Int J Clin Exp Pathol 2016;9(4):4706-4713
www.ijcep.com /ISSN:1936-2625/IJCEP0021324

Original Article

miR-383 inhibits proliferation and induces apoptosis via targeting HDAC2 in non-small cell lung cancer cells

Jinglian Wang, Bing Li

Department of Clinical Laboratory, Xinxiang Central Hospital, Xinxiang, Henan Province, China

Received December 7, 2015; Accepted February 17, 2016; Epub April 1, 2016; Published April 15, 2016

Abstract: Lung cancer is the most common cause of deaths among all cancers around the world and non-small cell lung cancer (NSCLC) accounts for approximately 85% of all lung cancer cases. In this study, we investigated the expression level of miR-383 and its biological function in NSCLC. Firstly, we observed miR-383 down-regulation and HDAC2 up-regulation in NSCLC by qRT-PCR and Western blot, respectively. Next, we found that HDAC2 levels were inversely correlated with miR-383 expression in NSCLC tumor tissues and identified HDAC2 as a direct target of miR-383 by luciferase reporter assay. Then, the functional in vitro assays indicated that ectopic overexpression of miR-383 inhibited proliferation and induced apoptosis in NSCLC A549 cells, while restoration of HDAC2 partially attenuated these effects of miR-383 on A549 cells. In addition, knockdown of miR-383 resulted in an increase in proliferation and a decrease in apoptosis of A549 cells. In conclusion, our findings revealed the suppressive role of miR-383 in the development of NSCLC and suggested that miR-383 may serve as a useful diagnostic marker and a potential therapeutic target in human lung cancer.

Keywords: miR-383, HDAC2, lung cancer, NSCLC, proliferation, apoptosis

Introduction

Lung cancer is the most common cause of deaths among all cancers around the world with approximately 1.3 million deaths per year, due to its high incidence, malignant behavior and poor prognosis [1]. Non-small cell lung cancer (NSCLC) represents the majority of lung cancers and accounts for approximately 85% of all lung cancer cases [2]. According to its histological features, NSCLC could be divided into three subtypes: squamous cell lung carcinoma, lung adenocarcinoma, and large-cell lung carcinoma [3]. Current treatment strategies in particular for NSCLC are surgical resection, chemotherapy and molecularly targeted therapy [4]. Despite the significant improvement in the diagnosis and treatment options, the prognosis of lung cancer patients still remains poor, and the five-year survival rate is less than 15% [5]. As in other cancers, NSCLC carcinogenesis is a complex process involving activation of many oncogenes and inactivation of tumor suppressor genes. Therefore, to identify some novel molecule correlated with the occurrence and development of NSCLC may be helpful for diagnosis and treatment of NSCLC patients.

MicroRNAs (miRNAs) are a class of small endogenous, non-coding RNAs with 18-25 nucleotides and post-transcriptionally regulate the expression of multiple genes by binding to their 3'-untranslated regions [6]. MiR-383 was firstly found to be down-regulated in patients with non-obstructive azoospermia and was recently reported to be associated with several human cancers [7]. For example, Li et al. showed that miR-383 was down-regulated in medulloblastoma (MB) samples and cell lines, and ectopic expression of miR-383 in MB cells led to suppression of cell growth, cell accumulation at sub-G1 phase and alteration of apoptosis-related proteins [8]. Xu et al. reported that miR-383 was down-regulated in glioma cells and could inhibit glioma cells proliferation, migration, and invasion [9]. Besides, Chen et al. demonstrated the down-regulation of miR-383 in hepatocellular carcinoma (HCC) and its suppressive effect on HCC cells proliferation [10]. However, the expression pattern and biological significance

of miR-383 in NSCLC has not yet been reported.

Epigenetic modification is very important in regulating genes transcription. Histone acetylation is regulated by two large families of enzymes-histone acetyltransferases (HATs) and histone deacetylases (HDACs), which modulate the chromatin structure by adding and removing acetyl groups from lysine residues in protein, respectively [11]. Histone acetylation and deacetylation regulated by HATs and HDACs play pivotal roles in cell proliferation, differentiation and cell cycle distribution [12, 13]. The 18 human HDAC members are classified into four classes based on their phylogenetic derivation and HDAC2 is a member of class II [14]. HDAC2 was showed to be involved in the development of several human cancers. It was reported that HDAC2 was overexpressed in oral cancer and its overexpression predicted a poor prognosis [15]. Increased expression of HDAC2 was also found in gastric cancer and gallbladder carcinoma [16, 17]. These results suggest that HDAC2 may play an important role in the development and progression of human cancers.

