

HOSTED BY



Contents lists available at ScienceDirect

Journal of Pharmacological Sciences

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

Full paper

Over-expression of microRNA-375 inhibits papillary thyroid carcinoma cell proliferation and induces cell apoptosis by targeting ERBB2



Xin-Zheng Wang^{a, *}, Ya-Kai Hang^b, Jin-Biao Liu^a, Yong-Qiang Hou^a, Ning Wang^a,
Ming-Jun Wang^a

^a Department III of General Surgery, The First Affiliated Hospital of Henan, University of Science and Technology, Luoyang City, Henan Province, PR China

^b Department of General Surgery, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, PR China

ARTICLE INFO

Article history:

Received 27 July 2015

Received in revised form

27 October 2015

Accepted 1 December 2015

Available online 12 December 2015

Keywords:

MicroRNA-375

Papillary thyroid carcinoma

ERBB2

Apoptosis

ABSTRACT

MicroRNAs (miRs) played important roles in the cell proliferation, apoptosis and other biological processes in cancer. In the present study we found that miR-375 was significantly down-regulated in human papillary thyroid carcinoma (PTC) tissues and cell lines. In this study we try to investigate the biological activity of miR-375 in human PTC cells and try to find the potential target of miR-375. Our study indicated that over-expression of miR-375 could inhibit the PTC cells proliferation and this inhibition was caused by the induction of cell apoptosis. In vivo animal study indicated that over-expression of miR-375 could significantly decrease the migration and invasion of human PTC cell in vivo. These results exhibit over-expression of miR-375 in human PTC cells could inhibit the process of human PTC. Further study demonstrated ERBB2 was a direct target of miR-375, over-expression of miR-375 decrease the both mRNA and protein expression of ERBB2 in human PTC cells. These data indicate miR-375 play important roles in the process and development of human PTC. These finds suggested that appropriate application of miR-375 regulation might be a new sight for the treatment of human PTC in the future.

© 2015 Japanese Pharmacological Society. Production and hosting by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

1. Introduction

MicroRNAs (miRs) are a series of small noncoding RNAs. MiRs constitute about three percent of the whole genome. About ninety percent genes could be regulated by miRs. MiRs could regulate the target genes through binding to the target genes' 3'-UTR (1, 2). They play important roles in the cell proliferation, apoptosis and other biological processes. Previous studies indicated that some miRs were abnormal expressed in tumor tissues and tumor cells including cell lines and primary cells. These genes regulated by the abnormal expressed miRs usually were oncogenes or tumor suppressor genes (3, 4), so the regulation of miRs might be a way to regulate tumor progress.

Thyroid cancer was formed from follicular or parafollicular thyroid cells, these two type cells could cause papillary or follicular cancers and anaplastic thyroid cancer (5). Eighty percent of the thyroid cancers were papillary thyroid carcinoma (PTC) and it was the most quickly increasing cancer in cancer patients (6). The present clinical treatment of PTC was surgical removal and radioactive iodine ablation, sometimes chemotherapy and radiotherapy also used in the treatment of PTC follow the initial surgical treatment. In order to find better therapeutic strategies to cure PTC, more understanding about the biological mechanism involved in PTC is becoming more and more important.

Previous studies indicated that miRs may play important roles in the pathogenesis of human PTC. Pallante *et al* reported that miR-221, -222 and -181b were up-regulated in human PTCs (7). Zhang *et al* reported that miR-21 was overexpressed in human PTCs, decrease the expression of miR-21 could inhibit the human PTC cells proliferation, invasion and induced the apoptosis through the regulation of programmed cell death 4 (8). He *et al* reported that miR-221, -222, and -146 were the 3 most up-regulated microRNAs in human PTC while miR-219, -138, -345

* Corresponding author. No. 24, Jinghua Street, Jianxi District, Luoyang City, Henan Province, PR China.

E-mail address: xinzheng_w@126.com (X.-Z. Wang).

Peer review under responsibility of Japanese Pharmacological Society.

and -26a were down-regulated in human PTCs (9). There was no report about the biological effects of miR-375 on human PTC, although it was found to be regulated in other tumors (10). In the present study we identified that the miR-375 was abnormal expressed in human PTCs, we hypothesize that the expression of miR-375 was associated the biological functions of human PTCs. In this study we try to investigate the biological effect of miR-375 on human PTC cells.

2. Materials and methods

2.1. PTC tissue samples

Patients were selected from The 1st Affiliated Hospital of Henan University of Science and Technology, 60 pairs of PTC and noncancerous normal tissue samples were surgically obtained from these PTC patients. These PTC tissues were diagnosed by an experienced pathologist. And these patients did not receive any other treatments before. Samples were stored at -80°C until the further study.

2.2. Animal

BALB/c nude mice (4–6 weeks) were purchased from Shanghai Laboratory Animal Center (SLAC) and maintained under specific pathogen free (SPF) conditions. All animal studies were in accordance with the Guide for the Care and Use of Laboratory Animals; all animal studies were performed according to the institutional ethical guidelines for animal experiment.

2.3. Cell culture

Human PTC cell TPC-1, K1 and normal thyroid cells Nthy-ori 3-1 cells were cultured in Dulbecco's Modified Eagle medium (DMEM) supplemented with 10% FBS. The cells were cultured in a humidified incubator supplemented with 5% CO_2 .

