Biochemical and Biophysical Research Communications 440 (2013) 604–610

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

MiR-92b regulates the cell growth, cisplatin chemosensitivity of A549 non small cell lung cancer cell line and target PTEN

Yan Li [a,1], Li Li [b,1], Yan Guan [a], Xiuju Liu [a], Qingyong Meng [c], Qisen Guo [a,*]

[a] Department of Medical Oncology, Shandong Cancer Hospital and Institute, Jinan University, Jinan, Shandong 250117, PR China
[b] Department of Pathology, Shandong University School of Medicine, Jinan, Shandong 250012, PR China
[c] The No. 2 People's Hospital of Jinan, Jinan, Shandong 250001, PR China

ARTICLE INFO

Article history:
Received 17 September 2013
Available online 4 October 2013

Keywords:
MiR-92b
PTEN
Cell growth
CDDP
NSCLC

ABSTRACT

MicroRNAs (miRNAs) have emerged to play important roles in tumorigenesis and drug resistance of human cancer. Fewer studies were explored the roles of miR-92b on human lung cancer cell growth and resistance to cisplatin (CDDP). In this paper, we utilized real-time PCR to verify miR-92b was significantly up-regulated in non-small cell lung cancer (NSCLC) tissues compared to matched adjacent normal tissues. *In vitro* assay demonstrated that knock-down of miR-92b inhabits cell growth and sensitized the A549/CDDP cells to CDDP. Furthermore, we found miR-92b could directly target PTEN, a unique tumor suppressor gene, which was downregulated in lung cancer tissues compared to the matched adjacent normal tissues. These data indicate that the miR-92b play an oncogene roles by regulates cell growth, cisplatin chemosensitivity phenotype, and could serve as a novel potential maker for NSCLC therapy.

1. Introduction

Lung cancer, including NSCLC, is the leading cause of cancer-related mortality men and women worldwide [1,2]. Patients with non-small cell lung cancer are mostly treated with platinum-based chemotherapy. However, the development of chemoresistance is a major obstacle limiting successful treatment [3]. Although recent advances in clinical and experimental oncology, the potential mechanism accounting for the tumorigenesis and drug resistance of NSCLC is still needed to be explored.

MicroRNAs (miRNAs) are a family of small, non-coding endogenous single RNA molecules that play important roles in gene expression through binding to the 3′-untranslated region (3′UTR) of target gene mRNA, leading to mRNA cleavage or translational repression [4]. MiRNAs are differentially expressed in human cancers and play important roles in carcinogenesis [5]. For instance, a number of differentially regulated miRNAs, such as miR-155, let-7a, miR-21 and miR-34a have been identified to be functionally associated with cancer cell proliferation, invasion and metastasis [6–9]. And numbers of miRNAs that deregulated in NSCLC were also revealed by microarray profiles in lung cancer tissues compared to normal tissues, including miR-92b [10–13]. However, there are fewer reports about the roles of miR-92b, especially in NSCLC cells.

In the present study, we demonstrated an up-regulation of miR-92b level in NSCLC compared to the matched normal tissues. Suppression of miR-92b inhabited NSCLC A549 cells proliferation, and sensitized the A549/CDDP cells to CDDP. And we also demonstrate that miR-92b is up-regulated in the drug-resistant A549/CDDP NSCLC cell line, compared with the parental A549 cell line. Subsequent experiments confirmed that miR-92b could regulate the expression of PTEN (phosphatase and tensin homolog deleted on chromosome ten) at both mRNA and protein levels through directly interacted with its 3′UTR. Our results suggest that miR-92b play roles in the development of drug resistance and growth malignant behavior in NSCLC cells, at least in part by targeting the tumor suppressor, PTEN.

2. Materials and methods

2.1. NSCLC cancer tissue and cell culture

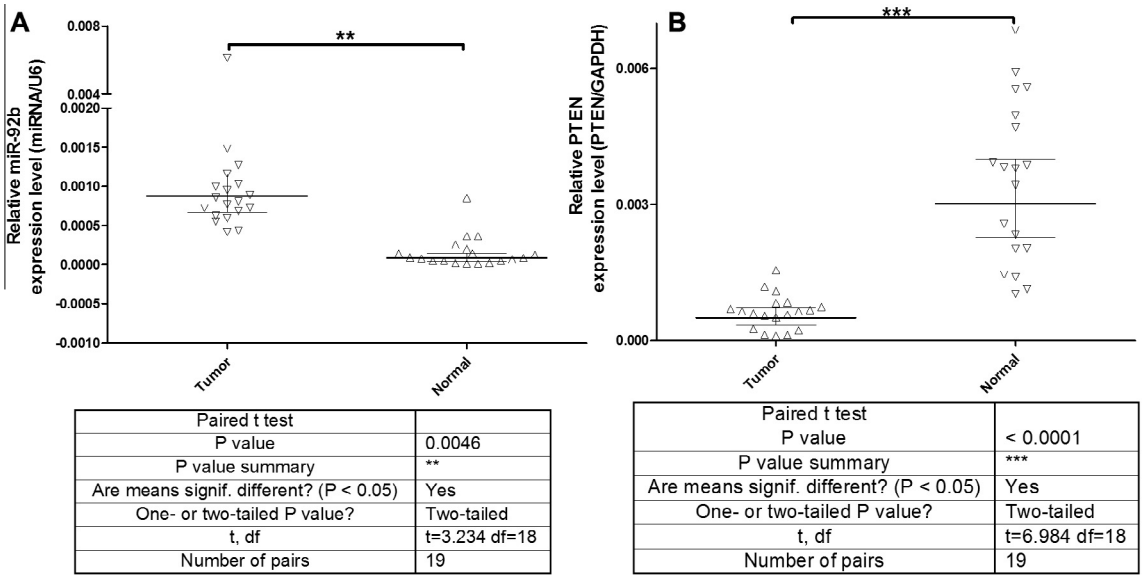
Paired NSCLC and adjacent non-tumor lung tissues were obtained from 19 consecutive patients who under-went primary surgical resection of NSCLC with informed consent at Shandong Cancer Hospital, China. Surgically laser capture micro-dissected NSCLC and adjacent normal tissues were immediately snap-frozen in liquid nitrogen and stored at −80 °C until total RNA was extracted. A549 cells were cultured in RPMI1640 medium, supplemented with 10% fetal bovine serum (FBS) and 1% PS (100 U/ml

