

Original Article

MiR-23a promotes cell proliferation and invasion in papillary thyroid carcinoma by targeting PTEN

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Abstract: Accumulating evidence has suggested that microRNAs (miRNAs) are involved in multiple processes in cancer development and progression by negatively regulating gene expression at posttranscriptional level. Recent studies have shown that miR-23a functions as an oncogene in many types cancer, but its role in papillary thyroid carcinoma (PTC) remains poorly understood. The aim of this study is to investigate the role and underlying molecular mechanism of miR-23a in PTC. Here, we demonstrated that miR-23a is frequently upregulated in PTC specimens compared with adjacent noncancerous tissues. Function assay showed that restoration of miR-23a in PTC cells markedly promoted cell proliferation, migration and invasion, and inhibited PTC apoptosis. Bioinformatics analysis showed phosphatase and tensin homolog (PTEN) as a potential target of miR-23a. Data from luciferase reporter assays further confirmed that miR-23a directly binds to the 3'UTR of PTEN messenger RNA (mRNA). Furthermore, we found that overexpression of miR-23a suppressed PTEN expression at both transcriptional and translational levels in PTC cells. We also confirmed that PTEN expression on mRNA level was decreased in PTC tissue, and was inversely correlated miR-23a expression in PTC tissue. These findings suggested that miR-23a, acting as an oncogenic regulator by directly targeting PTEN in PTC, is a useful potential treatment target of papillary thyroid carcinoma.

Keywords: Papillary thyroid carcinoma, miR-23a, PTEN, proliferation

Introduction

Papillary thyroid cancer (PTC), referred to as a differentiated neoplasia, is the most common histological type, accounting for approximately 80%-90% of all thyroid cancers cases [1, 2]. Despite highly curable and presenting a 10-year survival rate exceeding 90% [3], lymph node metastasis, especially in the neck, occurs in 20-50% of all patients and the overall recurrence rates may be as high as 35% [4], since clinical and biological behaviors cannot be properly predicted. Therefore, there is an urgent need for understanding the molecular mechanisms of PTC progression to find novel therapeutic strategies for this disease.

MicroRNAs (miRNAs) are a class of small non-coding RNA molecules that can regulate gene expression by binding to specific regions of target mRNAs, resulting in translational repression or degradation [5, 6]. In human cancers including thyroid cancer, it has been showed

that miRNAs play crucial roles in the regulation of cell proliferation, apoptosis, angiogenesis, and metastasis [7, 8]. Therefore, identification of novel miRNAs involved in PTC initiation and development might contribute to develop strategies for its diagnosis, treatment, and prognosis in the future.

miR-23a, located on human chromosome 19p13.2 [9], has been reported to play critical role in cell cycle, proliferation, differentiation, hematopoiesis, and cardiac hypertrophy in various cell types [10]. A larger number of studies have demonstrated that miR-23a is involved in the progression of various cancers, including lung cancer [11], liver cancer [12], colorectal cancer [13], gastric cancer [14] and prostate cancer [15]. However, the roles and the mechanisms of miR-23a in PTC poorly understood. Therefore, in the current study, we further validated the differential expression of miR-23a in PTC tissue and investigated the function and underlying mechanism of miR-23a in PTC.

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Materials and methods

Tissue sample

Twenty paired tissue specimens of human PTC and matched normal tissues were obtained from at Department of Thyroid Surgery, China-Japan Union Hospital of Jilin University (Changchun, China) from October 2012 to October 2014. The matched normal tissues were further identified by pathologist for their normal origin that they do not have tumor cells. All tissue samples were immediately snap-frozen in liquid nitrogen, and stored at -80°C until use. Informed consent was obtained from all patients. This study was approved by the Ethic Committee of Jilin University (Changchun, China).

Cell culture

The human PTC cell line, K1 cells were obtained from the Chinese Cell Bank of the Chinese Academy of Sciences (Shanghai, China), and were cultured in Dulbecco's modified Eagle's medium (DMEM, GIBCO, Carlsbad, CA) supplemented with 10% fetal bovine serum (FBS, Life Technologies, Inc., Grand Island, NY, USA), 100 U/ml penicillin and 100 mg/ml streptomycin at 37°C in a humidified atmosphere containing 5% CO₂.

