

Original Article

MicroRNA-451 functions as a tumor suppressor in thyroid cancer for multiple antitumor effects via directly targeting MIF

Wei Wan¹, Yan Tang², Yuhong Gao¹, Baoyu Li¹, Gang Ding¹

Departments of ¹General Surgery, ²Oncology, Yidu Central Hospital of Weifang, Weifang Medical University, China

Received October 4, 2015; Accepted December 5, 2015; Epub February 15, 2016; Published February 29, 2016

Abstract: Abnormal expression levels of microRNAs were found in various kinds of cancers, also including thyroid cancer, suggesting the important roles of miRNAs in cancer carcinogenesis and progression. In this study, we demonstrated for the first time that microRNA-451 was significantly down-regulated in papillary thyroid carcinomas (PTC) tissues and cell lines. Furthermore, our results showed that microRNA-451 inhibited PTC cell proliferation, migration and invasion. In addition, migration inhibitory factor (MIF) was identified as a bona fide target of microRNA-451 by computational analysis, followed by Dual-Luciferase report assay, quantitative Real-time PCR and western blot. Further mechanistic analysis demonstrated that knockdown of MIF could mimic the functions of microRNA-451 in PTC cells, rendering MIF as a functional target of microRNA-451. Taken together, these findings verified that microRNA-451 was a tumor suppressor for PTC and a favorable factor against PTC progression via directly targeting MIF. It could be investigated as targeted therapies for PTC.

Keywords: Thyroid cancer, MIF, microRNA-451, PTC

Introduction

Thyroid cancer, the most common kind of endocrine malignancy, accounts for approximately 93% of all endocrine system cancers [1]. In the past few decades, the incidence of thyroid cancer continues to increase [2]. In 2015, it is estimated that there would be 62,450 new cases and 1,950 deaths due to thyroid cancer in United States [3]. Thyroid cancer are mostly derived from parafollicular cells and follicular cells, and can be classified into four histological types: papillary thyroid carcinomas (PTC), follicular thyroid carcinoma (FTC), poorly differentiated carcinoma (PDTC) and undifferentiated anaplastic carcinoma (ATC) [4]. Among these four groups, PTC is the most common kind of thyroid cancer and comprises more than 80% of all thyroid cancer patients [5, 6]. Currently, the standard therapy treatments for PTC are surgical resection, radiotherapy and levothyroxine treatment [7]. Due to progresses in therapies, most PTC patients have a good prognosis. However, for PTC patients with metastasis, the prognosis is poor to standard treatments [8, 9].

The metastasis of thyroid cancer, which frequently involves bone and lung, is the major reason of poor prognosis [10]. Therefore, it is crucial to explore the molecular mechanisms of metastasis and investigate novel therapies to prevent metastasis for thyroid cancer patients.

MicroRNAs (miRNAs) are a group of non-coding, signal-stranded and naturally existing small RNAs (20-24 nucleotide in length) which negatively regulate expression of a great deal of genes through binding to the 3'untranslated regions (3'UTR) of genes in complementary base pairing manner and resulting in inhibition of gene translation [11-13]. A number of reports have suggested that miRNAs play essential roles in major physiological and pathological processes, such as cell differentiation, growth, apoptosis, cycle, angiogenesis, metabolism and metastasis [12, 14, 15]. Abnormal expression of miRNAs has been demonstrated to be associated with cancer carcinogenesis and development in many kinds of tumor, also including PTC [16, 17]. Recently, half of the human miRNAs are found to be located at cancer-asso-

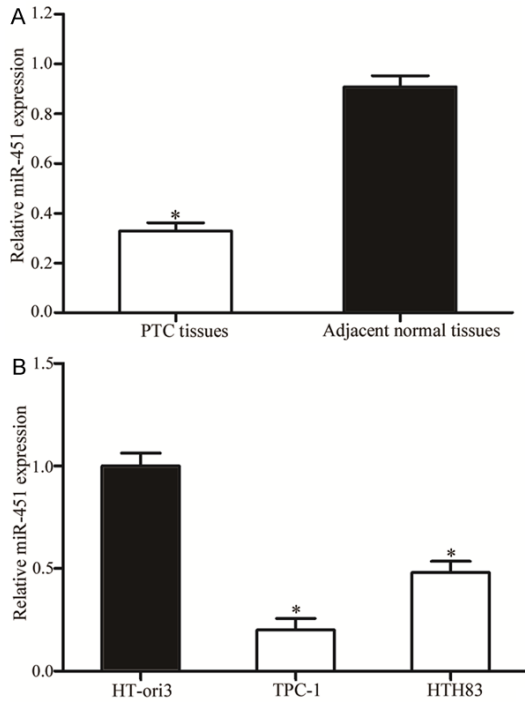


Figure 1. miR-451 expression in PTC tissues and cell lines. A. miR-451 was down-regulated in PTC tissues compared with matched adjacent normal thyroid papillary tissues. B. miR-451 was decreased in TPC-1 and HTH83 cell lines in comparison to HT-ori3. * $P < 0.05$ compared with their respective controls.

over-expressed by miR-451 mimics. B. miR-451 was down-regulated in TPC-1 and HTH83 cells after transfection with anti-miR-451.

ciated genomic regions which are often amplified, deleted, or rearranged in cancers. These findings verified that miRNAs may function as tumor suppressors or oncogens in cancer initial and progression, which indicated that miRNA-targeting therapeutic strategies are promising in cancer [18-20]. Therefore, in the future, miRNAs might investigate as a target for cancer therapy.