* Corresponding author. Address: Department of Medical Oncology, Shandong Cancer Hospital and Institute, Jinan University, No. 440, Jiyan Road, Jinan, Shandong 250117, PR China. Fax: +86 0531 67626322.
E-mail address: guoqisen0202@163.com (Q. Guo).
[1] These authors contribute equal to the work.

0006-291X/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2013.09.111

Table 1
Primers and sequence in this study.

Name	Primer	Sequence
U6	Forward	5′-GTGCTCGCTTCGGCAGCACATATAC-3′
U6	Reverse	5′-AAAAATATGGAACGCTCACGAATTTG-3′
GAPDH	Forward	5′-ATGTCGTGGAGTCTACTGGC-3′
GAPDH	Reverse	5′-TGACCTTGCCCACAGCCTTG-3′
MiR-92b	Forward	5′-TATTGCACTCGTCCCGGCCTCC-3′
MiR-92b	Reverse	5′-GTGCAGGGTCCGAGGT-3′
PTEN	Forward	5′-CCCAGTCAGAGGCGCTATGTGTAT-3′
PTEN	Reverse	5′-GTTCCGCCACTGAACATTGG-3′
MiR-92b inhibitor	Anti-sense miR-92b	2′-O-Me-GGAGGCCGGGACGAGUGCAAU



Paired t test	
P value	0.0046
P value summary	**
Are means signif. different? (P < 0.05)	Yes
One- or two-tailed P value?	Two-tailed
t, df	t=3.234 df=18
Number of pairs	19

Paired t test	
P value	< 0.0001
P value summary	***
Are means signif. different? (P < 0.05)	Yes
One- or two-tailed P value?	Two-tailed
t, df	t=6.984 df=18
Number of pairs	19

Fig. 1. MiR-92b was unregulated and PTEN was downregulated in NSCLC tissues. MiR-92b and PTEN expression level was analyzed by qRT-PCR in NSCLC tissues and adjacent non-tumor tissues. Data are presented as the means ± SD from three independent experiments, $^{*}p < 0.05$.

penicillin, 100 μg/ml streptomycin) and maintained in a humidified incubator with 5% CO2 at 37 °C.

2.2. MTT assay, miRNAs mimic and transfection

The transfected cells were plated into 96-well plates at a density of 5000 cells/well. At 48 h after transfection, the cells were incubated with MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) for 4 h at 37 °C. Then the cells were agitated with MTT solvent on an orbital shaker for 10 min avoiding light. The absorbance at 570 nm (OD570 nm) was measured with a spectrophotometer. The human miR-92b duplex mimics (miR-92b) and anti-sense miR-92b oligonucleotide duplex mimics were designed and provided by Ribobio (Guangzhou, Guangdong, China). 30–50% confluent cells were transfected with miRNAs by Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. The mimics sequences and primers used in this study was described in Table 1.

2.3. Colony formation assay

The cells were seeded into 12-well plate at a density of 200 cells/well after transfection. The medium was changed every three days. Approximately 10 days later, most of the cell clones contained more than 50 cells. The clones were washed with 1 times PBS and stained with crystal violet for about 5 min. Finally the clones were taken pictures and counted. The colony formation rate = (number of clones)/ (number of seeded cells) × 100%.

2.4. Induction of CDDP-resistant A549 cell lines and IC50 definition

The CDDP-resistant A549 cell lines were induced using progressive concentration of CDDP. Briefly, the A549 cells in logarithmic growth were treated with 0.5 μmol/L of CDDP. After 48 h, CDDP was withdrawn and cells were cultured without CDDP until they recovered. Then, the same treatment was performed, and when the cells were resistant to the current concentration, the CDDP concentration was gradually increased to 1–4 and finally to 6 μmol/L. When the induced cells survived in 6 μmol/L of CDDP for about 2 months with a normal activity, the cells were confirmed to be CDDP-resistant and named A549/CDDP. The resistance of the induced cells was measured by IC50. The concentration of CDDP that caused 50% inhibition of A549 cell activity was defined as IC50.

2.5. Annexin V and PI staining

Enumeration of apoptotic cells was done by using FITC conjugated Annexin V and PI (BD Pharmingen, San Jose, CA). Cells were washed twice in cold 1 times PBS and resuspended in Annexin V-binding buffer (BD Pharmingen) at a concentration of 3 × 106 per ml. This suspension (100 μl) was stained with 5 μl of Annexin V-FITC and 5 μl PI. These cells were gently vortexed and