2.4. Transfection of miR-375 mimic

MiR-375 mimics (mimic-miR375) and control mimics (mimic-control) were purchased from Invitrogen. Human PTC Cells were cultured in DMEM (10% FBS). Twenty-four hours later, cells were transfected with miR375 mimic or control by using Lipofectamine 2000 (Invitrogen) in antibiotic-free Opti-MEM medium (Gibco) according to the manufacturer's instruction. The concentration of miR375 mimic or control was 10 nM (final concentration). The culture medium was replaced with DMEM (10% FBS) 6 h after the transfection.

2.5. MiR-375 expression detection

Total RNA was extracted from human PTC tissues or PTC cells using TRIzol reagent (Invitrogen) as described by the manufacturer. Then the RNA was reverse transcribed using a TaqMan miRNA Reverse Transcription kit (Applied Biosystems) with a miRNA-specific looped RT primer. The quantitative real-time PCR for miR-375 was performed using TaqMan Universal PCR Master Mix (Applied Biosystems) with miRNA-specific TaqMan minor groove binder probes (Applied Biosystems). RNA U6 (Applied Biosystems) was used as an internal control. All qRT-PCR studies were performed in triplicate and the data were presented as mean \pm SD.

2.6. Cell proliferation assay

Cell proliferation assay was performed by the CCK8 assay as described in the manufacturer. First human PTC mock TPC-1, K1 cells or TPC-1, K1 cells with transfection were seeded into 96-well plates (5000 cells/well). 2 h before the designed time point, CCK8 reagent was added to these wells (1:10), then incubate the cells for another 2 h at 37°C . The absorbance (OD450) was expressed as the proliferation of the cells. All the cell proliferation studies were performed in triplicate, and the data were presented as mean \pm SD.

2.7. Colony formation assay

Approximately 500 mock TPC-1, K1 or transfected cells were placed in a fresh 6-well plate for 12 h and cultured in DMEM medium for another 2 weeks. Colonies were fixed with methanol and stained with 0.1% crystal violet in 20% methanol for 15 min. Then the number of colonies was counted. All the colony formulation studies were performed in triplicate and the data are presented as mean \pm SD.

2.8. Apoptosis assay

The human PTC mock TPC-1, K1 cells or TPC-1, K1 cells with transfection were cultured for 48 h and harvested. Cells were washed with PBS and stained with Annexin V and Propidium iodide. Then perform the apoptosis assay with an Annexin V-FITC apoptosis detection kit by using a flow cytometry (BD Biosciences) equipped with CellQuest software (BD Biosciences). The tests were performed in triplicate, and the data were presented as mean \pm SD.

2.9. Luciferase assay

The full length of the 3'-UTR from ERBB2 was cloned into the downstream region of the firefly luciferase gene using the pGL3-control vector. Mutant ERBB2 was used as corresponding controls. MiR-375 inhibitor and control were purchased from Invitrogen. The wild-type or mutant ERBB2 report vectors were co-transfected with the miR375 mimic or inhibitor into TPC-1 cells by Lipofectamine 2000. Cells were cultured in a humidified incubator supplemented with 5% CO_2 . 48 h later, the cells were harvested for luciferase assay. Dual-Luciferase Assay was performed by using the dual-luciferase reporter assay kit (Promega). The relative luciferase activity was normalized with renilla luciferase activity. The tests were performed in triplicate, and the data were presented as mean \pm SD.

2.10. Real-time PCR to detect the mRNA of ERBB2

The mRNA level of ERBB2 was detected by real time PCR. Total RNA was isolated from treated human PTC cells using TRIzol reagent (Invitrogen). cDNA was synthesized from 10 μg total RNA using a Reverse Transcription kit (Applied Biosystems). Taq man Real time PCR was performed as follow programs, 95°C for 10 min, annealing at required temperature for each primer for 15 s, and 72°C for 30 s, for 40 cycles. The primers used were listed below. ERBB2, F: -AGCCTTGCCCCATCAACTG-, R: -AATGCCAACCACCGCAGA-, probe: -CCACTCCTGTGTGGACCTGGATGACA-; β -actin, F: -ATCTGGACCA CACCTTCTACAAT-, R: -CCGTCACCGGAGTCCATCA-, probe: -TGACC CAGATCATGTTTGAGACCTTCAACAC. Probes were labeled with fluorescent reporter dyes FAM (ERBB2) or VIC (Actin). Actin was used as an internal control. All qRT-PCRs were performed in triplicate and the data were presented as mean \pm SD.

2.11. Western blot

Protein extract from transfected PTC cells were separated in a 10% SDS-polyacrylamide gel (Invitrogen) and electrophoretically transferred onto a PVDF membrane (GE). Membranes were blocked and incubated for 2 h with primer antibody ERBB2 and GAPDH (Cell Signaling Technology). After washing with TBST for 3 times, the membranes were incubated with horseradish peroxidase-linked antibody for 1 h. The membranes were washed and the proteins were visualized using ECL chemiluminescence and exposed to x-ray film. All of the samples were performed in triplicate.