In the present study, we focused on expression, functions and molecular mechanisms of miR-451 in PTC. We found that miR-451 was down-regulated in PTC tissues and cell lines. In addition, miR-451 directly targeted the 3'UTR of macrophage migration inhibitory factor (MIF) and decreased MIF mRNA and protein expression, thereby suppressing PTC cells proliferation, migration and invasion. These findings are a useful addition to our current understanding of the mechanism of PTC carcinogenesis and progression.

Material and methods

Ethics statement and clinical specimens

This consented thyroid cancer patient approved by the Institutional Review Board of the Hospital, according to its guidelines for the protection of human subjects. Informed consent was obtained from all thyroid cancer patients in written form, in according with the hospital's ethical guidelines.

Eight-six pairs of PTC tissues and corresponding adjacent normal thyroid papillary tissues were obtained from thyroid cancer patients who undergo surgery at Yidu Central Hospital of Weifang from 2011 to 2014. Patients involved in this study were not received other therapeutic treatments before surgery. All tissues were immediately snap frozen in liquid nitrogen after surgery and stored at -80°C refrigerator.

Cell culture and cell transfection

The human PTC cell lines (TPC-1 and HTH83), normal human thyroid cell line (HT-ori3) and HEK293T cell line were obtained from American Type Culture Collection (ATCC; Manassas, VA,

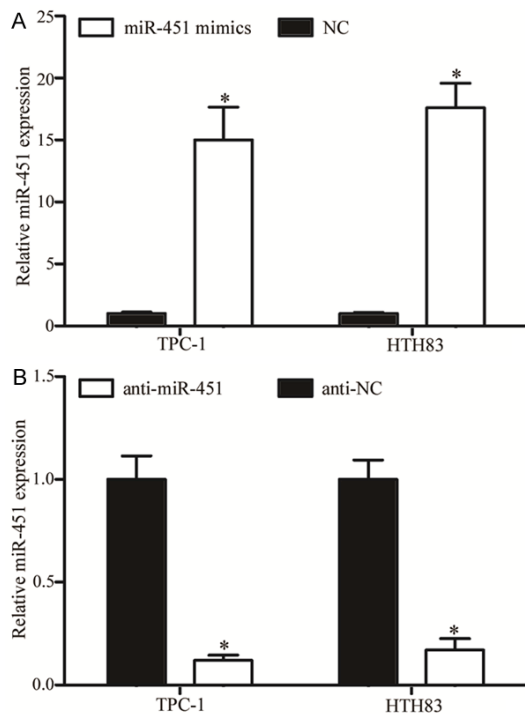


Figure 2. miR-451 expression in TPC-1 and HTH83 cells after transfection. A. The level of miR-451 was

