Vescovo VD and Denti MA. microRNA and Lung Cancer. Zhongguo Fei Ai Za Zhi 2010; 13: 380-385.

[12] Chellappan SP, Chen G, Umelo IA, Lv S, Teugels E, Fostier K, Kronenberger P, Dewaele A, Sadones J, Geers C and De Grève J. miR-146a Inhibits Cell Growth, Cell Migration and Induces Apoptosis in Non-Small Cell Lung Cancer Cells. PLoS One 2013; 8: e60317.

[13] Liu J, Lv WD and Zhao Q. Research Progress of microRNA in Revention and Treatment of Non-small Cell Lung Cancer. Prog Mod Biom 2015; 15: 1966-1970.

[14] Janikova M, Zizkova V, Skarda J,Kharaishvili G, Radova Land Kolar Z. Prognostic significance of miR-23b in combination with P-gp, MRP and LRP/MVP expression in non-small cell lung cancer. Neoplasma 2016; 63: 576-587.

[15] Donadelli M, Dando I, Fiorini C and Palmieri M. Regulation of miR-23b expression and its dual role on ROS production and tumour development. Cancer Lett 2014; 349: 107-113.

[16] Sun YP, Lu F, Han XY, Ji M, Zhou Y, Zhang AM, Wang HC, Ma DX and Ji CY. MiR-424 and miR-27a increase TRAIL sensitivity of acute myeloid leukemia by targeting PLAG1. Oncotarget 2016; 7: 25276-90.

[17] Grozio A, Paleari L, Catassi A, Servent D, Cilli M, Piccardi F, Paganuzzi M, Cesario A, Granone P, Mourier G and Russo P. Natural agents targeting the alpha7-nicotinic-receptor in NSCLC: a promising prospective in anti-cancer drug development. Int J Cancer 2008; 122: 1911-1915.

[18] Livak KJ and Schmittgen TD. Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the 2-ΔΔCT Method. Methods 2001; 25: 402-408.

[19] Zhang N, Su Y and Xu L. Targeting PKCepsilon by miR-143 regulates cell apoptosis in lung cancer. FEBS Lett 2013; 587: 3661-3667.

[20] Duong BN, Elliott S, Frigo DE, Melnik LI, Vanhoy L, Tomchuck S, Lebeau HP, David O, Beckman BS, Alam J, Bratton MR, McLachlan JA and Burow ME. AKT regulation of estrogen receptor beta transcriptional activity in breast cancer. Cancer Res 2006; 66: 8373-8381.

[21] Hutchinson J, Jin J, Cardiff RD, Woodgett JR and Muller WJ. Activation of Akt (protein kinase B) in mammary epithelium provides a critical cell survival signal required for tumor progression. Mol Cell Biol 2001; 21: 2203-2212.

[22] Ma Z, Zhu L, Luo X, Zhai S, Li P and Wang X. Perifosine enhances mTORC1-targeted cancer therapy by activation of GSK3beta in NSCLC cells. Cancer Biol Ther 2012; 13: 1009-1017.

[23] Mishra R. Glycogen synthase kinase 3 beta: can it be a target for oral cancer. Mol Cancer 2010; 9: 144.

[24] Seyhan AA. microRNAs with different functions and roles in disease development and as potential biomarkers of diabetes: progress and challenges. Mol Biosyst 2015; 11: 1217-1234.

[25] Calin GA, Sevignani C, Dumitru CD, Hyslop T, Noch E, Yendamuri S, Shimizu M, Rattan S, Bullrich F, Negrini M and Croce CM. Human microRNA genes are frequently located at fragile sites and genomic regions involved in cancers. Proc Natl Acad Sci U S A 2004; 101: 2999-3004.

[26] Cinegaglia NC, Andrade SC, Tokar T, Pinheiro M, Severino FE, Oliveira RA, Hasimoto EN, Cataneo DC, Cataneo AJ, Defaveri J, Souza CP, Marques MM, Carvalho RF, Coutinho LL, Gross JL, Rogatto SR, Lam WL, Jurisica I, Reis PP. Integrative transcriptome analysis identifies deregulated microRNA-transcription factor networks in lung adenocarcinoma. Oncotarget 2016; 7: 28920-34.