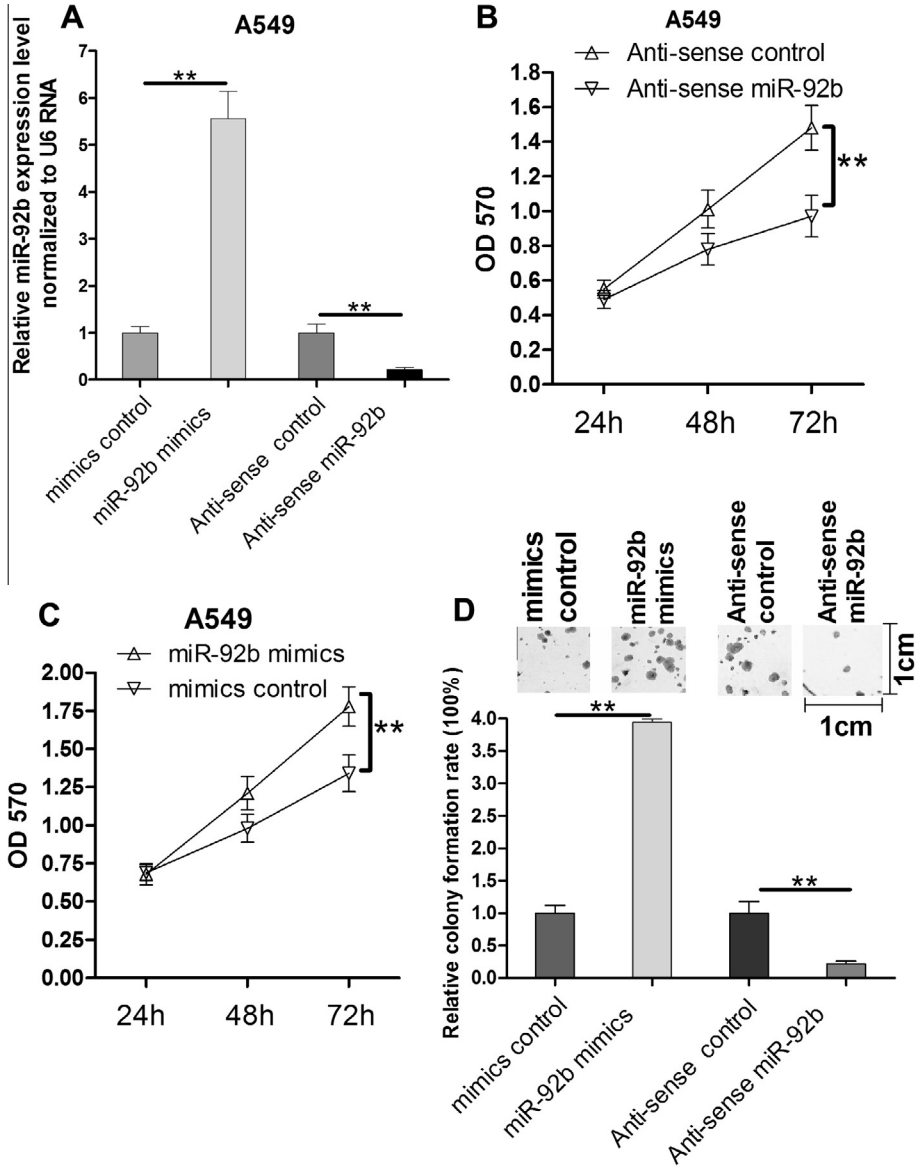


Fig. 2. Effects of miR-92b on the cell growth of A549 cells. (A) miR-92b expression level was analyzed by qRT-PCR. (B, C) MTT assay was performed to detect the effect of anti-sense miR-92b or miR-92b mimics on the cell viability in A549 cells with 24 h, 48 h and 72 h after transfection. (D) Colony formation assay was performed to detect the effect of miR-92b on cell growth in A549 cells. The colonies were stained with crystal violet and then counted. Representative pictures were shown, and data are presented as the means ± SD from three independent experiments, $^{**}p < 0.01$. Scale bar, 1 cm.

incubated for 15 min at room temperature in the dark. After addition of 400 μl of binding buffer to each tube, cells were analyzed by flow cytometry.

2.6. Dual luciferase activity assay

The 3′-UTR of human PTEN cDNA containing the putative target site for miR-92b was chemically synthesized and inserted into downstream of the luciferase gene in the pGL3-control vector (Promega, Madison, WI, USA). Twenty-four hours prior to transfection, the cells were plated at 1.5 × 105 cells/well in 24-well plates. Two hundred nanograms of pGL3-PTEN-3′-UTR plus 80 ng pRL-TK (Promega) were transfected in combination with 60 pmol of the miR-92b mimic, anti-sense miR-92b or controls using Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions. Luciferase activity was measured 24 h after transfection using the Dual Luciferase Reporter Assay System (Promega). Firefly luciferase activity was normalized to Renilla luciferase activity for each transfected well. Three independent experiments were performed in duplicate.

2.7. Western blot and immunofluorescence assays

The cells were harvested at 48 h after transfection and lysed by RIPA buffer for 30 min at 4 °C. 20 μg proteins were loaded into 15% SDS–PAGE for analysis. The first antibody was rabbit polyclonal anti-PTEN (Merck Millipore, Billerica, USA, 1:100 dilutions) and anti-GAPDH antibody (Abcam, San Francisco, USA, 1:1000 dilution). The secondary antibody was goat anti-rabbit IgG conjugated with HRP (horseradish peroxidase) with a dilution of 1:1000. The bound antibodies were detected using ECL Plus Western Blotting Detection system (GE Healthcare). GAPDH was used as an internal control to normalize PTEN expression level. Immunofluorescence assays was used to detect the PTEN expression level after transfected with anti-sense miR-92b 24 h later, the second antibody was donkey anti-rabbit which was labeled with Green fluorescein. The cell nuclear was stained by DAPI as a control. Pictures were

taken 24 h after transfection. Representative images from one of three independent experiments are shown. The fluorescence intensity was fixed quantity by Image-J software.