In the present study, we focused on the biological functions of miR-383 and its potential mechanism in NSCLC. Firstly, we detected the expression levels of miR-383 and HDAC2 in NSCLC clinical samples and cell lines, and further analyzed their correlation. Then, we proved HDAC2 as a direct target of miR-383 in HDAC2 by luciferase reporter assay and western blot. Finally, we investigated the biological effects of miR-383 and HDAC2 on NSCLC cells proliferation and apoptosis. Our findings collectively showed that miR-383 is down-regulated in NSCLC and can inhibit NSCLC cells proliferation via down-regulating HDAC2.

Materials and methods

Tissue collections

A total of 24 paired NSCLC and adjacent noncancerous lung tissues (more than 2 cm away from the cancer tissues and were pathologically confirmed as normal tissues) were obtained from patients who are diagnosed with NSCLC and underwent surgical resection in our department between May 2014 and March 2015. All the tissues were frozen in liquid nitrogen immediately and stored at -80°C until use. None of the patients received chemo- or radiotherapy before surgery. All of the patients provided written informed consent, and this study was approved by the Ethics Committees of our hospital.

Cell culture and transfection

Four NSCLC cell lines (SPC-A1, A549, H460 and HCC827) and normal GNHu27 cells were purchased from American Type Culture Collection (ATCC) (VA, USA). All of NSCLC cell lines were cultured in RPMI-1640 medium (HyClone) and the normal GNHu27 cells was cultured in Dulbecco's modified Eagle's medium (DMEM; Invitrogen, CA). The culture media were all supplemented with 10% fetal bovine serum, 100 U/ml of penicillin and 100 μg/ml of streptomycin. Cells were maintained at 37°C in a humidified atmosphere containing 5% CO_2. miR-383 mimics (miR-383), miR-383 inhibitors (anti-miR-383) and their negative controls (miR-NC and anti-miR-NC) were purchased from GenePharma (Shanghai, China). A HDAC2 expression plasmid (pcDNA3.1-HDAC2) containing the coding sequence was constructed using PCR-generated fragments and pcDNA3.1 (+) vector. The day before transfection, cells were seeded into 24-well plates and transfection was carried out with Lipofectamine 2000 following the manufacturer's instruction.

Quantitative real-time PCR

Total RNA was extracted from samples or cell lines using TRIzol reagent (Invitrogen, USA) according to the manufacturer's protocol and then reversely transcribed into cDNA. Real-time PCR was done by using the SYBR Green PCR Master Mix (Applied Biosystems) on an Applied Biosystems 7500 Real-time PCR system (Applied Biosystems, CA, USA). Small nuclear U6 RNA was used as an internal control and the relative miR-383 expression levels were normalized to that control by using comparative $2^{-\Delta\Delta Ct}$ method. The primers for miR-383 real time PCR is 5'-GTGCAGGGTCCGAGGT-3' (forward) and 5'-AGATCAGAAGGTGATTGTGGCT-3' (reverse). The experiment was independently repeated three times.

Western blot

Protein was extracted from tissues or treated cells using RIPA buffer (Beyotime) supplemented with protease inhibitors (Roche) and protein