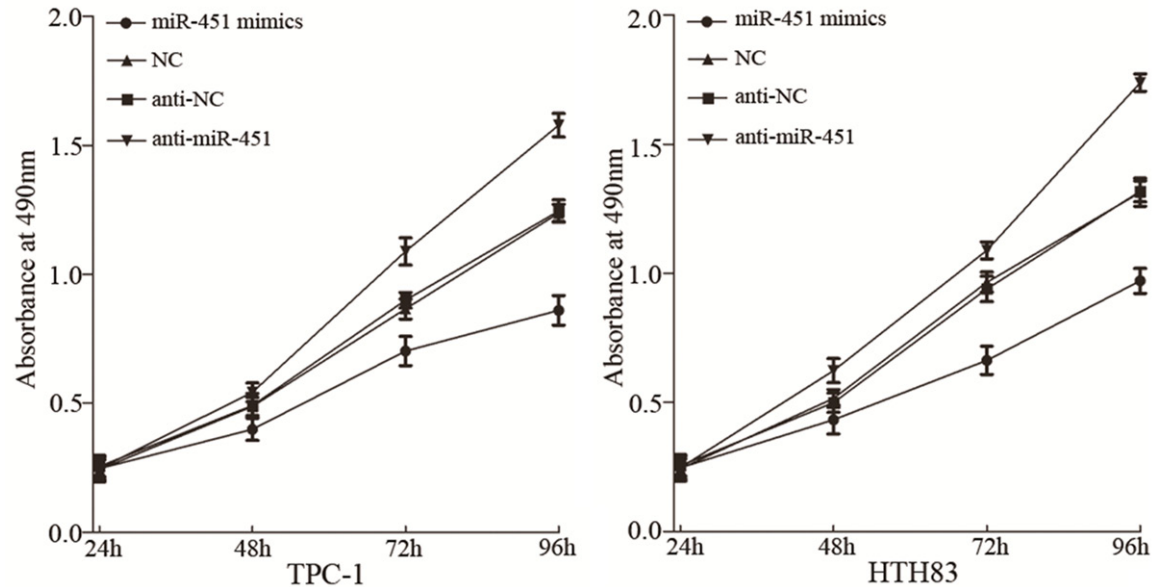


Figure 3. miR-451 inhibited PTC cells proliferation. MTT assays showed that miR-451 mimics suppressed TPC-1 and HTH83 cells proliferation, while anti-miR-451 enhanced TPC-1 and HTH83 cells growth. * $P < 0.05$ compared with their respective controls.

USA). HT-ori3 cell line was cultured in RPMI-1640 supplemented with 10% fetal bovine serum (FBS; Gibco, Grand Island, NY), 100 IU/ml penicillin (Gibco, Grand Island, NY) and 100 μ g/ml streptomycin (Gibco, Grand Island, NY). The other cell lines were maintained in Dulbecco's modified Eagle's medium (DMEM; Gibco, Grand Island, NY) with 10% FBS, 100 IU/ml penicillin and 100 μ g/ml streptomycin. All cell lines were incubated in a humidified 5% CO_2 cell incubator at 37°C.

miR-451 mimics, negative control (NC), anti-miR-451 inhibitor (anti-miR-451) and anti-NC inhibitor (anti-NC) were purchased from GenePharma (Shanghai, China). MIF siRNA and NC siRNA were obtained from Guangzhou RiboBio Co., Ltd (Guangzhou, China). Before transfection, cell culture medium was replaced using complete medium without antibiotics. Cell transfection was performed using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA), following to the manufacturer's instructions.

RNA isolation and quantitative real-time PCR (qRT-PCR)

Total RNA was extracted from human PTC tissues, corresponding adjacent normal thyroid papillary tissues, PTC cell lines and HT-ori3 using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. The RNA purity for each RNA samples was

determined by using an ultraviolet spectrometer for OD260, OD280, and OD230. M-MLV Reverse Transcription system (Promega, USA) was used for reverse transcribed. miR-451 level and MIF mRNA level was detected by qRT-PCR using SYBR Green PCR master mixture (Takara, China). U6 and GADPH were used as internal control for miR-451 and MIF mRNA, respectively.

MTT assay

Cell proliferation was evaluated using 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide (MTT; Sigma, St. Louis, MO, USA) assay. 3,000 cells were plated into 96-well plates for overnight incubation. Then, transfection was performed with Lipofectamine 2000 according to the manufacture's manual. After transfection 24 h, 48 h, 72 h and 96 h, MTT assays were conducted. In briefly, 20 μ l MTT (5 mg/mL) was added into each well and incubated at 37°C for 4 h. Cell culture medium was removed and formazan precipitates were dissolved in 150 μ l of DMSO. The viability was assessed by absorbance measurements with a plate reader at a wavelength of 490 nm. All the experiments were performed in triplicate.

Cell migration and invasion assay

Cell migration and invasion abilities was evaluated using transwell chambers (24-well insert,