2.8. Statistical analysis

All the data were shown as mean + SD and the experiments were repeated three times. The difference was determined by two-tailed students' *t*-test and $p < 0.05$ was considered statistically significant.

3. Result

3.1. MiR-92b was upregulated in NSCLC tissues

MicroRNA profiles have been demonstrated miR-92b was upregulated in the lung cancer tissues compared with normal lung tissues in the previous studies [10–13]. However, the precise expression level of miR-92b in NSCLC tissues remains unclear. To reach this goal, we performed qRT-PCR between the 19 pairs of NSCLC tissues and adjacent non-tumor tissues. As shown in Fig 1A, we discovered that miR-92b expression was increased in tumor tissues compared with the adjacent normal tissues. These data suggested that miR-92b may play oncogene roles in the NSCLC development.

3.2. Overexpression of miR-92b promotes, while knockdown of it inhabits A549 cell growth

MTT assay and colony formation assay were used to determine the effects of miR-92b in A549 cells. The expression of miR-92b mimics or anti-sense miR-92b was identified by qRT-PCR (Fig 2A). The Fig. 2B suggests transfection of A549 cells with anti-sense miR-92b caused suppression of A549 cell proliferation at 72 h post-infection when compared with controls. Meanwhile, miR-92b could promote A549 cell proliferation in Fig. 2C. To further confirm the effects of miR-92b on the cell growth of A549 cells. Colony formation assay was performed to detected colony formation ability of A549 cells. As expected, transfection of miR-92b can increase the colony formation ability of A549 cells about 3folds while blocking of it decrease about 80% when compared with controls. This implied that miR-92b can regulate cell growth ability significantly in NSCLC A549 cell lines.

3.3. MiR-92b regulates the resistance of NSCLC A549 cells to CDDP

To determine whether miR-92b is involved in the development of drug resistance in NSCLC cells, the level of miR-92b was analyzed in the A549/CDDP cells compared with the parent cell line, A549. Real-time PCR for miR-92b verified that miR-92b was significantly upregulated in the A549/CDDP cells, and the increased fold-change was 9.25 ± 0.75 (Fig. 2A). In the A549 cells, IC50 revealed that the A549 cells transfected with the miR-92b mimic exhibited a significantly increaseing resistance to CDDP compared with the controls (Fig. 2B), whereas the A549/CDDP cells transfected with the anti-sense miR-92b exhibited a significantly decreasing resistance to CDDP compared with the controls (Fig. 2C). These results suggest that miR-92b regulates the resistance of NSCLC A549 cells to CDDP.

3.4. Anti-miR-92b sensitizes A549/CDDP cells to CDDP-induced apoptosis

To determine whether miR-92b plays a role in the development of drug resistance, at least in part through the modulation of apoptosis, we evaluated CDDP-induced apoptosis following the transfection of A549/CDDP cells with anti-sense miR-92b or anti-sense control. In the A549/CDDP cells, a marked increasing in apoptosis rate (1.6–36.6%), as assessed by flow cytometry, was observed in the anti-sense miR-92b transfected cells following treatment with CDDP, compared with the control group (Fig. 3D). These results suggest anti-miR-92b sensitizes A549/CDDP cells to CDDP-induced apoptosis.

3.5. Indentify PTEN is a target of miR-92b

We consensus analysis the miR-92b targets using multi-miRNA target prediction algorithms including Targetscan, Target-Miner, miRDB, DIANA LAB and RNA22, and find PTEN, which are reported play important roles in the pathogenesis and drug resistance of melanoma cells [14,15], has a high possibility to be a candidate target of miR-92b. Generally, four of the five algorithms predicted