Real-time quantitative RT-PCR

Total RNAs were isolated from cultured cells and clinical samples by TRIzol reagent (Invitrogen Life Technologies, USA) according to manufacturer's instructions. The miR-23a and PTEN were quantified as previously described, respectively [16, 17]. U6 snRNA or GAPDH expression was used as an endogenous control. Relative gene expression levels were calculated using the 2^{-ΔΔCt} method.

Cell transfection

miR-23a mimics (miR-23a), and corresponding miRNA negative control (miR-NC), miR-23a inhibitors (Anti-miR-23a) and corresponding miRNA control (Anti-miR-NC), were brought from GenePharma (Shanghai, China), and were transiently transfected into K1 cells using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol.

Transfection efficiencies were determined in every experiment at 48 h after transfection.

Cell proliferation assay

Cells were plated in 96-well plates at 1 × 10³ per well in a final volume of 100 μl and cultured for 24 h-72 h after transfection, then cell proliferation was determined using the MTT assay according to the manufacturer's protocol. The absorbance was detected at 490 nm on a Bio-Rad iMark Microplate Absorbance Reader (BD Biosciences, Mansfield, MA, USA).

Cell apoptosis assay

The cell apoptosis was determined in K1 cells transfected with miR-23a or Anti-miR-23a by using Annexin V/propidium iodide detection kit (Keygene, Nanjing, China) according to the manufacturer's protocol under a FACS Calibur flow cytometer (BD Biosciences). The apoptosis ratio was calculated using CellQuest software (BD Biosciences).

Transwell cell migration and invasion assays

Migration and invasion of K1 cells were measured using a 24-well Transwell plate (8 μm pore size, Corning Costar, USA) at 24 h after transfection. For migration assays, 5 × 10⁴ transfected cells in serum-free DMEM medium were placed into the non-coated membrane in the top chamber in and the lower chamber was filled with 0.6 ml DMEM medium containing 10% FBS. For invasion assays, the chamber inserts were coated with 200 mg/ml BD Matrigel (BD Biosciences, San Jose, CA, USA) and dried overnight. Then 5 × 10⁴ transfected cells in serum-free DMEM medium were placed into the top chamber coated with Matrigel and the lower chamber was filled with 0.6 ml DMEM medium containing 10% FBS. After incubation at 37°C for 48 h, cells adhering to the lower membrane were stained with 0.1% crystal violet in 20% methanol, imaged under light microscope (200×, Olympus, Tokyo, Japan). The stained cells were counted from five random fields under microscope.

Luciferase reporter assay

The PTEN 3'UTR fragment containing putative binding sites for miR-23a was amplified using PCR and inserted into downstream of the lucif-

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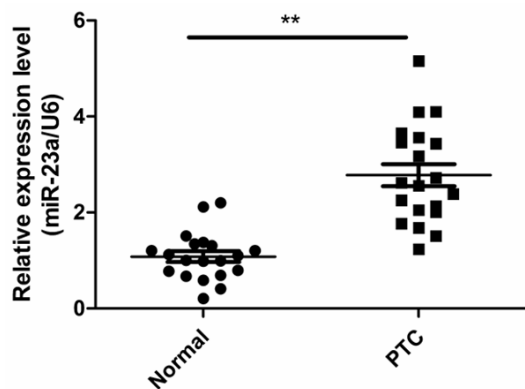


Figure 1. The expression of miR-23a is upregulated in human PTC tissues. Relative levels of miR-23a were determined in 20 PTC tissues and matched adjacent normal tissues by quantitative RT-PCR (qRT-PCR). U6 was used as loading control. * $P < 0.05$, ** $P < 0.01$ versus normal tissues.

erase gene in the pGL3-luciferase reporter plasmid (Promega, Madison, WI, USA), named as PTEN-3'UTR-WT. The corresponding mutant were created by substituting the seed region of the miR-23a binding site, and inserted into downstream of the luciferase gene in the pGL3-luciferase reporter plasmid (Promega, Madison, WI, USA), designated PTEN-3'UTR-MUT.