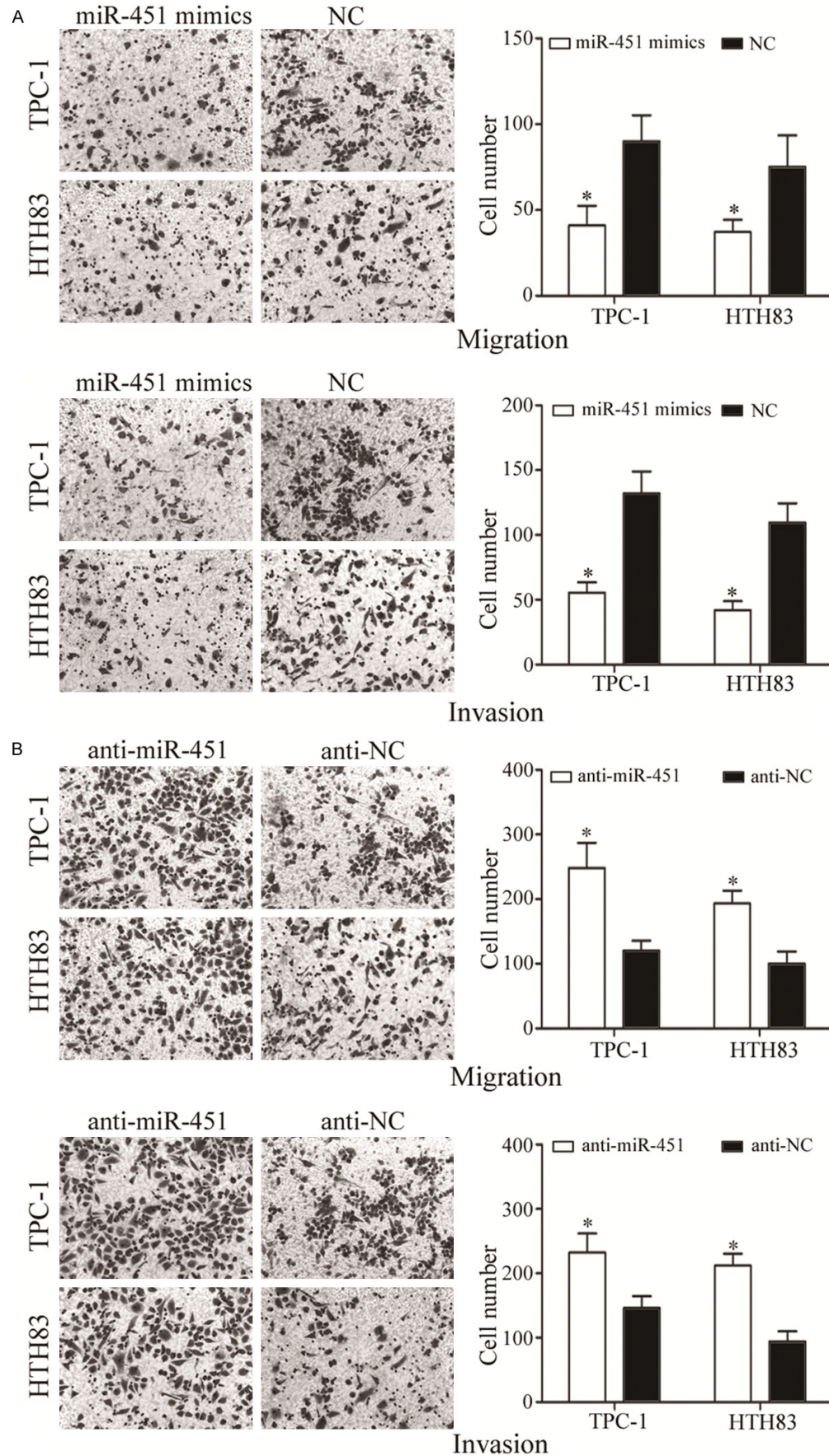


Figure 4. miR-451 inhibited PTC cells migration and invasion. A. miR-451 mimics inhibited TPC-1 and HTH83 cells migration and invasion abilities. B. Down-regulation of miR-451 resulted into a significant promotion of cell migration and invasion in TPC-1 and HTH83 cells. * $P < 0.05$ compared with their respective controls.

Corning, Inc., Corning, NY) with 8 μ m pore size polycarbonate membrane. Cell migration and invasion assays were performed following the same procedure, with the exception that transwell chamber membranes were coated with Matrigel (BD Biosciences, San Jose, CA) for invasion assays. After transfection 48 h, cells were collected and re-suspended as single-cell solutions in DMEM medium without FBS. 5×10^4 cells in 200 μ l medium was plated into the upper chamber, while 500 μ l DMEM medium supplemented with 20% FBS was added into the lower chamber as a chemoattractant. After incubation 24 h at 37°C in a 5% CO₂ incubator, cells were fixed, stained with 0.5% crystal violet and washed with PBS for three times. Cells on the top of membrane were carefully removed with a cotton swab. Then, chambers were subjected to an inverted microscope (Olympus Corporation, Tokyo, Japan) by counting five random fields per membrane. All experiments were repeated at least three times.

Western blot

The effect of miR-451 on MIF protein expression was assessed using western blot. In the present study, Primary antibodies, goat anti-human monoclonal MIF and mouse anti-human GAPDH were purchased from Santa Cruz Biotech. After transfection 72 h, the protein was extracted from cells using cold RIPA lysis buffer (Beyotime Institute of Biotechnology, China). The concentration of total protein was measured with Bicinchoninic Acid Protein assay kit (BCA; Thermo Fisher Scientific, Inc., Rockford, IL, USA). In briefly, equal quantities of protein were separated by 10% SDS-PAGE (Beyotime Institute of Biotechnology, China) and transferred electrically to polyvinylidene difluoride (PVDF; Millipore, USA) membranes. After blocking with 5% non-fat dry milk in TBST, the membranes were incubated with primary antibodies for overnight at 4°C. Then, membranes were washed with TBST for three times and incubated with corresponding secondary antibodies (Beyotime Institute of Biotechnology, China). The bands were visualized with ECL solution (Pierce, Rockford, IL, USA). The protein intensities were quantified with AlphaEaseFC software. GAPDH was used as an internal control.