2.12. Experimental metastasis assay in vivo

To investigate the effect of miR-375 on human PTC cell migration and invasion in vivo, mock TPC-1 cells, mimic-miR375 transfected TPC-1 cells and mimic-control transfected TPC-1 cells were injected into the left ventricle of BALB/c nude mice (3,000,000 cells in 0.2 ml normal saline) by using a 26-gauge needle, as previously described (11–13). Successful left ventricle injection was monitored by the pulsatile flow of red blood into the needle hub indicating correct placement. There were 8 mice in one group. 4 weeks later, mice were sacrificed, thyroid glands were harvested and the number of metastatic tumor nodules in each thyroid gland was counted. The data were presented as mean \pm SEM.

2.13. Statistical analysis

The data were expressed as the mean \pm SEM or mean \pm SD. Statistical analysis was performed by t-test using SPSS 13.0 to evaluate the significance of differences between groups.

3. Results

3.1. MiR-375 level in human PTC tissues and cell lines

Previous studies indicated that miR-375 was abnormally expressed in many types of human cancer, and they were involved in the proliferation, apoptosis and development of human cancer (14–16). In this study we first examine the expression of miR-375 in PTC tissues. 60 pairs of PTC and noncancerous normal tissue samples were submitted to qRT-PCR assay. RNAs extracted from PTC tissues were reverse transcript, the level of miR-375 was detected by qPCR. U6 was used as the internal control. The results indicated that the miR-375 gene expression in human PTC patient tissues was significantly decreased compared to normal tissues ($p < 0.01$) (Fig. 1A). Next we try to test the level of miR-375 in human PTC cell lines. The level of miR-375 in human PTC cells (TPC-1 and K1) and normal thyroid cells Nthy-ori 3-1 were tested by qRT-PCR. The results indicated that the levels of miR-375 in TPC-1 and K1 cells were also significant down-regulated compared with the level in Nthy-ori 3-1 cells ($p < 0.01$) (Fig. 1B). These results indicate that deregulation of miR-375 may play important roles in the PTC progression and development.

3.2. Manipulation of miR-375 expression in PTC cells

To selectively regulate the expression level of miR-375 in human PTC cells, miR-375 mimic transfection assay was performed. TPC-1 and K1 cells were transfected with mimic-miR375 or mimic-control and cultured. The level of miR-375 in the treated cells was tested by qRT-PCR. After the transfection of mimic-miR375, the expression of miR-375 increased significantly compare to mock cells, while the level of miR-375 was not changed in the cells transfected with mimic-control ($p < 0.01$) (Fig. 2). In order to

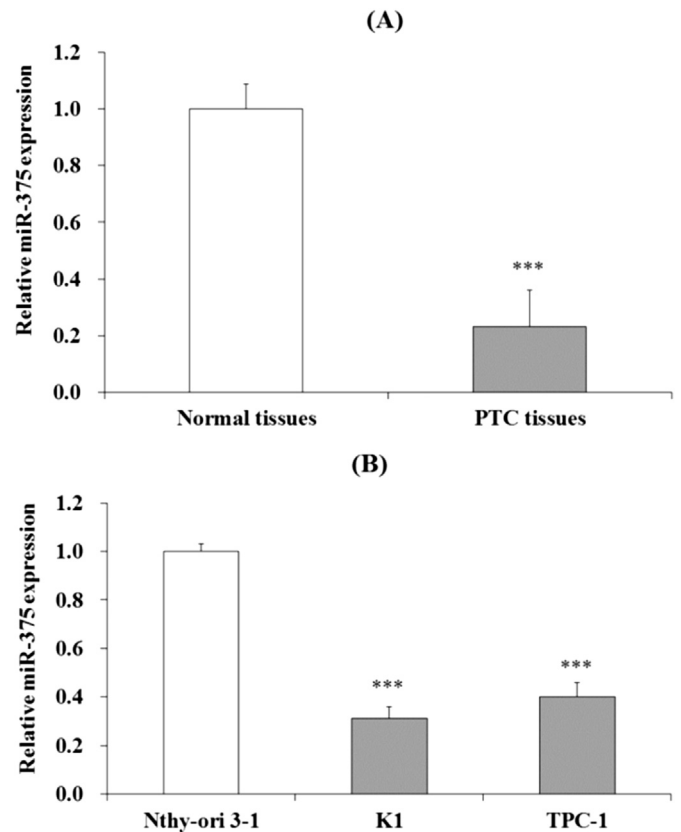


Fig. 1. qRT-PCR analysis of miR-375 in human PTC tissues and cell lines. (A) Analysis of miR-375 expression in human PTC tissues. Data are presented as fold-changes in human PTC tissues relative to normal tissues. (B) Analysis of miR-375 expression human PTC cell lines (K1, TPC-1) compared with the normal thyroid gland cell line (Nthy-ori3-1). All experiments were performed in triplicate and the data were presented as mean \pm SD, *** $p < 0.001$.

investigate the biological effect of miR-375 in human PTC cells, we manipulated the level of miR-375 by mimic transfection assay in the followed study.