For luciferase activity assay, the 1×10^5 K1 cells were seeded into 24-well plates and cultured for 24 h, then were co-transfected with wild-type (WT) or mutant (Mut) 3'UTR of PTEN and miR-23a or miR-NC. At 48 h after transfection, the dual luciferase activities were examined using the Dual-Luciferase Reporter Assay System (Promega, WI, USA), according to the manufacturer's instructions.

Western blot

The transfected cells were collected, washed and lysed with RIPA buffer (Beyotime, Shanghai, China). Total concentrations of protein were measured by using the bicinchoninic acid protein assay kit (Beyotime). Equivalent quantities (40 μ g) of protein were separated by using 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto PVDF membranes (Santa Cruz Biotechnology, Inc, USA). The membranes were blocked with 5% non-fat milk in Tris buffered saline for 2 h at room temperature and incubated overnight at 4°C with primary antibodies against PTEN (1:1000, Santa Cruz)

and GAPDH (1:5000, Santa Cruz). The membranes were washed 3 times in Tris-Tween-20 and incubated with the corresponding horseradish peroxidase (HRP)-conjugated secondary antibody (1:5000, Santa Cruz) for 1 h at room temperature. Proteins were visualized with chemiluminescence detection (Signagen, Rockville, MD, USA).

Statistical analysis

All data at least from three independent experiments were expressed as mean \pm SD (standard deviation). Statistical analysis between two samples was performed assessed by two-tailed Student's t test. The relationship between PTEN and miR-23a expressions was tested with two-tailed Pearson's correlation. $P < 0.05$ was considered to be statistically significant.

Results

Expression of miR-23a is upregulated in PTC tissues

The expression of miR-23a level in 20 PTC tissues and paired adjacent normal tissues were determined by quantitative RT-PCR (qRT-PCR). As shown in **Figure 1**, the expression of miR-23a in PTC tissues was significantly upregulated compared with corresponding adjacent normal tissues ($P < 0.01$).

miR-23a promoted cell proliferation and inhibited apoptosis of PTC cells

To unveil the biological effects of miR-23a in thyroid carcinoma cells, the PTC-derived cell line, K1 cells were transfected with miR-23a or anti-miR-23a, then biological function of miR-23a in the K1 cells were evaluated. QRT-PCR analysis confirmed that transfected miR-23a mimic caused miR-23a upregulation, while transfected anti-miR-23a led to miR-23a expression downregulation in K1 cells (**Figure 2A**). WST-1 assay demonstrated that upregulation of miR-23a in K1 cells significantly increased cell proliferation, while downregulation of miR-23a in K1 cells significantly decreased cell proliferation (**Figure 2B**). Flow cytometer assay demonstrated that upregulation of miR-23a in K1 cells significantly decreased cell apoptosis, while downregulation of miR-23a in K1 cells significantly induced cell apoptosis (**Figure 2C**).

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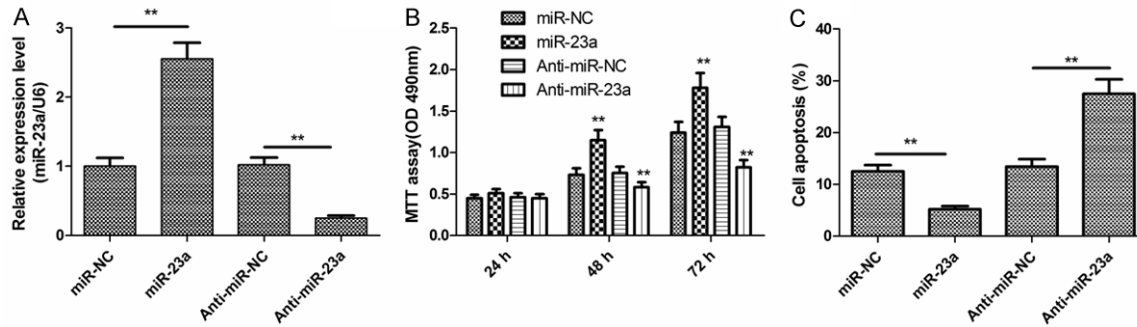


Figure 2. MiR-23a inhibits proliferation, and induces apoptosis in PTC cells. (A) QRT-PCR analyzed miR-23a expression in K1 cells transfected with miR-23a mimic or Anti-miR-23a. (B and C) Cell proliferation (B), and apoptosis (C) were determined in K1 cells transfected with miR-23a or Anti-miR-23a. * $P < 0.05$, ** $P < 0.01$. versus miR-NC or anti-miR-NC.