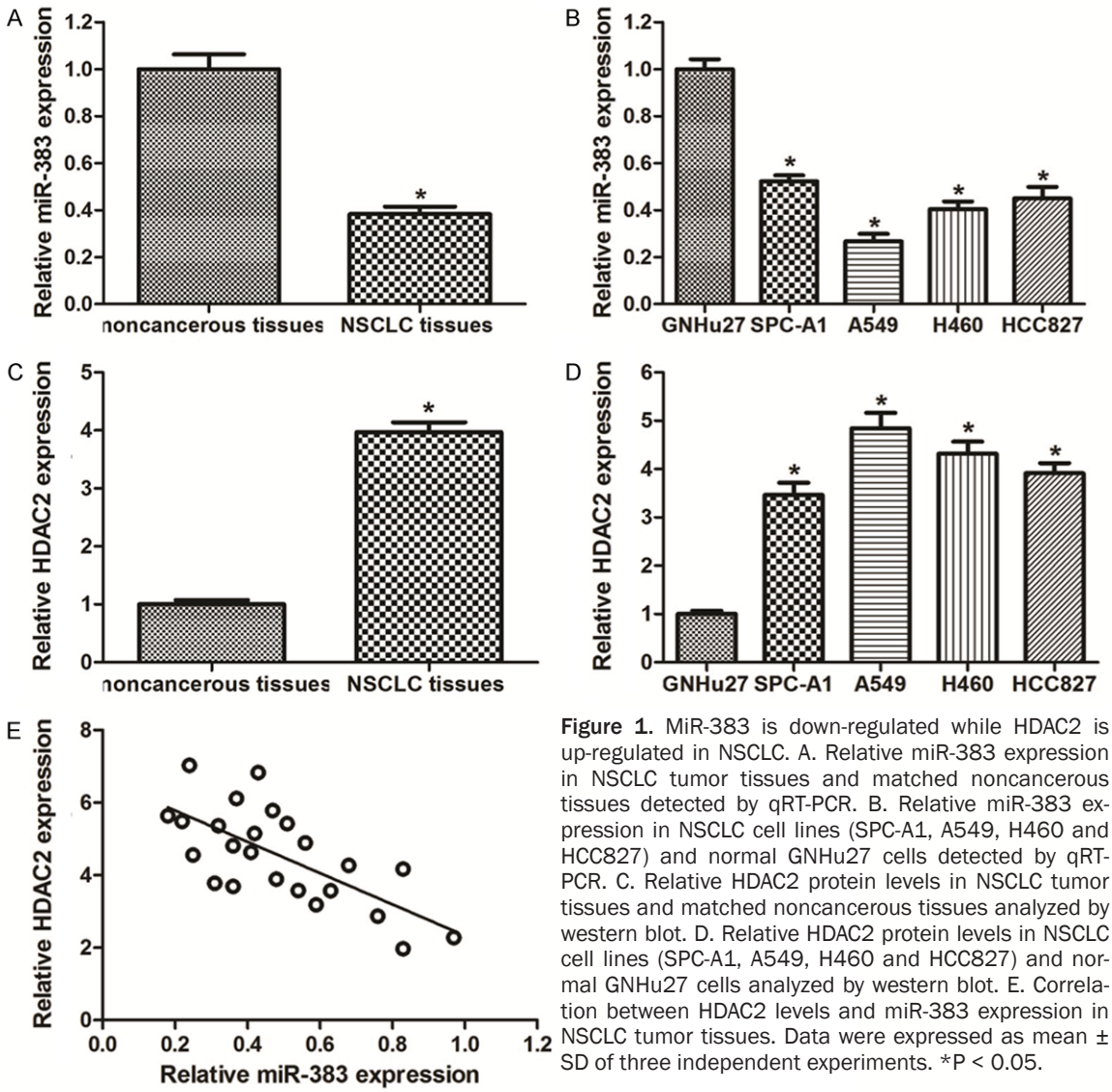

Figure 1. MiR-383 is down-regulated while HDAC2 is up-regulated in NSCLC. A. Relative miR-383 expression in NSCLC tumor tissues and matched noncancerous tissues detected by qRT-PCR. B. Relative miR-383 expression in NSCLC cell lines (SPC-A1, A549, H460 and HCC827) and normal GNHu27 cells detected by qRT-PCR. C. Relative HDAC2 protein levels in NSCLC tumor tissues and matched noncancerous tissues analyzed by western blot. D. Relative HDAC2 protein levels in NSCLC cell lines (SPC-A1, A549, H460 and HCC827) and normal GNHu27 cells analyzed by western blot. E. Correlation between HDAC2 levels and miR-383 expression in NSCLC tumor tissues. Data were expressed as mean ± SD of three independent experiments. *P < 0.05.

concentration was measured using the BCA Protein Assay kit (Beyotime). Equal amounts of protein samples were separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene fluoride (PVDF) membranes (Millipore, USA). Afterwards, membranes were blocked with 5% non-fat milk at room temperature for 1 h and incubated with primary antibody against HDAC2 or β-actin (Abcam, USA) at 4°C overnight. The next day, the membranes were washed with PBS and then incubated with secondary antibody (Santa Cruz, USA) at room temperature for 2 h. Finally, protein bands were detected by enhanced chemi-luminescence (ECL; Santa Cruz Biotechnology) according to the manufacturer's protocol. Three independent experiments were performed.