3.3. Up-regulation of miR-375 decrease human PTC cell proliferation

The effect of miR-375 on human PTC cell proliferation was examined by CCK8 assay. TPC-1 and K1 cells transfected with miR-

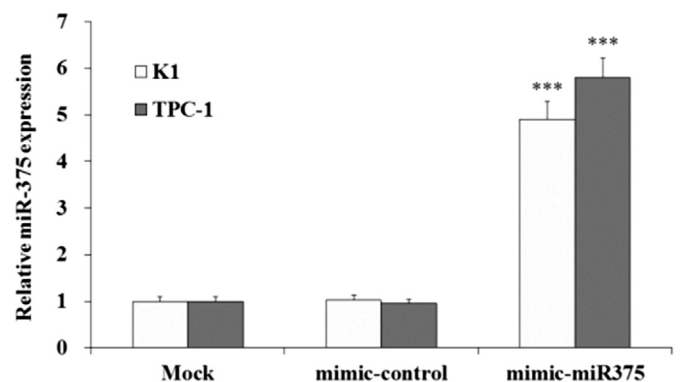


Fig. 2. qRT-PCR analysis of miR-375 expression in mock PTC cells or PTC cells transfected with mimic-miR375 or mimic-control. All experiments were performed in triplicate and the data were presented as mean \pm SD. *** $p < 0.001$.

375 mimic or control were cultured for 48 h, the cell proliferation were tested by CCK8. The results indicated that up-regulation of miR-375 significantly decreased the proliferation of TPC-1 and K1 cells compared with mock cells. The cells transfected with mimic-control shows no changes ($p < 0.01$) (Fig. 3A and B). To further confirm the effect of miR-375 on human PTC cell proliferation, colony-forming growth assay was performed. The results of colony-formation assays demonstrated that clonogenic survival was decreased after the transfected with mimc-miR375 in TPC-1 and K1 cells compared with mock cells, while there was no effect in the cells transfected with mimic-control ($p < 0.01$) (Fig. 3C). Taken together, these results indicated that induce the expression of miR-375 in human PTC cells could induce growth inhibition in TPC-1 and K1 cells.

3.4. Up-regulation of miR-375 increase the apoptosis of human PTC cell

Previous studies indicated that cell apoptosis may contribute to the cell growth inhibition (17). To study whether the growth inhibition of human PTC cell was caused by the induction of cell apoptosis, we performed Flow-cytometric analysis of TPC-1 and K1 cells with the transfection with mimic-miR375 or mimic-control. The apoptotic rate of TPC-1 mimic-miR375 cells was about 5.8% and the rate of K1 mimic-miR375 cells was about 6.4% while the rate were 0.7% or 0.5% in mock TPC-1 or K1 cells. The results indicated that the apoptotic rate of PTC cells transfected with mimic-miR375 was significantly increased than the rate of mock PTC cells, while the rate in TPC-1 (0.8%) and K1 (0.6%) cells transfected with mimic-control was no affected ($p < 0.01$) (Fig. 4). Therefore over-expression of miR-375 could induce the apoptosis of human PTC cell TPC-1 and K1.

3.5. ERBB2 is a direct target of miR-375

To investigate the mechanism of miR-375 on human PTC cells, we searched the potential target genes by using commonly cited programs (TargetScan, PicTar and miRanda). The predicted results indicated that ERBB2 was a direct target of miR-375 (Fig. 5A). In order to validate whether ERBB2 is the right target gene of miR-375, human PTC TPC-1 cells were co-transfected with pGL3-ERBB2 and mimic-miR375/miR-375 inhibitor, then the dual luciferase assay was performed. The luciferase results indicated that overexpression of miR-375 in TPC-1 cells co-transfected with ERBB2 WT led to a reduction of luciferase, while there was no reduction of luciferase in the TPC-1 cells co-transfected with ERBB2 Mut. When co-transfected miR-375 inhibitor with ERBB2 WT in TPC-1 cells led to luciferase increase (Fig. 5B). These results indicated that ERBB2 was the direct target of miR-375. Further study indicated that while transfected the PTC cells (K1 and TPC-1) with mimic-miR375 or mimic-control, the both mRNA and protein level of ERBB2 in mimic-miR375 group was decreased significantly compared to mimic-control (Fig. 5C and D). Consistently, induction of microRNA-375 led to a decreased expression of ERBB2 in human PTC cells.

3.6. Up-regulation of miR-375 suppresses the migration and invasion of human PTC cell in vivo

To investigate the biological effect of miR-375 on human PTC cell in vivo, human PTC TPC-1 mock cells, TPC-1 cells transfected with mimic-miR375 or mimic-control were injected into nude mice via tail vein. Four weeks later, mice were sacrificed, tissues were harvest and the number of metastatic nodules was counted. In TPC-1 mock group and the mimic-control group, there were some large