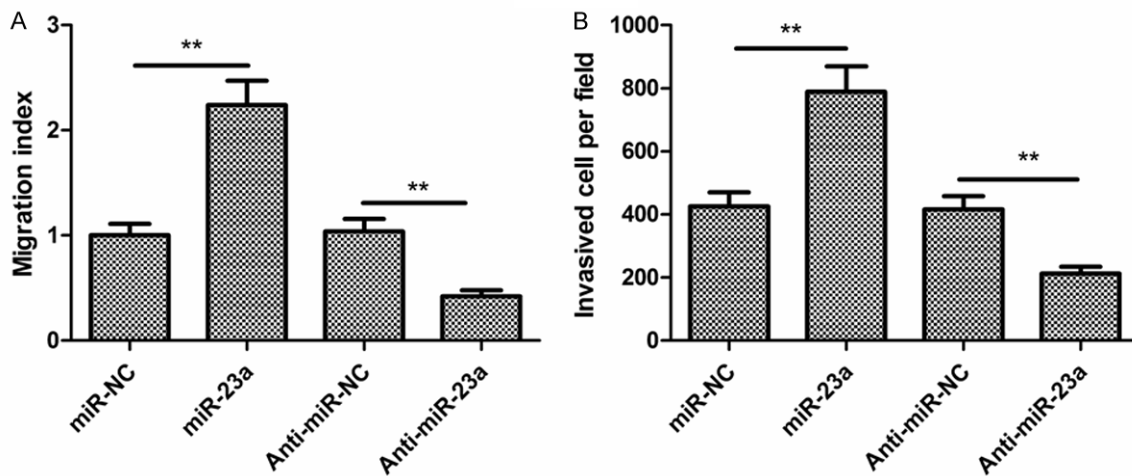


Figure 3. MiR-23a inhibits migration and invasion of PTC cells. (A and B) Cell migration (A) and invasion (B) were determined in K1 cells transfected with miR-23a or Anti-miR-23a by transwell chamber assay. $P < 0.05$, ** $P < 0.01$ versus miR-NC or anti-miR-NC.

MiR-23a promoted cell migration and invasion of PTC cells

To test whether miR-23a effect on PTC cells migration and invasion, cell migration and invasion assays were performed in K1 cells transfected with miR-23a or Anti-miR-23a by transwell assay. It was found that upregulation of miR-23a in K1 cells significantly increased cell migration (Figure 3A) and invasion (Figure 3B) capabilities, while downregulation of miR-23a in K1 cells obviously decreased cell migration (Figure 3A) and invasion (Figure 3B) capabilities.

PTEN is a direct target of miR-23a

Potential targets of miR-23a were predicted using bioinformatic databases such as Target-

Scan, miRanda and PicTar, among targets gene, PTEN, a tumor-suppressor gene, was selected as a potential target for miR-23a since there was a putative miR-23a-binding seed sequence within the 3'UTR of PTEN mRNA (Figure 4A). Luciferase reporter assay further confirmed that overexpression of miR-23a in K1 cells decreased the luciferase activity, whereas underexpression of miR-23a showed a significant increase in luciferase activity (Figure 4B). No luciferase activity change was observed when the cells were transfected with the Mut PTEN reporter plasmids (Figure 4B). In addition, qRT-PCR and western blot assays demonstrated that upregulation of miR-23a in K1 cells could decrease PTEN expression on mRNA level (Figure 4C) and protein level (Figure 4D), while underexpression of miR-23a in K1 cells