Luciferase reporter assay

The 3'-UTR of HDAC2 containing the potential binding sites of miR-383 or the mutant HDAC2 3'-UTR was amplified by PCR and then cloned into XhoI/NotI restriction sites of psiCHECK2 vector (Promega, WI, USA). For the luciferase reporter assay, cells were cultured in 24-well plates, and then co-transfected with either miR-383 mimics or NC and psiCHECK2 containing wild-type (WT) or mutated (Mut) HDAC2 3'-UTR. After 48 hours of transfection, luciferase activity was measured using the Dual

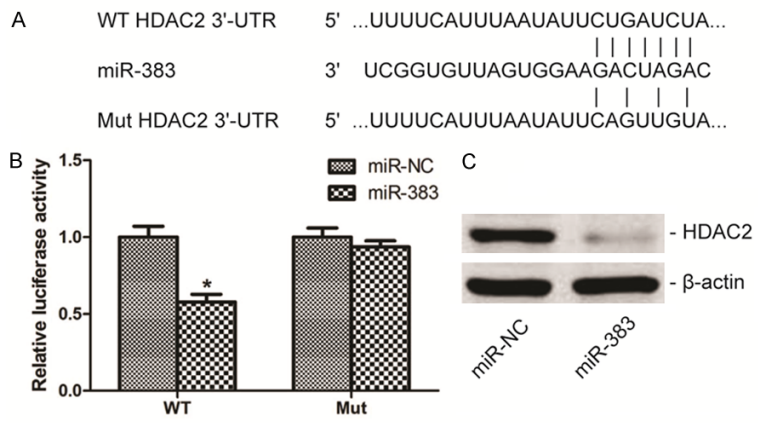

Figure 2. HDAC2 is a direct target of miR-383 in NSCLC. A. Putative miR-383 binding sites on 3'-UTR of HDAC2 mRNA predicted by Targetscan. B. Relative luciferase activity of A549 cells co-transfected with miR-383 mimics (miR-383) or its negative control (miR-NC) and wild-type (WT) or mutated (Mut) 3'-UTR of HDAC2. C. HDAC2 expression in A549 cells transfected with miR-383 mimics or its negative control showed by western blot. Data were expressed as mean ± SD of three independent experiments. *P < 0.05.

Luciferase Reporter Assay System (Promega Corporation, WI, USA) with the manufacturer's instructions. This experiment was run in triplicate for each sample.

MTT assay

Cell proliferation was analyzed by the 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay according to the manufacturer's guideline. Briefly, after 48 hours of transfection, A549 cells were harvested and seeded into 96-well plates at a density of 2 × 10^3 cells per well. After incubation at 37°C for different time (0 h, 24 h, 48 h, 72 h, 96 h), 20 μL MTT (0.5 mg/ml, Sigma) was added into each well and then further incubated for 4 h. Afterwards, medium was discarded and 100 μL of DMSO (Sigma) was added to stop the reaction. Finally, the absorbance was measured at 490 nm using a microplate reader. Each experiment was performed in triplicates.

Apoptosis assay

Cell apoptosis was evaluated using flow cytometry with FITC-Annexin V Apoptosis Detection Kit (BD Biosciences) according to the manufacturer's protocol. Briefly, treated cells were collected by centrifugation and resuspended at a concentration of 1 × 10^6 cells/mL. Then, cells were incubated with Annexin V and PI for 15 min in the dark. Cell apoptosis was analyzed by FACS Caliber flow cytometer (BD Bioscience). This experiment was repeated at least three times.

Statistical analysis

All experiments were repeated at least three times and data were expressed as means ± SD. Statistical analyses were performed by using the SPSS 17.0 statistical software (Chicago, IL). The significance of differences between groups was compared by Student's t test (two groups) or one-way analysis of variance (ANOVA, more than two groups). $P < 0.05$ was considered to indicate statistical significance.

Results

miR-383 is down-regulated while HDAC2 is up-regulated in NSCLC

We detected miR-383 expression by qRT-PCR in 24 paired NSCLC tissues and NSCLC cell lines. Our results revealed that miR-383 expression was significantly down-regulated in NSCLC tissues or cell lines (SPC-A1, A549, H460 and HCC827) compared with the adjacent noncancerous lung tissues or normal GNHu27 cells (**Figure 1A**, **1B**). Whereas, the results of western blot indicated that HDAC2 is significantly up-regulated in NSCLC tissues or cell lines compared to the adjacent noncancerous lung tissues or normal GNHu27 cells (**Figure 1C**, **1D**). Furthermore, we observed an obvious inverse correlation between HDAC2 levels and miR-383 expression in NSCLC tumor tissues (**Figure 1E**). These results indicated that miR-383 and HDAC2 are both involved in the development of NSCLC.