Dual-Luciferase report assay

To explore whether miR-451 directly targeted 3'UTR of MIF, Dual-Luciferase report assays (Promega, Madison, WI, USA) were performed. HEK293T cells were seeded into 24-well plates and cultured until the density reached 70%-80%. HEK293T cells were transfected with PGL3-MIF-3'UTR Wt or PGL3-MIF-3'UTR Mut, and co-transfection with miR-451 mimics or NC using Lipofectamine 2000 according to the manufacturer's instructions. After incubation 48 h, Dual-Luciferase report assays were performed. Firefly activities and renilla luciferase activities were detected for each well. The renilla luciferase activities were used as an internal control. All the experiments were performed in triplicate.

Statistical analysis

Data were expressed as mean \pm S.D., and compared using SPSS 17 software (SPSS Inc., Chicago, IL, USA). Differences were considered significant at P value less than 0.05.

Results

miR-451 expression level in PTC tissues and cell lines

To measure miR-451 expression in PTC, we monitored its expression in PTC tissues and corresponding adjacent normal thyroid papillary tissues using qRT-PCR. The qRT-PCR analysis showed that miR-451 was obviously down-regulated in PTC tissues compared with matched adjacent normal thyroid papillary tissues (shown in **Figure 1A**). We then measured miR-451 expression in PTC cell lines and a normal human thyroid cell line HT-ori3. We found that the expression level of miR-451 was decreased in TPC-1 and HTH83 cell lines in comparison to HT-ori3 (shown in **Figure 1B**).

miR-451 expression level in TPC-1 and HTH83 cells after transfection

To further investigate miR-451 roles in PTC, miR-451 was up-regulated and down-regulated in PTC cells. After transfection 48 h, qRT-PCR was performed to measure miR-451 expression level. As shown in **Figure 2A**, the level of