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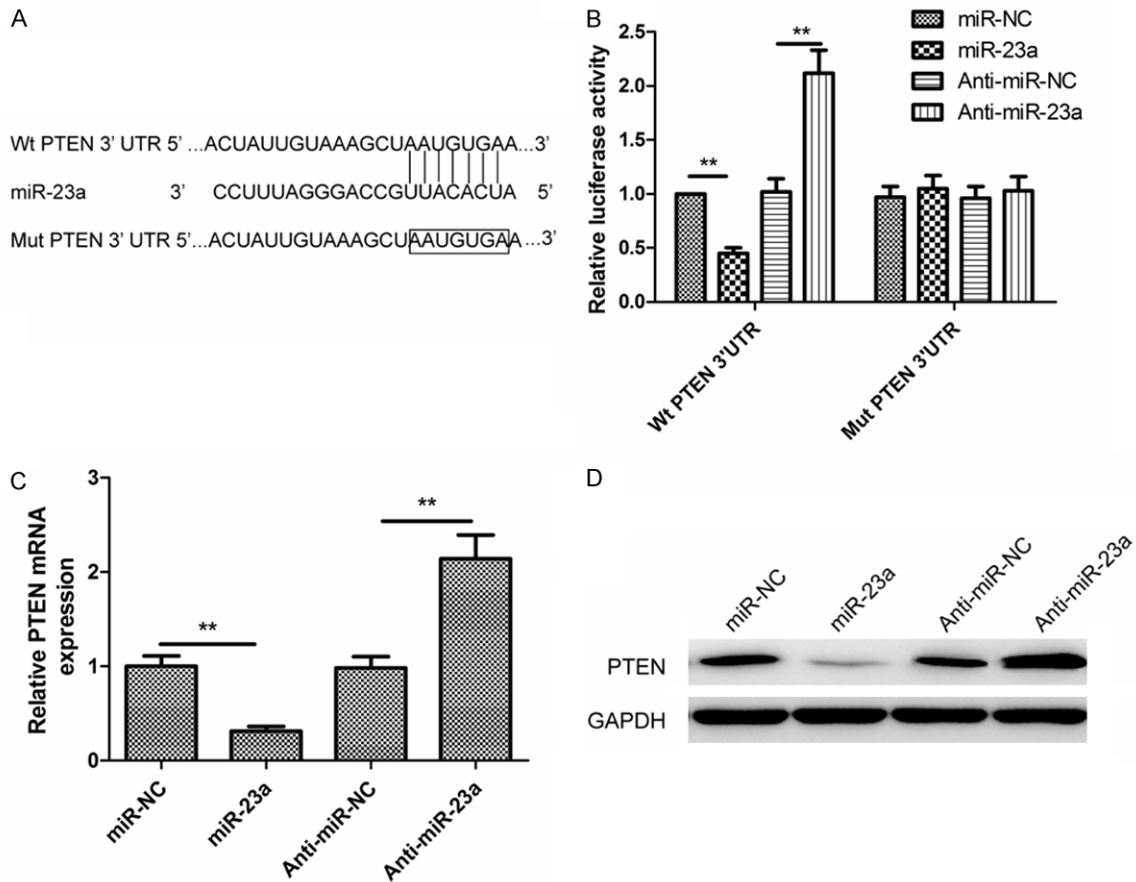


Figure 4. PTEN is a direct target of miR-23a. (A) The predicted binding sites for miR-23a in the 3'UTR of PTEN and the mutations in the binding sites are shown. (B) Relative luciferase activity was determined in K1 cells co-transfection with wide-type or mutant-type 3' UTR PTEN reporter plasmids and miR-23a/Anti-miR-23a. Wt: wide-type, Mut: mutant-type. (C and D) PTEN mRNA expression level (C) and protein expression level (D) were determined in K1 cells after transfected with miR-23a or Anti-miR-23a by qRT-PCR and Western blot. GAPDH was used to an internal control.

has opposite effect. These results indicate that PTEN is a miR-23a target in PTC.

MiR-23a expression is inversely correlated with PTEN expression

Knowing PTEN is a target of miR-23a, the expression of PTEN in 20 PTC tissues and paired adjacent normal tissues were determined by qRT-PCR. We found that PTEN mRNA expression levels were decreased in PTC tissues compared to matched adjacent normal tissues (**Figure 5A**). In addition, the relationship between PTEN and miR-23a expressions was tested with two-tailed Pearson's correlation, found that PTEN expression was negatively correlated with miR-23a expression in PTC tissues (**Figure 5B**; $r=-0.66$, $P<0.01$).