HDAC2 is a direct target of miR-383 in NSCLC

Based on the bioinformatics analysis using three different programs (miRanda, Pictar and targetscan), HDAC2 mRNA has a putative miR-383 binding site in its 3'-UTR (**Figure 2A**), which suggests that HDAC2 mRNA is a potential target of miR-383. To confirm that, we conducted luciferase reporter assay and western blot. As

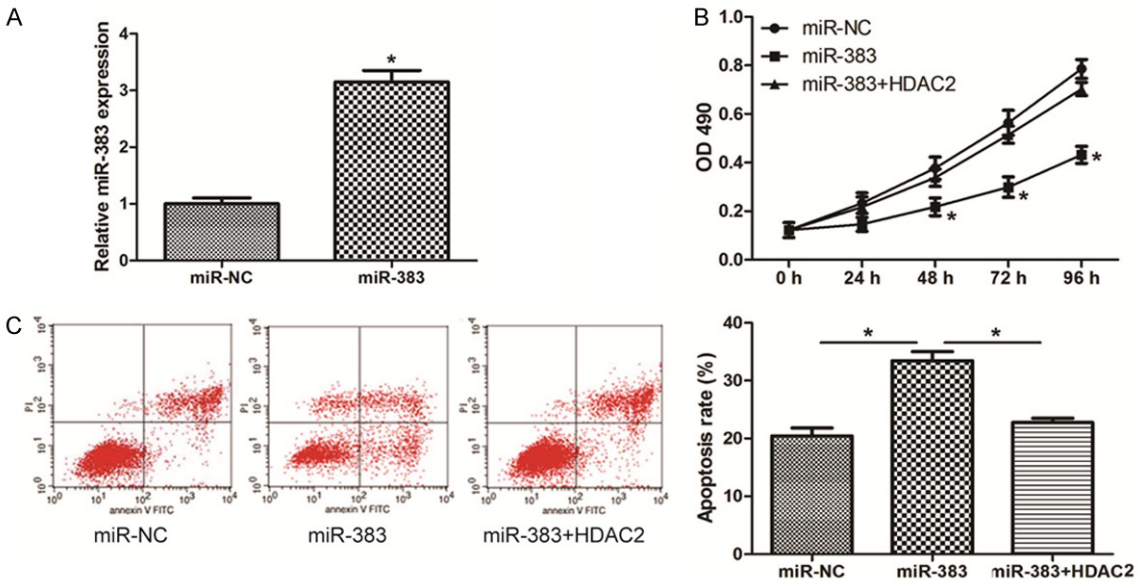

Figure 3. miR-383 inhibits proliferation and induces apoptosis via targeting HDAC2 in NSCLC. A. Relative miR-383 expression in A549 cells when transfected with miR-383 mimics (miR-383) or its negative control (miR-NC) detected by qRT-PCR. B. Proliferation of transfected A549 cells evaluated by MTT assay. C. Apoptosis rate of transfected A549 cells measured by flow cytometry. Data were expressed as mean ± SD of three independent experiments. *P < 0.05.

shown in **Figure 2B**, a significantly down-regulation of luciferase activity was observed in A549 cells when cotranfected with miR-383 mimics and the wild type (WT) but not the mutant (Mut) 3'-UTR of HDAC2. Besides, our results of western blot showed that miR-383 overexpression significantly inhibited HDAC2 protein levels in A549 cells (**Figure 2C**). These results collectively proved HDAC2 as a direct target of miR-383 in NSCLC.

miR-383 inhibits proliferation and induces apoptosis via targeting HDAC2 in NSCLC

To investigate the biological function of miR-383 in NSCLC. A549 cells were transfected with miR-383 mimics (miR-383) or its negative control (miR-NC) and the efficiency was examined by qRT-PCR (**Figure 3A**). MTT assay was conducted to analyze the proliferation of transfected cells, data showed that overexpression of miR-383 significantly inhibited A549 cells proliferation (**Figure 3B**). Results of flow cytometry indicated that the apoptosis rate of A549 cells was significantly higher when transfected with miR-383 mimics (**Figure 3C**). However, when co-transfected with miR-383 mimics and pcDNA3.1-HDAC2, we found that restoration of HDAC2 partially attenuated the effects of miR-383 on A549 cells proliferation and apoptosis (**Figure 3B**, **3C**). All these results suggest that miR-383 inhibits proliferation and induces apoptosis via targeting HDAC2 in NSCLC.

Knockdown of miR-383 promotes proliferation and suppresses apoptosis in A549 cells

To further confirm the effects of miR-383 on A549 cells proliferation and apoptosis, cells were transfected with miR-383 inhibitors (anti-miR-383) or the negative control (anti-miR-NC) and qRT-PCR was used to test the transfection efficiency (**Figure 4A**). Our results showed that the proliferation of A549 cells was promoted while the apoptosis was suppressed when transfected with miR-383 inhibitors (**Figure 4B**, **4C**). These results confirmed the effects of miR-383 on A549 cells proliferation and apoptosis.