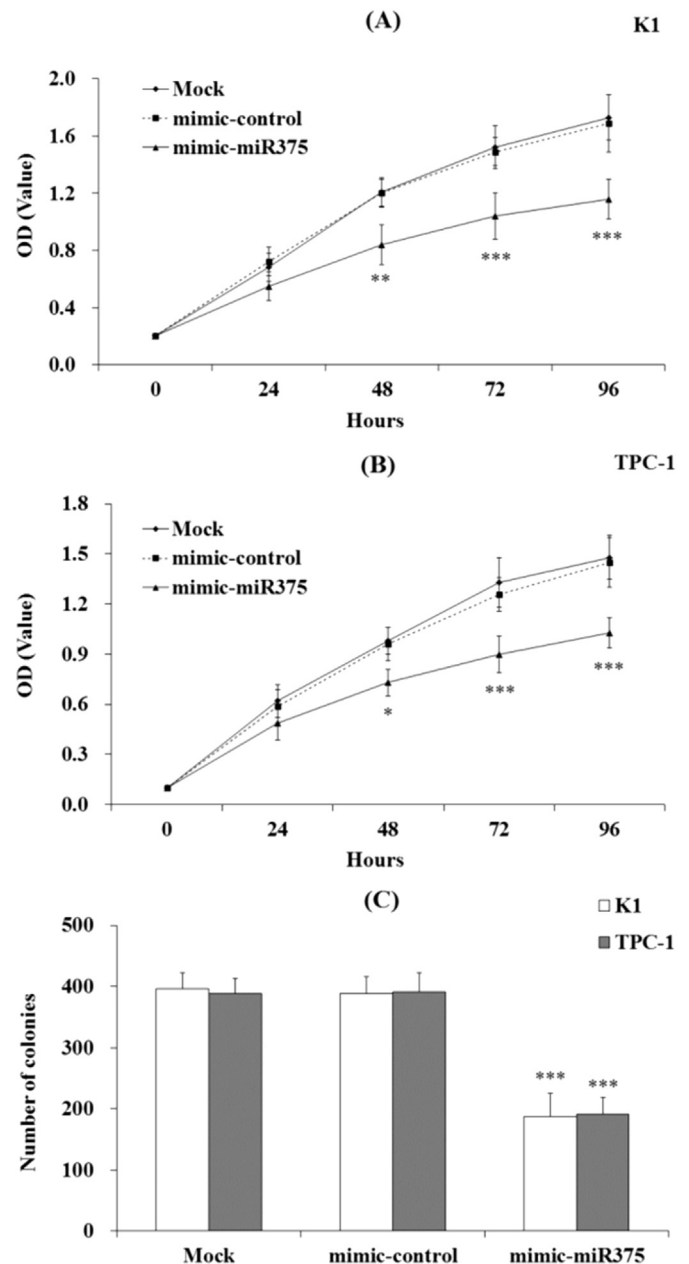


Fig. 3. Effect of miR-375 on PTC cell proliferation. (A) Mock K1 cells or K1 transfected with mimic-miR375 or mimic-control cells were seeded into 96-well plates. CCK8 assay was performed to determine the cell proliferation. (B) Mock TPC-1 cells or TPC-1 transfected with mimic-miR375 or mimic-control cells were seeded into 96-well plates. CCK8 assay was performed to determine the cell proliferation. (C) Colony-forming growth assays were performed to determine the proliferation of PTC cells. The colonies were counted. All experiments were performed in triplicate and the data were presented as mean \pm SD. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

tumor clusters; while no large tumor was found in the mimic-miR375 group. The tumor number results indicated that transfected with miR-375 significantly decrease the migration and invasion of human PTC cell in vivo (Fig. 6).

4. Discussion

MiRs are a class of non-coding endogenous RNAs in plants and animals. They were about 22 nucleotides in length and played important roles in the transcriptional regulation of gene