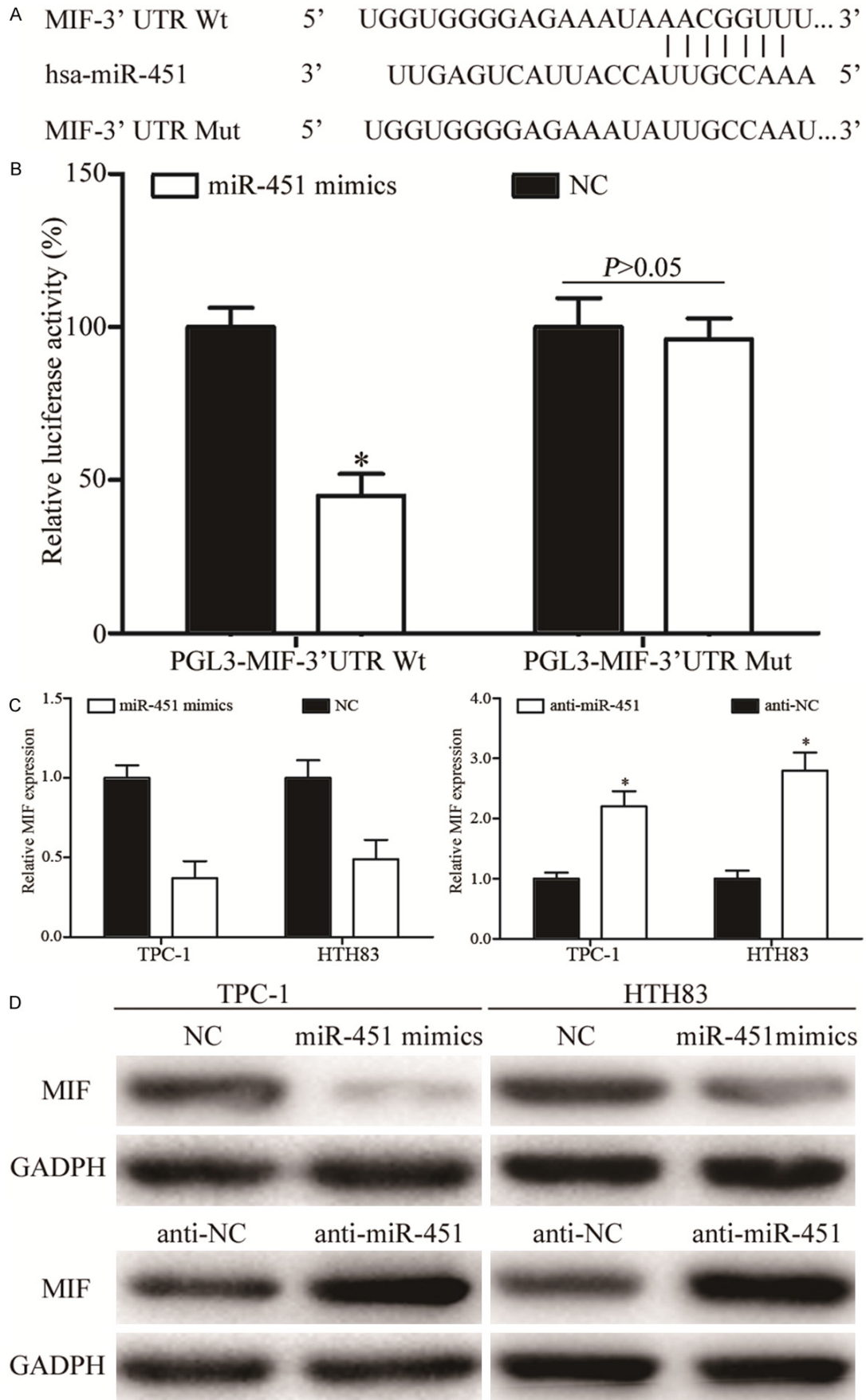


Figure 5. miR-451 directly targeted MIF in vitro. A. The miR-451 binding site in the 3'UTR of MIF and the MIF 3'-UTR mutant sequence. B. miR-451 significantly decreased the luciferase activity that carried MIF-3'UTR Wt but not MIF-3'UTR Mut. C. MIF was significantly down-regulated at mRNA level in TPC-1 and HTH83 cells after transfection with miR-451 mimics. Meanwhile, anti-miR-451 improved MIF mRNA expression level in TPC-1 and HTH83 cells. D. miR-451 mimics inhibited MIF protein expression and anti-miR-451 enhanced MIF expression. *P<0.05 compared with their respective controls.

miR-451 was over-expressed by miR-451 mimics. Meanwhile, miR-451 was down-regulated in TPC-1 and HTH83 cells after transfection with anti-miR-451 (shown in **Figure 2B**).

miR-451 inhibited TPC-1 and HTH83 cells proliferation

The effect of miR-451 on PTC cells proliferation was evaluated by MTT assay. As shown in **Figure 3**, the absorbance at 490 nm was decreased in miR-451 mimics-transfected TPC-1 and HTH83 cells compared with NC group. The absorbance was higher in TPC-1 and HTH83 cells transfected with anti-miR-451 than the NC inhibitor group. Hence, miR-451 functioned as a tumor growth suppressor in PTC.

miR-451 inhibited TPC-1 and HTH83 cells migration and invasion

Transwell chambers were adopted to explore the effect of altering miR-451 expression in PTC cells migration and invasion abilities. We found that miR-451 mimics inhibited TPC-1 and HTH83 cell migration and invasion abilities (shown in **Figure 4A**). Moreover, down-regulation of miR-451 resulted into a significant promotion of cell migration and invasion abilities in TPC-1 and HTH83 cells (shown in **Figure 4B**). Thus, miR-451 inhibited PTC cells migration and invasion.

miR-451 directly targeted MIF in vitro

miRanda (<http://www.microrna.org>), TargetScan (<http://www.targetscan.org/>) and miRDB (mirdb.org/miRDB/) were used to predict direct targets of miR-451. In those targets, MIF got our attention. As shown in **Figure 5A**, MIF contained a miR-451 seed match at position 102-108 of the MIF 3'UTR.

To determine whether miR-451 interacted directly with MIF, Dual-Luciferase report assays were performed in HEK293T cell lines. As shown in **Figure 5B**, co-transfection of miR-451 mimics and the PGL3-MIF-3'UTR Wt led to a

significant down-regulation of luciferase activity. However, co-transfection of miR-451 mimics and PGL3-MIF-3'UTR Mut did not cause a decrease in luciferase activity.

To explore whether miR-451 targeted MIF, we measured MIF expression at mRNA and protein level in TPC-1 and HTH83 cells following transfection with miR-451 mimics and anti-miR-451. qRT-PCR results showed that MIF was significantly down-regulated at mRNA level in TPC-1 and HTH83 cells after transfection with miR-451 mimics. Meanwhile, anti-miR-451 improved MIF mRNA expression level in PTC cells (shown in **Figure 5C**). Western blot showed that, compared with control group, miR-451 mimics inhibited MIF protein expression and anti-miR-451 enhanced MIF expression (shown in **Figure 5D**). Taken together, these results indicated that miR-451 directly targeted MIF.

MIF was involved in miR-451-mediated tumor suppression functions in TPC-1 and HTH83 cells

MIF siRNA was used to knockdown MIF expression and measured its functions in PTC cells proliferation, migration and invasion. After transfection with MIF siRNA or NC siRNA, western blot was performed to measure MIF protein expression. As shown in **Figure 6A**, MIF was significantly down-regulated in MIF siRNA-transfected TPC-1 and HTH83 cells.