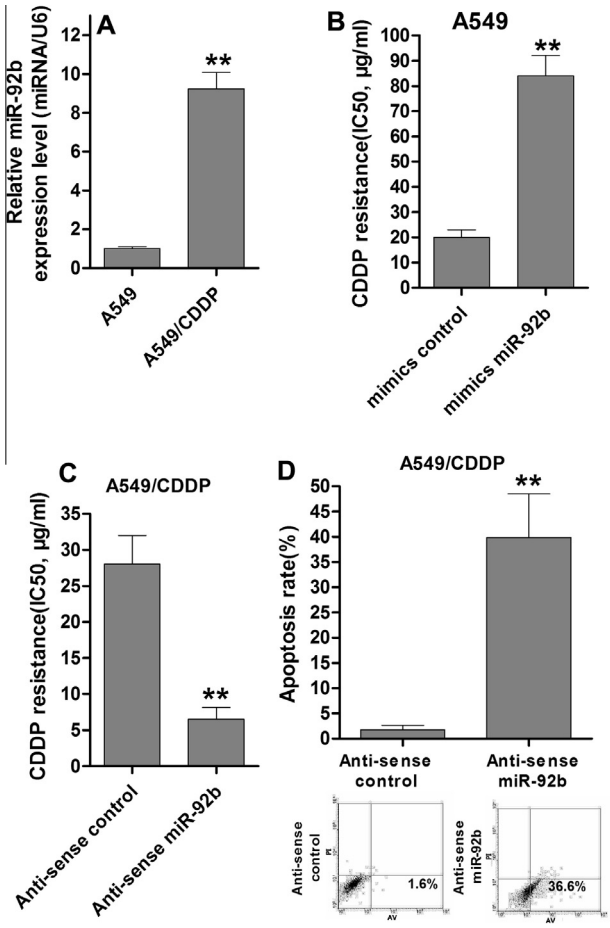


Fig. 3. MiR-92b regulates the resistance of NSCLC A549 cells to CDDP. (A) Real-time quantification of miR-92b by stem-loop reverse transcription and real-time PCR (RT-PCR) showed that miR-92b was upregulated in the A549/CDDP cisplatin (CDDP)-resistant cell line compared with the A549 cell line. Triplicate assays were performed for each RNA sample, and the relative amount of miR-92b was normalized to U6 snRNA. Data are presented as fold-changes of miR-92b levels in the A549 cells relative to the A549/CDDP cells, which was set as 1 (means ± SD). $^{**}p < 0.01$. (B) In the A549 cells, the cells transfected with the miR-92b mimic exhibited significantly increased resistance to CDDP. (C) In the A549/CDDP cells, the cells transfected with the anti-sense miR-92b exhibited significantly decreased resistance to CDDP. $^{**}p < 0.01$. (D) In the A549/CDDP cells, apoptosis was evaluated by flow cytometry. The results revealed a marked increase in apoptosis in anti-sense miR-92b-transfected cells following treatment with CDDP, compared with the anti-sense control-transfected cells. Representative flow cytometry report is shown. The results shown represent the means ± SD from three independent experiments. $^{**}p < 0.01$.

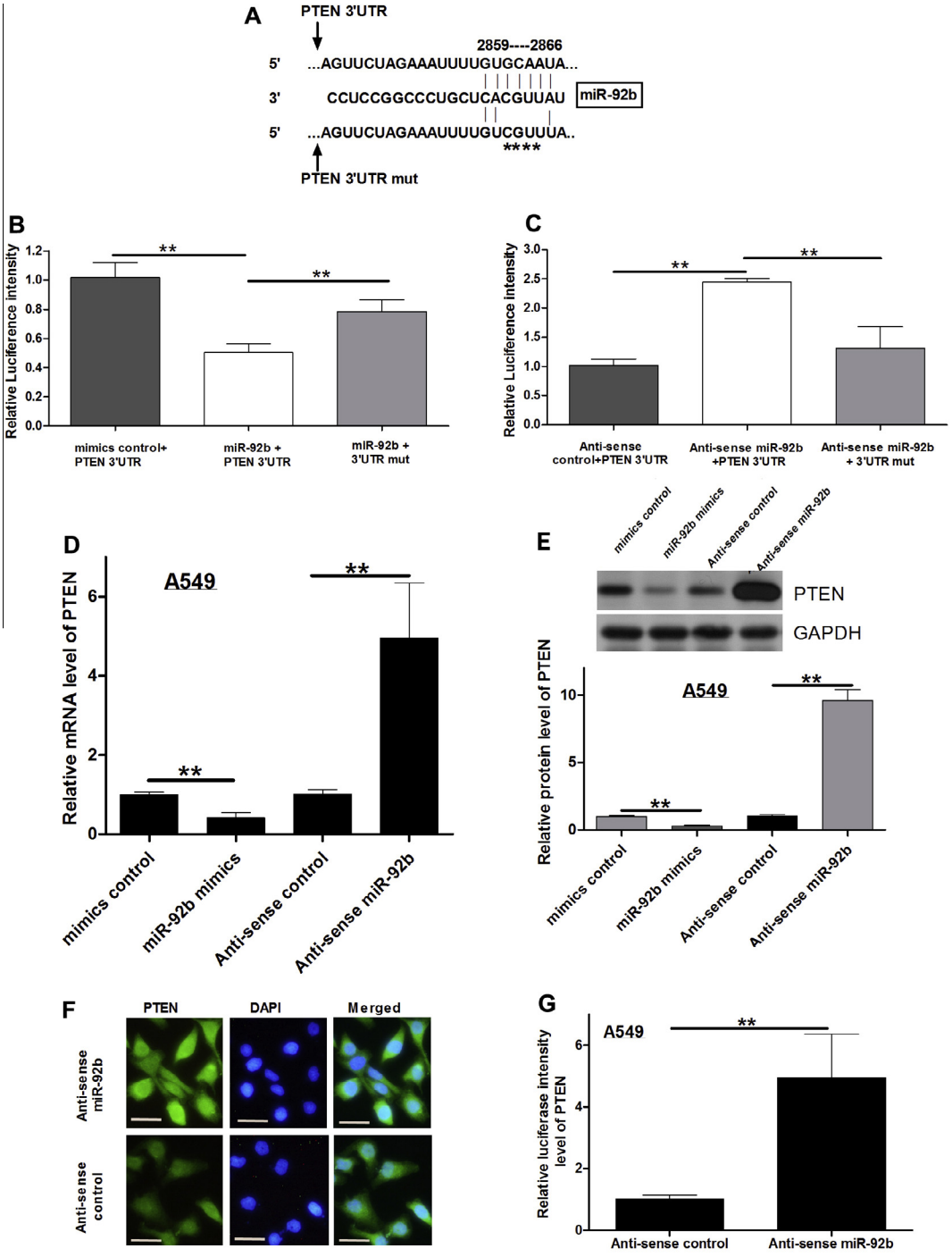


Fig. 4. Predict PTEN was potential targets for miR-92b and its validation. (A) Showed the algorithms between miR-92b and the 3′UTR of PTEN and also the mutant PTEN 3′UTR (four nucleotides within binding sites were mutated). (B,C) Luciference reporter assay was performed to detect the effect of miR-92b and anti-sense miR-92b on the Luciference intensity controlled by 3′UTR of PTEN. $^{**}p < 0.01$. (D) qRT-PCR was performed to detect the effect of miR-92b on PTEN mRNA expression. (E) Western blot was preformed to detect the effect of miR-92b on the protein levels of PTEN. (F,G) Immuneofluorescence assay was preformed to detect the effect of anti-sense miR-92b on the expression levels of PTEN. Representative pictures were shown, and data are presented as the means ± SD from three independent experiments. Scale bar, 20 μm. ** $p < 0.01$.