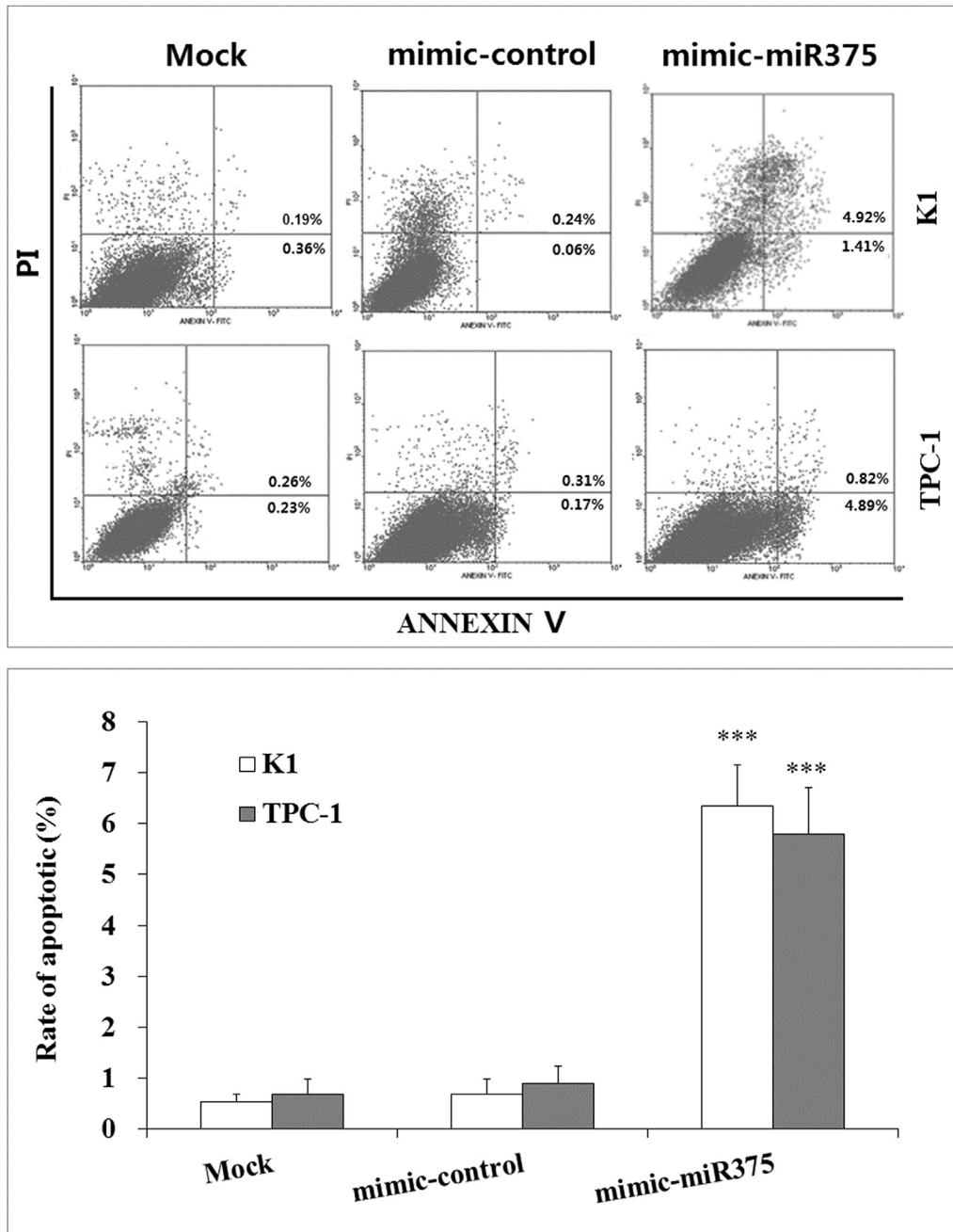


Fig. 4. Effect of miR-375 on PTC cell apoptosis. Flow cytometry analysis of apoptosis in K1 and TPC-1 cells transfected with mimic-miR375 or mimic-control. All experiments were performed in triplicate and the data were presented as mean \pm SD. *** $p < 0.001$.

expression. So far, about 28645 miRNAs has been identified in plants, animals and virus (18). There were contributed to inhibition of translation or the degradation of mRNAs, then regulation of target gene expression (19). Previous studies indicated that miRNAs could act as oncogenes or tumor suppressor genes in the processes of tumorigenesis (20–23). A few abnormal expressed miRs were identified in human PTC; they played important roles in the processed of human PTC (9). MiR-375 was identified on human chromosome 2, it was first found in the pancreas and it was between CRYBA2 and CCDC108 (24, 25). However the roles of miR-375 in human PTC were not fully understood.

In this study we try to investigate the biological effect of miR-375 in human PTC cells. In our work, we found miR-375 was

down-regulated in human PTC tissues, further study indicated the level of miR-375 was also down-regulated in human PTC cell lines. In this study we transfected the PTC cells with mimic-miR375 to up-regulate the expression of miR-375, the qRT-PCR study confirmed the expression of miR-375 was increased while transfected with mimic-miR375. Then we studied the biological effect of miR-375 on the human PTC cell lines, we found that over-expression of miR-375 expression would inhibit the proliferation of human PTC cell lines by the induction of cell apoptosis. Animal study indicated that transfected with mimic-miR375 significantly decrease the migration and invasion of human PTC cell in vivo. These results exhibit induction of miR-375 in human PTC cells could inhibit the process of human PTC.