MTT assay revealed that the growth rate of MIF siRNA-transfected TPC-1 and HTH83 cells was obviously decreased compared with that of controls (shown in **Figure 6B**). Furthermore, migration and invasion assays showed that MIF siRNA group markedly inhibited cell migration and invasion abilities of TPC-1 and HTH83 cells compared to cells transfected with NC siRNA (shown in **Figure 6C**). These results demonstrated that MIF siRNA could mimic the functions of miR-451 in TPC-1 and HTH83 cells, rendering MIF as a functional target of miR-451 in PTC.

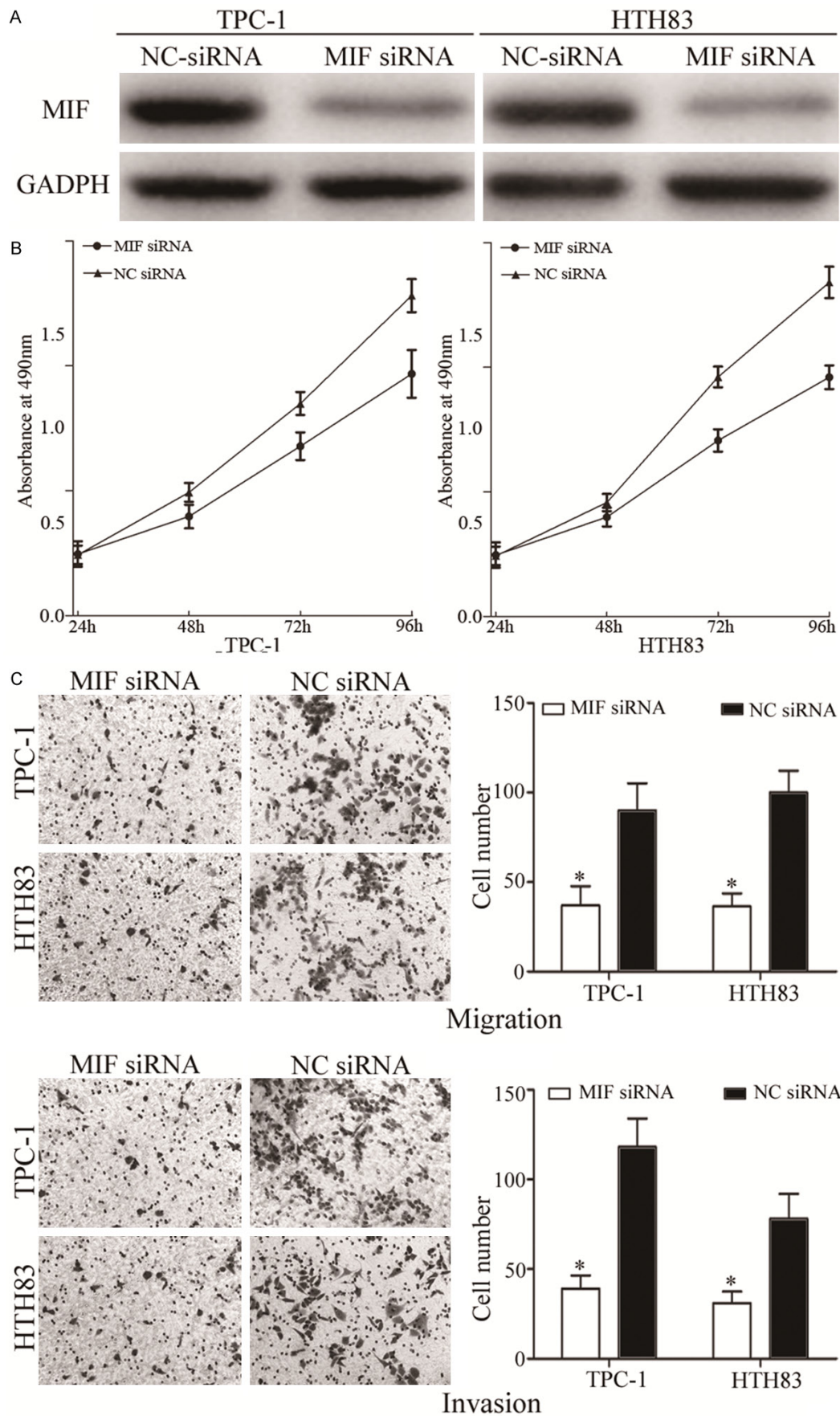


Figure 6. Effects of MIF in TPC-1 and HTH83 cell proliferation, migration and invasion. A. MIF was significantly down-regulated in MIF siRNA-transfected TPC-1 and HTH83 cells. B. MTT assays showed that growth rate of MIF siRNA-transfected TPC-1 and HTH83 cells was obviously decreased compared with that of controls. C. Cell migration and invasion assays revealed that MIF siRNA group markedly inhibited cell migration and invasion abilities of TPC-1 and HTH83 cells compared to cells transfected with NC siRNA. * $P < 0.05$ compared with their respective controls.