Discussion

Accumulating evidence has suggested that aberrant expression of miRNAs involved in PTC procession and metastasis [7]. Recently, a number of miRNAs have been found to play crucial roles in PTC Initiation and development [8]. For example, Guan et al reported that miR-144 expression was downregulated in thyroid cancer and that miR-144 inhibited thyroid cancer cell invasion and migration via directly targeting ZEB1 and ZEB2 [18]. Ma et al reported that miR-34a can significantly promote cell proliferation and inhibit apoptosis in papillary thyroid carcinoma by targeting growth arrest specific1 (GAS1) via PI3K/Akt/Bad pathway [19]. Chou et al found that restoration of miR-146b significantly promotes cell migration and invasive-

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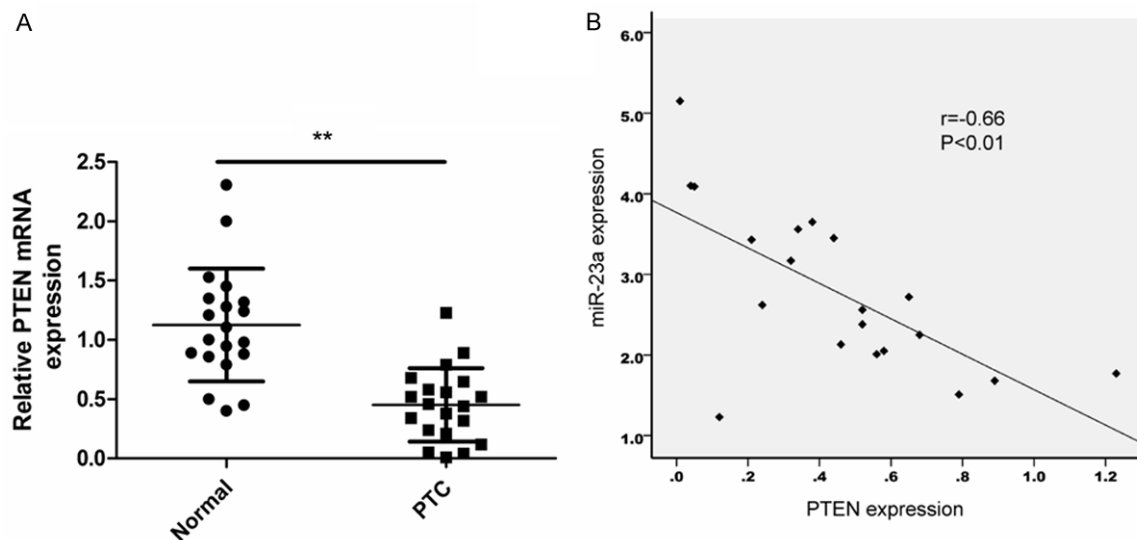


Figure 5. PTEN expression is inversely correlated with miR-23a expression in PTC tissues. A. PTEN mRNA expression in 20 cases of PTC tissue and matched normal tissues were determined by qRT-PCR. GAPDH was used as an internal control. ** $P < 0.01$ versus normal tissues. B. The reverse relationship between PTEN mRNA expression and miR-23a expression was determined by Pearson's correlation.

ness and increases resistance to chemotherapy-induced apoptosis in papillary thyroid carcinoma [20]. In the present study, our results showed that miR-23a expression was downregulated in PTC tissues compared to corresponding adjacent normal tissue. Forced overexpression of human miR-23a in PTC cells could promote cell proliferation, migration and invasion, as well as decreased cell apoptosis. These results suggested that miR-137 could act as a potential therapeutic target for PTC.