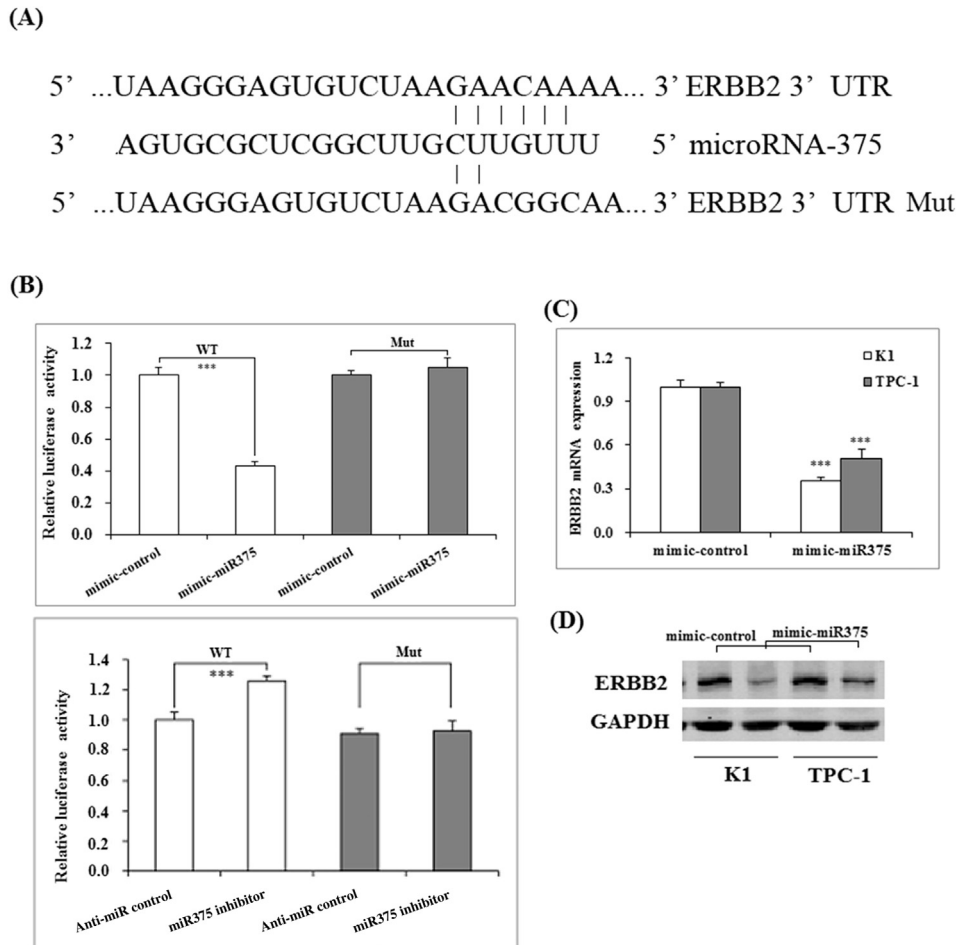


Fig. 5. ERBB2 is a direct target gene of miR-375. (A) Prediction of miR-375 binding sites in the 3'UTRs of human ERBB2 genes. (B) Confirm ERBB2 is the direct target gene of miR-375. Cells were co-transfected with wild type or mutant ERBB2 and mimic-miR375, miR375 inhibitor or control, the dual-luciferase assay was performed. (C) ERBB2 mRNA expression level was decreased by the transfection of mimic-miR375, as detected by qRT-PCR analysis. (D) ERBB2 protein expression level was decreased by the transfection of mimic-miR375, as detected by Western blot analysis. All experiments were performed in triplicate and the data were presented as mean \pm SD. *** $p < 0.001$.

ERBB2 is a member of EGFR family; it is associated in many cancers, it could induce the proliferation of cancer cells (26). It was overexpressed in many cancer patients (27). ERBB2 was associated with Ras-MAPK and PI3K-Akt pathway, overexpression of ERBB2 could increase cell proliferation and decrease cell

apoptosis, high ERBB2 expression would deactivate the sensitivity of cells to the chemotherapy and radiotherapy. In this study we found that ERBB2 is a target of miR-375 by using commonly cited programs, and dual luciferase assay results confirmed that ERBB2 is a direct target of miR-375. Further studies showed that induction of miR-375 could decrease the expression of ERBB2 in human PTC cells. But how did miR-375 inhibit PTC cell proliferation and induce apoptosis, whether the apoptosis was associated with Ras-MAPK or PI3K-Akt pathway was still unknown. To better understand the action mechanism of miR-375, further studies are needed.

In conclude, the present study indicated that miR-375 was down-regulated in human PTC tissues and cell lines. Overexpression of miR-375 could decrease the PTC cell proliferation, induce PTC cell apoptosis and inhibit the migration and invasion in vivo. Besides, we demonstrated the miR-375 regulate the PTC cell behavior through directly targeting ERBB2, an inverse relationship between miR-375 and ERBB2. These suggested that appropriate application of miR-375 regulation might be a new sight for human PTC treatment in the future.

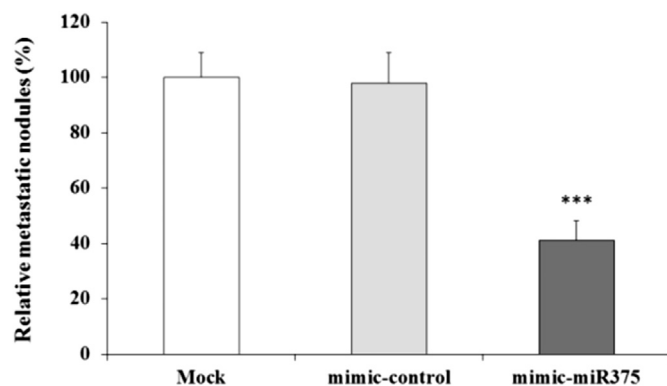


Fig. 6. Effects of miR-375 on the migration and invasion of PTC cells in vivo. The mock and transfected cells were injected intravenously via tail vein respectively. 28 days later, mice were sacrificed and the relative metastatic nodules were calculated. All experiments were performed in triplicate and the data were presented as mean \pm SEM. *** $p < 0.001$.

Conflict of interest

The authors declare that there are no conflicts of interest.