Discussion

Lung cancer, mainly refers to non-small cell lung cancer (NSCLC), has become one of the most serious threats to human health all over the word, especially in china with its incidence increasing every year [18]. What worse, nearly 77% of NSCLC patients are not diagnosed until spreading beyond the primary site and around 55% of patients have metastatic disease at

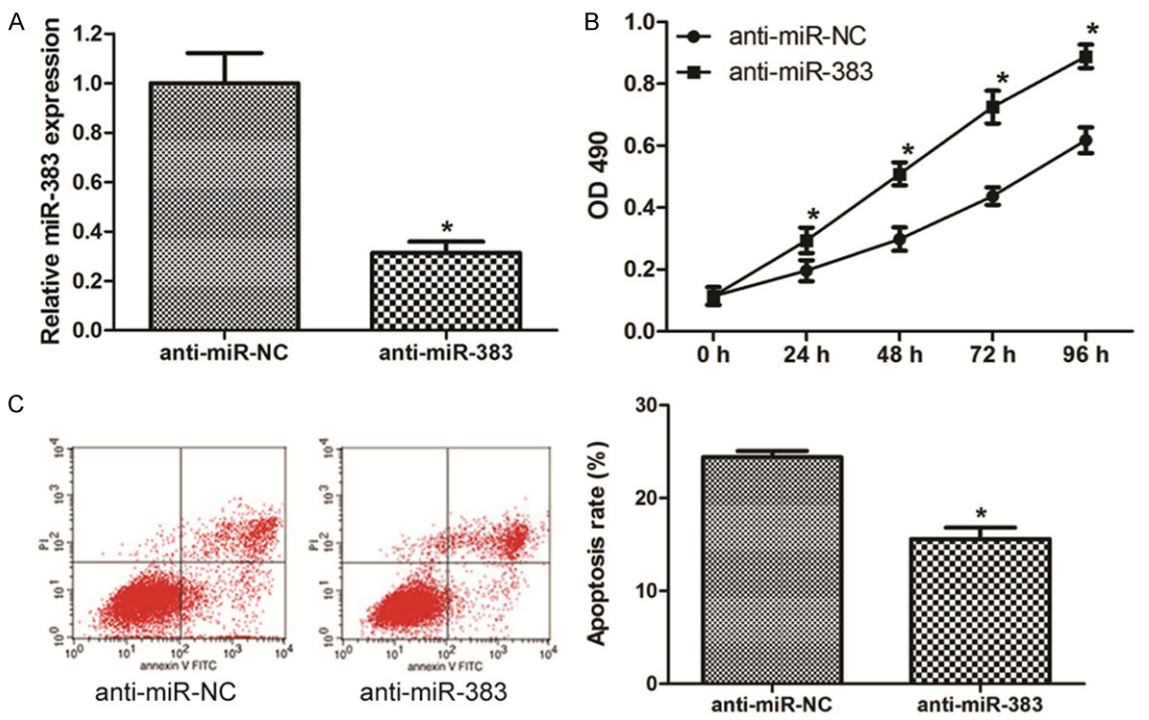

Figure 4. Knockdown of miR-383 promotes proliferation and suppresses apoptosis in A549 cells. A. Relative miR-383 expression in A549 cells when transfected with miR-383 inhibitors (anti-miR-383) or the negative control (anti-miR-NC) detected by qRT-PCR. B. Proliferation of transfected A549 cells evaluated by MTT assay. C. Apoptosis rate of transfected A549 cells measured by flow cytometry. Data were expressed as mean ± SD of three independent experiments. *$P < 0.05$.

diagnosis [19]. In recent years, numerous miRNAs are reported to be involved in the development of NSCLC. For example, miR-30d-5p was showed to be downregulated in NSCLC tissues, and could significantly inhibit the growth, cell cycle distribution, and motility of NSCLC cells [20]. miR-92a was found to be significantly upregulated in NSCLC tissues and could promote growth, metastasis, and chemoresistance in NSCLC cells by targeting PTEN [21]. It was also indicated that miR-148b expression was decreased in tumor tissues and its low level was associated with poor survival in NSCLC patients [22].