Recent studies have found that miR-23a expression is up-regulated in lung cancer [11], liver cancer [12], colorectal cancer [13], gastric cancer [14] and prostate cancer, which suggested that miR-23a could act as oncogene. On the contrary, miR-23a is downregulated and plays a suppressive role in acute promyelocytic leukemia (APL) [21] and oral squamous cell carcinoma (OSCC) [22]. These inconsistent results suggested that dysregulation of miR-23a in various cancers may be dependent on the cellular microenvironment, details tumor type. However, the biological role of miR-23a in PTC remains unclear. Here, we investigated the expression of miR-23a in 20 PTC sample and their paired adjacent normal tissues by qRT-PCR, and found that miR-23a was significantly upregulated in PTC clinical specimens. In addition, we also found that miR-23a promoted PTC

cell growth, migration and invasion by targeting PTEN. These results suggest that miR-23a acts as a potential oncogene in PTC.

Phosphatase and tensin homolog deleted on chromosome 10 (PTEN), located on human chromosome 10q23.3, has been found to act as tumor suppressor gene frequently loss in various human cancers, including PTC [23]. It has been demonstrated that PTEN plays a suppressive role in cancer cell proliferation, migration and invasion by blocking the PI3K/Akt pathway [24]. Recently, a report showed that PTEN was a target gene of miR-23a in osteosarcoma cell [25]. Consistent with this result, our results further confirmed that PTEN was a target gene of miR-23a in PTC cells. In addition, we also showed that PTEN expression on mRNA level was upregulated in PTC tissues and its expression inversely correlated with miR-23a expression. These findings might suggest that miR-23 function as a tumor suppressive miRNA in PTC at least partly through targeting of PTEN.

In summary, the present study first showed that the expression of miR-23a was upregulated in PTC tissues, and its overexpression drastically promoted cell proliferation, migration and invasion, decreased cell apoptosis. In addition, PTEN was confirmed as a target of miR-23a, and its expression was negatively correlated

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with miR-23a expression in PTC tissues. These results indicate that miR-23a functions as an oncogene in PTC and represents a potential therapeutic target for the treatment of PTC.

Disclosure of conflict of interest

None.