there have putative target sites in an 8-mer seed match manner. To confirm whether the 3′UTR of PTEN was a functional target of miR-92b in NSCLC, we set a Luciference reporter system. The alignment of miR-93 with the PTEN 3′UTR insert is illustrated in Fig 4A (seed sequence was 2859–2866). Meanwhile, we also constructed a mutated 3′UTR vector, which contains four mutated nucleotides in the seed sequence (Fig. 4A asterisk). When miR-92b was overexpression by miR-92b mimics, the Luciferase expression level was significantly lower than the control group. However, the Luciferase intensity with the mutated 3′UTR was not affected by miR-92b (Fig. 4B). When blocking the expression of miR-92b with anti-sense miR-92b, we get an increased luciferase intensity in A549 cells (Fig. 4C). So we concluded that PTEN was a direct target of miR-92b in A549 cells.

3.6. MiR-92b down-regulates PTEN expression at mRNA and protein level in A549 cells

Next, qRT-PCR and assays and Western blotting were performed to determine the regulation of endogenous PTEN by miR-92b.

Compared with the controls, miR-92b significantly decrease the PTEN mRNA expression level (Fig. 4D). And the PTEN protein level was also significantly decreased by transfection of miR-92b (Fig. 4E). At the same time, compared with anti-sense controls, anti-sense miR-92b upregulated the expression of PTEN in A549 cells (Fig. 4D, E). Immunofluorescence assay was also suggested anti-sense miR-92b can increased the expression of PTEN in A549 cells (enhanced luciferase intensity showing in Fig. 4F,G). These results strongly suggest that miR-92b negatively regulates PTEN expression in NSCLC A549 cells.

3.7. The PTEN was inversely expressed with miR-92b in NSCLC and normal tissues

At last, we conjectured the expression of PTEN in the 19 paired NSCLC and normal lung tissues, which was performed as well as the miR-92b. To expected, PTEN was expressed at a low level in the 19 tumor tissues compared to the corresponding normal tissues (Fig. 1B). It demonstrate PTEN was downregulated while miR-92b was upregulated in the NSCLC tumor tissues, that maybe explained by PTEN was negatively regulated by miR-92b in the process of NSCLC tumorigenesis. But the person correlation coefficient between the expression of miR-92b and PTEN was not get a statistic significance (date was not shown). That may attribute to the larger numbers of molecules in regulation of the expression of PTEN. However, these results suggest the upregulation of miR-92b at least partly caused to the downregulation of PTEN in NSCLC.

4. Discussion

Extensive researches have indicated miRNAs to be critical regulators in cancer-related processes, such as miR-21, miR-155 and miR-34a [7,16,17]. MiRNAs are thought to function as either tumor suppressors or oncogenes though target oncogenes or tumor suppressor genes during tumorigenesis and development of cancers [18–20]. In NSCLC tissues, many onco-miRs/tumor suppressor-target or tumor suppressor-miRs/onco-target pathways have been demonstrated to participate in the tumorigenesis of lung cancer, including miR7/BCL2 axis, miR-99b/FGFR3 axis, miR-101/EZH2 axis, miR-192/RB1 axis and miR-196/HOXA5 axis. [21–25]. However, miRNA/target network was so complex that more and more miRNA/target axis needs to be elucidated in lung cancer especially NSCLC. To the present, miR-92b was investigated to associated with intestinal epithelial differentiation in Caco2-BBE cells by targeting PepT1, regulated neuroblastoma tumorigenesis by targeting DKK3, involved in cell cycle regulation in human embryonic stem cells by targeting p57, and promoted proliferation and invasion in GBM cells by targeting Nemo-like kinase [26–29]. In the present study, we observed a significant inhibition on cell growth by suppression of miR-92b, while a significant promotion by overexpression of miR-92b by MTT and colony formation assays (Fig. 2). These further suggesting miR-92b to be an oncogene in NSCLC cell lines.

As one of the first-line chemotherapeutic agents for the treatment of NSCLC, Cisplatin (CDDP) is a platinum-based compound that forms intra- and inter-strand adducts with DNA [30,31]. Despite tremendous efforts, CDDP treatment often results in the development of drug resistance, leading to therapeutic failure, and the molecular mechanisms leading to CDDP chemoresistance are poorly understood. Recent studies demonstrated that the acquired drug resistance of cancer cells is related to deregulation of miRNAs such as miR21, miR-503, miR-181a and miR-620 [32–34]. To explore whether the unregulated oncogene miR-92b was involved in the NSCLC cells resistant to CDDP, we examined the miR-92b expression in CDDP resistant A549 cells (A549/CDDP) and found that miR-92b was upregulated in the A549/CDDP cells. Further functional studies demonstrate miR-92b can regulate the resistance of NSCLC A549 cells to CDDP (Fig. 3).

Because the fundamental function of miRNAs is to regulate their targets by direct cleavage of the mRNA or by inhibition of protein synthesis [4], we further predicted a number of additional miR-92b targets in NSCLC. Based on consensus analysis multi-miRNA target prediction algorithms, we focus on the tumor suppressor gene PTEN, which has been suggested plays important roles not only in cell cycle detention and apoptosis, but also in regulation of cell adherence, migration, differentiation and has the function of enhancing the sensitivity of cancer cells to certain anticancer agents [35,36]. The following validation study investigated miR-92b can directly target PTEN in A549 cells. Overexpression of miR-92b significantly reduced the endogenous PTEN mRNA and protein levels, while inhabitation of miR-92b has the opposite effects. Therefore, these results suggested that miR-92b decreased the expression of PTEN through direct 3′UTR interactions in NSCLC A549 cells, which may explained the miR-92b mediated regulation mechanism of A549 cell growth and resistant to CDDP.