[27] Cinegaglia NC, Andrade SC, Tokar T, Pinheiro M, Severino FE, Oliveira RA, Hasimoto EN, Cataneo DC, Cataneo AJ, Defaveri J, Souza CP, Marques MM, Carvalho RF, Coutinho LL, Gross JL, Rogatto SR, Lam WL, Jurisica I, Reis PP. Integrative transcriptome analysis identifies deregulated microRNA-transcription factor networks in lung adenocarcinoma. Oncotarget 2016; 7: 28920-34.

[28] Bandi N, Zbinden S, Gugger M, Arnold M, Kocher V, Hasan L, Kappeler A, Brunner T and Vassella E. miR-15a and miR-16 are implicated in cell cycle regulation in a Rb-dependent manner and are frequently deleted or down-regulated in non-small cell lung cancer. Cancer Res 2009; 69: 5553-5559.

[29] Donadelli M and Palmieri M. Roles for microRNA 23b in regulating autophagy and development of pancreatic adenocarcinoma. Gastroenterology 2013; 145: 936-938.

[30] Pençe S, Özbek E, Ozan TN and Ersoy TN. Deregulation of seven CpG island-harboring miRNAs in bladder cancer: miR-155 and miR-23b as the most promising oncomiRs. CellMolBiol (Noisy-le-Grand, France) 2016; 62: 25-30.

[31] Qiu Q, Wei Y, Mercatali L, Lbriahim T, Amadori D and Kang Y. The MicroRNA-23b/27b/24 Cluster Promotes Breast Cancer Lung Metastasis by Targeting Metastasis-suppressive Gene Prosaposin. Bio Chem 2014; 289: 21888-21895.

[32] Ishteiwy RA, Ward TM, Dykxhoorn DM and Burnstein KL. The microRNA -23b/-27b cluster suppresses the metastatic phenotype of castration-resistant prostate cancer cells. PLoS One 2012; 7: e52106.

[33] Zhang H, Hao Y, Yang J, Zhou Y, Li J, Yin S, Sun C, Ma M, Huang Y and Xi JJ. Genome-wide functional screening of miR-23b as a pleiotropic modulator suppressing cancer metastasis. Nat Commun 2011; 2: 554.

[34] Pellegrino L, Stebbing J, Braga VM, Frampton AE, Jacob J, Buluwela L, Jiao LR, Periyasamy M, Madsen CD, Caley MP, Ottaviani S, Roca-Alonso L, El-Bahrawy M, Coombes RC, Krell J and Castellano L. miR-23b regulates cytoskeletal remodeling, motility and metastasis by directly targeting multiple transcripts. Nucleic Acids Res 2013; 41: 5400-5412.

[35] Begum SH, Hayashi M, Ogawa T, Jabboure FJ, Brait M, Izumchenko E, Tabak S, AhrendtSA, Westra WH, Koch W, Sidransky D, Hoque MO. An integrated genome-wide approach to discover deregulated microRNAs in non-small cell lung cancer: Clinical significance of miR-23b-3p deregulation. Sci Rep 2015; 5: 13236.

[36] Chandarlapaty S, Sawai A, Scaltriti M, Rodrik-Outmezguine V, Grbovic-Huezo O, Serra V, Majumder PK, Baselga J and Rosen N. AKT inhibition relieves feedback suppression of receptor tyrosine kinase expression and activity. Cancer Cell 2011; 19: 58-71.

[37] Cross DA, Alessi DR, Cohen P, Andjelkovich M and Hemmings BA. Inhibition of glycogen synthase kinase-3 by insulin mediated by protein kinase B. Nature 1995; 378: 785-789.

[38] Luo J. Glycogen synthase kinase 3beta (GSK3beta) in tumorigenesis and cancer chemotherapy. Cancer Lett 2009; 273: 194-200.