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References

- [1] Brown RL, de Souza JA and Cohen EE. Thyroid cancer: burden of illness and management of disease. *J Cancer* 2011; 2: 193-199.
- [2] Pitoia F, Bueno F, Urciuoli C, Abelleira E, Cross G and Tuttle RM. Outcomes of patients with differentiated thyroid cancer risk-stratified according to the American thyroid association and Latin American thyroid society risk of recurrence classification systems. *Thyroid* 2013; 23: 1401-1407.
- [3] Mazzaferri EL and Jhiang SM. Long-term impact of initial surgical and medical therapy on papillary and follicular thyroid cancer. *Am J Med* 1994; 97: 418-428.
- [4] Grant CS. Recurrence of papillary thyroid cancer after optimized surgery. *Gland Surg* 2015; 4: 52-62.
- [5] Bushati N and Cohen SM. MicroRNA functions. *Annu Rev Cell Dev Biol* 2007; 23: 175-205.
- [6] Hwang HW and Mendell JT. MicroRNAs in cell proliferation, cell death, and tumorigenesis. *Br J Cancer* 2006; 94: 776-780.
- [7] Aragon Han P, Weng CH, Khawaja HT, Nagarajan N, Schneider EB, Umbricht CB, Witwer KW and Zeiger MA. MicroRNA expression and association with clinicopathologic features in papillary thyroid cancer: a systematic review. *Thyroid* 2015; 25: 1322-9.
- [8] de la Chapelle A and Jazdzewski K. MicroRNAs in thyroid cancer. *J Clin Endocrinol Metab* 2011; 96: 3326-3336.
- [9] Fujita PA, Rhead B, Zweig AS, Hinrichs AS, Karolchik D, Cline MS, Goldman M, Barber GP, Clawson H, Coelho A, Diekhans M, Dreszer TR, Giardine BM, Harte RA, Hillman-Jackson J, Hsu F, Kirkup V, Kuhn RM, Learned K, Li CH, Meyer LR, Pohl A, Raney BJ, Rosenbloom KR, Smith KE, Haussler D and Kent WJ. The UCSC Genome Browser database: update 2011. *Nucleic Acids Res* 2011; 39: D876-882.
- [10] Chhabra R, Dubey R and Saini N. Cooperative and individualistic functions of the microRNAs in the miR-23a~27a~24-2 cluster and its implication in human diseases. *Mol Cancer* 2010; 9: 232.
- [11] Cao M, Seike M, Soeno C, Mizutani H, Kitamura K, Minegishi Y, Noro R, Yoshimura A, Cai L and Gemma A. MiR-23a regulates TGF-beta-induced epithelial-mesenchymal transition by targeting E-cadherin in lung cancer cells. *Int J Oncol* 2012; 41: 869-875.
- [12] Bao L, Zhao J, Dai X, Wang Y, Ma R, Su Y, Cui H, Niu J, Bai S, Xiao Z, Yuan H, Yang Z, Li C, Cheng R and Ren X. Correlation between miR-23a and onset of hepatocellular carcinoma. *Clin Res Hepatol Gastroenterol* 2014; 38: 318-330.
- [13] Jahid S, Sun J, Edwards RA, Dizon D, Panarelli NC, Milsom JW, Sikandar SS, Gumus ZH and Lipkin SM. MiR-23a promotes the transition from indolent to invasive colorectal cancer. *Cancer Discov* 2012; 2: 540-553.
- [14] Liu X, Liu Q, Fan Y, Wang S, Liu X, Zhu L, Liu M and Tang H. Downregulation of PPP2R5E expression by miR-23a suppresses apoptosis to facilitate the growth of gastric cancer cells. *FEBS Lett* 2014; 588: 3160-3169.
- [15] Wen YC, Lee WJ, Tan P, Yang SF, Hsiao M, Lee LM and Chien MH. By inhibiting snail signaling and miR-23a-3p, osthole suppresses the EMT-mediated metastatic ability in prostate cancer. *Oncotarget* 2015; 6: 21120-21136.
- [16] Wang G, Li B, Fu Y, He M, Wang J, Shen P and Bai L. MiR-23a suppresses proliferation of osteosarcoma cells by targeting SATB1. *Tumour Biol* 2015; 36: 4715-4721.
- [17] 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].
- [18] Guan H, Liang W, Xie Z, Li H, Liu J, Liu L, Xiu L and Li Y. Down-regulation of miR-144 promotes thyroid cancer cell invasion by targeting ZEB1 and ZEB2. *Endocrine* 2015; 48: 566-574.
- [19] Ma Y, Qin H and Cui Y. MiR-34a targets GAS1 to promote cell proliferation and inhibit apoptosis in papillary thyroid carcinoma via PI3K/Akt/Bad pathway. *Biochem Biophys Res Commun* 2013; 441: 958-963.
- [20] Chou CK, Yang KD, Chou FF, Huang CC, Lan YW, Lee YF, Kang HY and Liu RT. Prognostic implications of miR-146b expression and its functional role in papillary thyroid carcinoma. *J Clin Endocrinol Metab* 2013; 98: E196-205.

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- [21] Saumet A, Vetter G, Bouttier M, Portales-Casamar E, Wasserman WW, Maurin T, Mari B, Barbry P, Vallar L, Friederich E, Arar K, Cassinat B, Chomienne C and Lecellier CH. Transcriptional repression of microRNA genes by PML-RARA increases expression of key cancer proteins in acute promyelocytic leukemia. *Blood* 2009; 113: 412-421.
- [22] Kozaki K, Imoto I, Mogi S, Omura K and Inazawa J. Exploration of tumor-suppressive microRNAs silenced by DNA hypermethylation in oral cancer. *Cancer Res* 2008; 68: 2094-210.
- [23] Wang G, Li B, Fu Y, He M, Wang J, Shen P and Bai L. MiR-23a suppresses proliferation of osteosarcoma cells by targeting SATB1. *Tumour Biol* 2015; 36: 4715-4721.
- [24] Morani F, Pagano L, Prodam F, Aimaretti G and Isidoro C. Loss of expression of the oncosuppressor PTEN in thyroid incidentalomas associates with GLUT1 plasmamembrane expression. *Panminerva Med* 2012; 54: 59-63.
- [25] Zhang S and Yu D. PI (3) king apart PTEN's role in cancer. *Clin Cancer Res* 2010; 16: 4325-4330.