Interestingly, the role of histone deacetylases (HDACs) in NSCLC has also been investigated during the past decades. Several HDAC inhibitors are showed to have anti-tumor effect in NSCLC. Brazelle et al. indicated that HDAC inhibitor LBH589, scriptaid, valproic acid, apicidin, and MS-275 could induce cell death in NSCLC cells [23]. The suppressive effect on NSCLC cells growth was also observed by using other HDAC inhibitors such as honokiol [24] and trichostatin A [25]. As a member of HDAC family, HDAC2 was identified as an oncogenic regulator in NSCLC by deregulating expression of apoptosis and cell cycle proteins [26]. In liver cancer, HDAC2 was demonstrated to be regulated by miR-145 and play a pivotal role in the development of hepatocellular carcinoma [27].

In our study, we firstly explored miR-383 expression levels and HDAC2 protein patterns in paired NSCLC tissues or NSCLC cell lines, respectively. We founded that miR-383 is significantly down-regulated while HDAC2 is significantly up-regulated in NSCLC tumor tissues and cancer cell lines when compared with normal controls. Additionally, we observed an obvious inverse correlation between HDAC2 levels and miR-383 expression in NSCLC tissues. These results suggest that miR-383 and HDAC2 are both altered in NSCLC and may be involved in its development. Next, luciferase reporter assay and western blot were performed to elucidate the exactly correlation of miR-383 and HDAC2 in NSCLC. Our results showed that HDAC2 is a direct target of miR-383 in NSCLC.

Then, we explored the biological function of miR-383 on NSCLC cells proliferation and apoptosis. After transfected with miR-383 mimics, A549 cells showed a significant decrease in cell proliferation and a significant increase in apoptosis. However, restoration of HDAC2 partially attenuated the effects of miR-383 on A549 cells. These data indicate that miR-383 may inhibit proliferation and induce apoptosis via targeting HDAC2 in NSCLC cells. To further confirm the function of miR-383 in NSCLC, we transfected A549 cells with miR-383 inhibitors and found an opposite effects of miR-383 mimics on A549 cells proliferation and apoptosis.

In conclusion, the current study provides evidence that miR-383 is dramatically down-regulated while HDAC2 is significantly up-regulated in NSCLC. In addition, miR-383 can inhibit proliferation and induce apoptosis via targeting HDAC2 in NSCLC. Our findings suggest that miR-383 down-regulation may play important roles in the development of NSCLC and miR-383 may serve as a useful diagnostic marker and a potential therapeutic target in human lung cancer.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Bing Li, Department of Clinical Laboratory, Xinxiang Central Hospital, Xinxiang 453000, Henan Province, China. E-mail: suli1763@126.com

References

[1] Jemal A, Bray F, Center MM, Ferlay J, Ward E and Forman D. Global cancer statistics. CA Cancer J Clin 2011; 61: 69-90.

[2] Subramaniam S, Thakur RK, Yadav VK, Nanda R, Chowdhury S and Agrawal A. Lung cancer biomarkers: State of the art. J Carcinog 2013; 12: 3.

[3] Wang M, Zhang Q, Wang J and Zhai Y. MicroRNA-498 is downregulated in non-small cell lung cancer and correlates with tumor progression. J Cancer Res Ther 2015; 11 Suppl 1: C107-111.

[4] Camidge DR, Pao W and Sequist LV. Acquired resistance to TKIs in solid tumours: learning from lung cancer. Nat Rev Clin Oncol 2014; 11: 473-481.

[5] da Cunha Santos G, Shepherd FA and Tsao MS. EGFR mutations and lung cancer. Annu Rev Pathol 2011; 6: 49-69.

[6] Yu LD, Jin RL, Gu PC, Ling ZH, Lin XJ and Du JY. Clinical significance of microRNA-130b in osteosarcoma and in cell growth and invasion. Asian Pac J Trop Med 2015; 8: 752-756.

[7] Lian J, Zhang X, Tian H, Liang N, Wang Y, Liang C, Li X and Sun F. Altered microRNA expression in patients with non-obstructive azoospermia. Reprod Biol Endocrinol 2009; 7: 13.

[8] Li KK, Pang JC, Lau KM, Zhou L, Mao Y, Wang Y, Poon WS and Ng HK. MiR-383 is downregulated in medulloblastoma and targets peroxiredoxin 3 (PRDX3). Brain Pathol 2013; 23: 413-425.

[9] Xu D, Ma P, Gao G, Gui Y, Niu X and Jin B. MicroRNA-383 expression regulates proliferation, migration, invasion, and apoptosis in human glioma cells. Tumour Biol 2015; 36: 7743-7753.

[10] Chen L, Guan H, Gu C, Cao Y, Shao J and Wang F. miR-383 inhibits hepatocellular carcinoma cell proliferation via targeting APRIL. Tumour Biol 2015; [Epub ahead of print].