In summary, this study further extends the biological role of miR-92b in NSCLC A549 cells and for the first time identifies PTEN as a novel target of miR-92b. These findings may provide a basic rationale for the use of miR-92b in the treatment of NSCLC.

Acknowledgments

This work was supported by Shandong Provincial scientific and technological project (No. 2010GSF10258).

References

[1] S. Subramaniam, R.K. Thakur, V.K. Yadav, R. Nanda, S. Chowdhury, A. Agrawal, Lung cancer biomarkers: state of the art, J. Carcinog. 12 (2013) 3.
[2] A. Jemal, M.J. Thun, L.A. Ries, H.L. Howe, H.K. Weir, M.M. Center, E. Ward, X.C. Wu, C. Eheman, R. Anderson, U.A. Ajani, B. Kohler, B.K. Edwards, Annual report to the nation on the status of cancer, 1975–2005, featuring trends in lung cancer, tobacco use, and tobacco control, J. Natl Cancer Inst. 100 (2008) 1672–1694.
[3] G. Szakacs, J.K. Paterson, J.A. Ludwig, C. Booth-Genthe, M.M. Gottesman, Targeting multidrug resistance in cancer, Nat. Rev. Drug Discov. 5 (2006) 219–234.
[4] D.P. Bartel, MicroRNAs: genomics, biogenesis, mechanism, and function, Cell 116 (2004) 281–297.
[5] A. Esquela-Kerscher, F.J. Slack, Oncomirs–microRNAs with a role in cancer, Nat. Rev. Cancer 6 (2006) 259–269.
[6] Y. Akao, S. Noguchi, A. Iio, K. Kojima, T. Takagi, T. Naoe, Dysregulation of microRNA-34a expression causes drug-resistance to 5-FU in human colon cancer DLD-1 cells, Cancer Lett. 300 (2011) 197–204.
[7] H. Shibuya, H. Iinuma, R. Shimada, A. Horiuchi, T. Watanabe, Clinicopathological and prognostic value of microRNA-21 and microRNA-155 in colorectal cancer, Oncology 79 (2010) 313–320.
[8] C.C. Yu, Y.W. Chen, G.Y. Chiou, L.L. Tsai, P.I. Huang, C.Y. Chang, L.M. Tseng, S.H. Chiou, S.H. Yen, M.Y. Chou, P.Y. Chu, W.L. Lo, MicroRNA let-7a represses chemoresistance and tumourigenicity in head and neck cancer via stem-like properties ablation, Oral Oncol. 47 (2011) 202–210.
[9] E. Tili, J.J. Michaille, D. Wernicke, H. Alder, S. Costinean, S. Volinia, C.M. Croce, Mutator activity induced by microRNA-155 (miR-155) links inflammation and cancer, Proc. Natl. Acad. Sci. U. S. A. 108 (2011) 4908–4913.
[10] U. Vosa, T. Vooder, R. Kolde, K. Fischer, K. Valk, N. Tonisson, R. Roosipuu, J. Vilo, A. Metspalu, T. Annilo, Identification of miR-374a as a prognostic marker for survival in patients with early-stage nonsmall cell lung cancer, Genes Chromosom. Cancer 50 (2011) 812–822.
[11] L. Yu, N.W. Todd, L. Xing, Y. Xie, H. Zhang, Z. Liu, H. Fang, J. Zhang, R.L. Katz, F. Jiang, Early detection of lung adenocarcinoma in sputum by a panel of microRNA markers, Int. J. Cancer 127 (2010) 2870–2878.
[12] P. Guan, Z. Yin, X. Li, W. Wu, B. Zhou, Meta-analysis of human lung cancer microRNA expression profiling studies comparing cancer tissues with normal tissues, J. Exp. Clin. Cancer Res. 31 (2012) 54.
[13] R. Wang, Z.X. Wang, J.S. Yang, X. Pan, W. De, L.B. Chen, MicroRNA-451 functions as a tumor suppressor in human non-small cell lung cancer by targeting ras-related protein 14 RAB14, Oncogene 30 (2011) 2644–2658.
[14] X. Fu, J. Tian, L. Zhang, Y. Chen, Q. Hao, Involvement of microRNA-93, a new regulator of PTEN/Akt signaling pathway, in regulation of chemotherapeutic drug cisplatin chemosensitivity in ovarian cancer cells, FEBS Lett. 586 (2012) 1279–1286.