References

- (1) Chi SW, Zang JB, Mele A, Darnell RB. Argonaute HITS-CLIP decodes microRNA-mRNA interaction maps. *Nature*. 2009;460:479–486.
- (2) Hale BJ, Yang CX, Ross JW. Small RNA regulation of reproductive function. *Mol Reprod Dev*. 2014;81:148–159.
- (3) Srivastava A, Goldberger H, Dimtchev A, Ramalinga M, Chijioko J, Marian C, et al. MicroRNA profiling in prostate cancer—the diagnostic potential of urinary miR-205 and miR-214. *PLoS One*. 2013;8:e76994.
- (4) Hodge LS, Elsawa SF, Grote DM, Price-Troska TL, Asmann YW, Fonseca R, et al. MicroRNA expression in tumor cells from Waldenstrom's macroglobulinemia reflects both their normal and malignant cell counterparts. *Blood Cancer J*. 2011;1:e24.
- (5) Carling Tobias, Udelsman Robert. Thyroid cancer. *Annu Rev Med*. 2014;65:125–137.
- (6) Siegel R, Naishadham D, Jemal A. Cancer statistics. *CA Cancer J Clin*. 2012;2012(62):10–29.
- (7) Pallante P, Visone R, Ferracin M. MicroRNA deregulation in human thyroid papillary carcinomas. *Endocr Relat Cancer*. 2006;13:497–508.
- (8) Zhang J, Yang Y, Liu Y, Fan Y, Liu Z, Wang X, et al. MicroRNA-21 regulates biological behaviors in papillary thyroid carcinoma by targeting programmed cell death 4. *J Surg Res*. 2014;189:68–74.
- (9) He H, Jazdzewski K, Li W, Liyanarachchi S, Nagy R, Volinia S, et al. The role of microRNA genes in papillary thyroid carcinoma. *Proc Natl Acad Sci U. S. A*. 2005;102:19075–19080.
- (10) Tsukamoto Y, Nakada C, Noguchi T, Tanigawa M, Nguyen LT, Uchida T, et al. MicroRNA-375 is downregulated in gastric carcinomas and regulates cell survival by targeting PDK1 and 14-3-3zeta. *Cancer Res*. 2010;70:2339–2349.
- (11) Kang Y. Analysis of cancer stem cell metastasis in xenograft animal models. *Methods Mol Biol*. 2009;568:7–19.
- (12) Chan CM, Jing X, Pike LA, Zhou Q, Lim DJ, Sams SB, et al. Targeted inhibition of Src kinase with dasatinib blocks thyroid cancer growth and metastasis. *Clin Cancer Res*. 2012;18:3580–3591.
- (13) Ma Qingping, Jiang Qianqian, Pu Qiang, Zhang Xuechao, Yang Weihuan, Wang Yu, et al. MicroRNA-143 inhibits migration and invasion of human non-small-cell lung cancer and its relative mechanism. *Int J Biol Sci*. 2013;9:680–692.
- (14) Ding L, Xu Y, Zhang W, Deng W, Si M, Du Y, et al. MiR-375 frequently down-regulated in gastric cancer inhibits cell proliferation by targeting JAK2. *Cell Res*. 2010;20:784–793.
- (15) Liu L, Yu X, Guo X, Tian Z, Su M, Long Y, et al. miR-143 is downregulated in cervical cancer and promotes apoptosis and inhibits tumor formation by targeting Bcl-2. *Mol Med Rep*. 2012;5:753–760.
- (16) Wang F, Li Y, Zhou J, Xu J, Peng C, Ye F, et al. miR-375 is down-regulated in squamous cervical cancer and inhibits cell migration and invasion via targeting transcription factor SP1. *Am J Pathology*. 2011;179:2580–2588.
- (17) Wang Y, Lee CG. MicroRNA and cancer—focus on apoptosis. *J Cell Mol Med*. 2009;13:12–23.
- (18) Zhang Xiaomin, Azhar Gohar, Williams Emmanuel D, Rogers Steven C, Wei Jeanne Y. MicroRNA clusters in the adult mouse heart: age-associated changes. *BioMed Res Int*. 2015;2015:12. <http://dx.doi.org/10.1155/2015/732397>. Article ID 732397.
- (19) Sun K, Lai EC. Adult-specific functions of animal microRNAs. *Nat Rev Genet*. 2013;14:535–548.
- (20) Van Kouwenhove M, Kedde M, Agami R. MicroRNA regulation by RNA-binding proteins and its implications for cancer. *Nat Rev Cancer*. 2011;11:644–656.
- (21) Kasinski AL, Slack FJ. Epigenetics and genetics. MicroRNAs en route to the clinic: progress in validating and targeting microRNAs for cancer therapy. *Nat Rev Cancer*. 2011;11:849–864.
- (22) Iorio MV, Croce CM. MicroRNA dysregulation in cancer: diagnostics, monitoring and therapeutics. A comprehensive review. *EMBO Mol Med*. 2012;4:143–159.
- (23) Valencia-Sanchez MA, Liu J, Hannon GJ, Parker Roy. Control of translation and mRNA degradation by miRNAs and siRNAs. *Gene Dev*. 2006;20:515–524.
- (24) Baroukh NN, Van Obberghen E. Function of microRNA-375 and microRNA-124a in pancreas and brain. *FEBS J*. 2009;276:6509–6521.
- (25) Poy MN, Eliasson L, Krutzfeldt J, Kuwajima S, Ma X, Macdonald PE. A pancreatic islet-specific microRNA regulates insulin secretion. *Nature*. 2004;432:226–230.
- (26) Muthuswamy SK, Li D, Lelievre S, Bissell MJ, Brugge JS. ErbB2, but not ErbB1, reinitiates proliferation and induces luminal repopulation in epithelial acini. *Nat Cell Biol*. 2001;3:785–792.
- (27) Bang YJ. Advances in the management of HER2-positive advanced gastric and gastroesophageal junction cancer. *J Clin Gastroenterol*. 2012;46:637–648.