[11] Fan J, Alsarraf O, Dahrouj M, Platt KA, Chou CJ, Rice DS and Crosson CE. Inhibition of HDAC2 protects the retina from ischemic injury. Invest Ophthalmol Vis Sci 2013; 54: 4072-4080.

[12] Marks P, Rifkind RA, Richon VM, Breslow R, Miller T and Kelly WK. Histone deacetylases and cancer: causes and therapies. Nat Rev Cancer 2001; 1: 194-202.

[13] Witt O, Deubzer HE, Milde T and Oehme I. HDAC family: What are the cancer relevant targets? Cancer Lett 2009; 277: 8-21.

[14] Yang XJ and Seto E. The Rpd3/Hda1 family of lysine deacetylases: from bacteria and yeast to mice and men. Nat Rev Mol Cell Biol 2008; 9: 206-218.

[15] Chang HH, Chiang CP, Hung HC, Lin CY, Deng YT and Kuo MY. Histone deacetylase 2 expression predicts poorer prognosis in oral cancer patients. Oral Oncol 2009; 45: 610-614.

[16] Song J, Noh JH, Lee JH, Eun JW, Ahn YM, Kim SY, Lee SH, Park WS, Yoo NJ, Lee JY and Nam SW. Increased expression of histone deacetylase 2 is found in human gastric cancer. APMIS 2005; 113: 264-268.

[17] Du X, Zhao H, Zang L, Song N, Yang T, Dong R, Yin J, Wang C and Lu J. Overexpression of histone deacetylase 2 predicts unfavorable prognosis in human gallbladder carcinoma. Pathol Oncol Res 2013; 19: 397-403.

[18] Chen W, Zheng R, Zhang S, Zhao P, Zeng H and Zou X. Report of cancer incidence and mortality in China, 2010. Ann Transl Med 2014; 2: 61.

[19] Chang A. Chemotherapy, chemoresistance and the changing treatment landscape for NSCLC. Lung Cancer 2011; 71: 3-10.

[20] Chen D, Guo W, Qiu Z, Wang Q, Li Y, Liang L, Liu L, Huang S, Zhao Y and He X. MicroRNA-30d-

5p inhibits tumour cell proliferation and motility by directly targeting CCNE2 in non-small cell lung cancer. Cancer Lett 2015; 362: 208-217.
[21] Ren P, Gong F, Zhang Y, Jiang J and Zhang H. MicroRNA-92a promotes growth, metastasis, and chemoresistance in non-small cell lung cancer cells by targeting PTEN. Tumour Biol 2015; [Epub ahead of print].
[22] Ge H, Li B, Hu WX, Li RJ, Jin H, Gao MM and Ding CM. MicroRNA-148b is down-regulated in non-small cell lung cancer and associated with poor survival. Int J Clin Exp Pathol 2015; 8: 800-805.
[23] Brazelle W, Kreahling JM, Gemmer J, Ma Y, Cress WD, Haura E and Altiok S. Histone deacetylase inhibitors downregulate checkpoint kinase 1 expression to induce cell death in non-small cell lung cancer cells. PLoS One 2010; 5: e14335.
[24] Singh T, Prasad R and Katiyar SK. Inhibition of class I histone deacetylases in non-small cell lung cancer by honokiol leads to suppression of cancer cell growth and induction of cell death in vitro and in vivo. Epigenetics 2013; 8: 54-65.
[25] Chen CQ, Chen CS, Chen JJ, Zhou LP, Xu HL, Jin WW, Wu JB and Gao SM. Histone deacetylases inhibitor trichostatin A increases the expression of Dleu2/miR-15a/16-1 via HDAC3 in non-small cell lung cancer. Mol Cell Biochem 2013; 383: 137-148.
[26] Jung KH, Noh JH, Kim JK, Eun JW, Bae HJ, Xie HJ, Chang YG, Kim MG, Park H, Lee JY and Nam SW. HDAC2 overexpression confers oncogenic potential to human lung cancer cells by deregulating expression of apoptosis and cell cycle proteins. J Cell Biochem 2012; 113: 2167-2177.
[27] Noh JH, Chang YG, Kim MG, Jung KH, Kim JK, Bae HJ, Eun JW, Shen Q, Kim SJ, Kwon SH, Park WS, Lee JY and Nam SW. MiR-145 functions as a tumor suppressor by directly targeting histone deacetylase 2 in liver cancer. Cancer Lett 2013; 335: 455-462.