[15] M. Tamura, J. Gu, H. Tran, K.M. Yamada, PTEN gene and integrin signaling in cancer, J. Natl Cancer Inst. 91 (1999) 1820–1828.
[16] R. Hummel, D.J. Hussey, J. Haier, MicroRNAs: predictors and modifiers of chemo- and radiotherapy in different tumour types, Eur. J. Cancer 46 (2010) 298–311.
[17] K. Bhatt, L. Zhou, Q.S. Mi, S. Huang, J.X. She, Z. Dong, MicroRNA-34a is induced via p53 during cisplatin nephrotoxicity and contributes to cell survival, Mol. Med. 16 (2010) 409–416.
[18] A.A. Shah, P. Leidinger, N. Blin, E. Meese, MiRNA: small molecules as potential novel biomarkers in cancer, Curr. Med. Chem. 17 (2010) 4427–4432.
[19] F. Meng, S.S. Glaser, H. Francis, S. DeMorrow, Y. Han, J.D. Passarini, A. Stokes, J.P. Cleary, X. Liu, J. Venter, P. Kumar, S. Priester, L. Hubble, D. Staloch, J. Sharma, C.G. Liu, G. Alpini, Functional analysis of microRNAs in human hepatocellular cancer stem cells, J. Cell Mol. Med. 16 (2012) 160–173.
[20] T.A. Farazi, J.I. Spitzer, P. Morozov, T. Tuschl, MiRNAs in human cancer, J. Pathol. 223 (2011) 102–115.
[21] X.H. Liu, K.H. Lu, K.M. Wang, M. Sun, E.B. Zhang, J.S. Yang, D.D. Yin, Z.L. Liu, J. Zhou, Z.J. Liu, W. De, Z.X. Wang, MicroRNA-196a promotes non-small cell lung cancer cell proliferation and invasion through targeting HOXA5, BMC Cancer 12 (2012) 348.
[22] H.M. Cho, H.S. Jeon, S.Y. Lee, K.J. Jeong, S.Y. Park, H.Y. Lee, J.U. Lee, J.H. Kim, S.J. Kwon, E. Choi, M.J. Na, J. Kang, J.W. Son, MicroRNA-101 inhibits lung cancer invasion through the regulation of enhancer of zeste homolog 2, Exp. Ther. Med. 2 (2011) 963–967.
[23] J. Kang, S.Y. Lee, S.Y. Lee, Y.J. Kim, J.Y. Park, S.J. Kwon, M.J. Na, E.J. Lee, H.S. Jeon, J.W. Son, MicroRNA-99b acts as a tumor suppressor in non-small cell lung cancer by directly targeting fibroblast growth factor receptor 3, Exp. Ther. Med. 3 (2012) 149–153.
[24] S. Xiong, Y. Zheng, P. Jiang, R. Liu, X. Liu, Y. Chu, MicroRNA-7 inhibits the growth of human non-small cell lung cancer A549 cells through targeting BCL-2, Int. J. Biol. Sci. 7 (2011) 805–814.
[25] S. Feng, S. Cong, X. Zhang, X. Bao, W. Wang, H. Li, Z. Wang, G. Wang, J. Xu, B. Du, D. Qu, W. Xiong, M. Yin, X. Ren, F. Wang, J. He, B. Zhang, MicroRNA-192 targeting retinoblastoma 1 inhibits cell proliferation and induces cell apoptosis in lung cancer cells, Nucleic Acids Res. 39 (2011) 6669–6678.
[26] G. Dalmasso, H.T. Nguyen, Y. Yan, H. Laroui, M.A. Charania, T.S. Obertone, S.V. Sitaraman, D. Merlin, MicroRNA-92b regulates expression of the oligopeptide transporter PepT1 in intestinal epithelial cells, Am. J. Physiol. Gastrointest. Liver Physiol. 300 (2011) G52–59.
[27] B.H. Haug, J.R. Henriksen, J. Buechner, D. Geerts, E. Tomte, P. Kogner, T. Martinsson, T. Flaegstad, B. Sveinbjornsson, C. Einvik, MYCN-regulated miRNA-92 inhibits secretion of the tumor suppressor DICKKOPF-3 (DKK3) in neuroblastoma, Carcinogenesis 32 (2011) 1005–1012.
[28] K. Wang, X. Wang, J. Zou, A. Zhang, Y. Wan, P. Pu, Z. Song, C. Qian, Y. Chen, S. Yang, Y. Wang, MiR-92b controls glioma proliferation and invasion through regulating Wnt/beta-catenin signaling via Nemo-like kinase, Neuro Oncol. 15 (2013) 578–588.
[29] S. Sengupta, J. Nie, R.J. Wagner, C. Yang, R. Stewart, J.A. Thomson, MicroRNA 92b controls the G1/S checkpoint gene p57 in human embryonic stem cells, Stem cells 27 (2009) 1524–1528.
[30] J.C. Reed, Mechanisms of apoptosis avoidance in cancer, Curr. Opin. Oncol. 11 (1999) 68–75.
[31] I. Judson, L.R. Kelland, New developments and approaches in the platinum arena, Drugs 59 (Suppl. 4) (2000) 29–36. discussion 37–28.
[32] W. Gao, X. Lu, L. Liu, J. Xu, D. Feng, Y. Shu, MiRNA-21: a biomarker predictive for platinum-based adjuvant chemotherapy response in patients with non-small cell lung cancer, Cancer Biol. Ther. 13 (2012) 330–340.
[33] T. Qiu, L. Zhou, T. Wang, J. Xu, J. Wang, W. Chen, X. Zhou, Z. Huang, W. Zhu, Y. Shu, P. Liu, MiR-503 regulates the resistance of non-small cell lung cancer cells to cisplatin by targeting Bcl-2, Int. J. Mol. Med. 32 (2013) 593–598.
[34] L. Galluzzi, E. Morselli, I. Vitale, O. Kepp, L. Senovilla, A. Criollo, N. Servant, C. Paccard, P. Hupe, T. Robert, H. Ripoche, V. Lazar, A. Harel-Bellan, P. Dessen, E. Barillot, G. Kroemer, MiR-181a and miR-630 regulate cisplatin-induced cancer cell death, Cancer Res. 70 (2010) 1793–1803.
[35] M. Tanaka, H.B. Grossman, *In vivo* gene therapy of human bladder cancer with PTEN suppresses tumor growth, downregulates phosphorylated Akt, and increases sensitivity to doxorubicin, Gene Ther. 10 (2003) 1636–1642.
[36] L.C. Cantley, B.G. Neel, New insights into tumor suppression: PTEN suppresses tumor formation by restraining the phosphoinositide 3-kinase/AKT pathway, Proc. Natl. Acad. Sci. U. S. A. 96 (1999) 4240–4245.