Discussion

In recent years, abnormal expression level of miRNAs were found in various kinds of cancers, also including thyroid cancer, suggesting the important roles of miRNAs in cancer initial and progression [21, 22]. Hence, miRNAs could be investigated as a new therapeutic treatment targets and as a powerful intervention means in cancers. In the human genome, miR-451 is located on chromosome 17 at 17q11.2 [23]. It was reported that miR-451 was down-regulated in many kind of human cancers, such as bladder cancer [24], hepatocellular carcinoma [25], lung cancer [26], osteosarcoma [27], nasopharyngeal carcinoma [28], esophageal carcinoma [29], glioma [30] and colorectal carcinoma [31]. However, up to date, miR-451 has not been studied in PTC. In this study, we found that miR-451 was down-regulated in PTC tissues and cell lines. It suggested that miR-451 may act as a tumor suppressor in PTC.

miR-451 has been demonstrated as a tumor suppressor. For example, in bladder cancer, decreased expression level of miR-451 was obviously correlated with histological differentiation degree and TNM stage. Ectopic expression of miR-451 suppressed bladder cancer cell growth, migration, invasion and enhanced apoptosis through regulating EMT [24]. In hepatocellular carcinoma, reduced miR-451 expression was associated with advanced clinical stage, metastasis and worse disease-free or overall survival. In functional studies, miR-451 decreased hepatocellular carcinoma cell proliferation, migration and invasion in vitro, metastasis in vivo, induced G0/G1 arrest and promoted apoptosis through negative regulation of c-Myc, ATF2, caspase-3, MMP-9 and IKK- β [25, 32-34]. In osteosarcoma, Zhang et al. reported that low expression of miR-451 was correlated with metastasis and recurrence. Yuan and his colleagues found that miR-451 down-regulation more frequently occurred in osteosarcoma tissues with advanced clinical stage, positive distant metastasis and poor response to neoadjuvant chemotherapy. In addition, enforced miR-451 expression in osteosarcoma inhibited cell growth, migration, invasion, tumorigenesis

and induced apoptosis, G1 cell cycle arrest via targeting PGE2, CCND1, CXCL16 and LRH-1 [27, 35-37]. These findings suggested that miR-451 played important functions in these cancers, and may function as a potential therapeutic target for these cancers.

In our study, we revealed that miR-451 significantly inhibited PTC cells proliferation, migration and invasion. Since miR-451 contributed to PTC carcinogenesis and progression, we next set out to explore the molecular mechanisms contributed to miR-451 functions in PTC. In this study, MIF was identified as a direct target of miR-451. Firstly, bioinformatics analysis indicated that MIF contained a miR-451 seed match at position 102-108 of the MIF 3'UTR. Secondly, Dual-Luciferase report assay showed that miR-451 directly targeted MIF 3'UTR. Thirdly, MIF was down-regulated at mRNA and protein level after transfection with miR-451 mimics, whereas MIF was up-regulated after transfection with anti-miR-451. Finally, functions of MIF siRNA in proliferation, migration and invasion was similar to miR-451 in PTC cells, rendering MIF was a direct target of miR-451 in vitro.

MIF, a proinflammatory cytokine first described and named in the 1950s, is located on chromosome 22 [38]. The MIF protein is relatively small (12.5 kDa), lacking a conventional N-terminal leader sequence and is therefore released from the cell by a leaderless secretion pathway [39]. An expanding body of evidences found that MIF is expressed in many kinds of cells, such as macrophage cells [40], lymphocytes [41], neutrophils [42], eosinophils [43], and so on. In addition, accumulated recent studies have demonstrated the essential functions of MIF in cancers [44]. Up-regulation of MIF has been found in various kinds of human cancer, including breast cancer [45], lung cancer [46] and gastric cancer [47]. Moreover, some work groups reported that MIF expression was correlated with prognosis in cancers. For example, in head and neck cancer, high MIF expression level was correlated with higher lymph node metastasis and reduced survival [48]. In primary nasopharyngeal carcinoma,

high expression level of MIF was significantly associated with increased microvessels, lymph node metastasis, overall survival, disease-specific survival and locoregional failure of nasopharyngeal carcinoma patients. MIF was also found up-regulated in thyroid cancer. Knock-down of MIF suppressed thyroid cancer cell proliferation, colony formation ability, migration, invasion, and vascular endothelial growth factor secretion [49]. Therefore, it is worthwhile to investigate novel targeted therapy against MIF in thyroid cancer. In this study, we revealed that miR-451 negatively regulated MIF to inhibit PTC cell growth and metastasis. It could be investigated as a targeted therapy for PTC.

In conclusion, miR-451 was down-regulated in PTC tissues and cell lines. In addition, enforced miR-451 expression significantly inhibited PTC cell growth, migration and invasion. Moreover, MIF was identified as a direct target of miR-451 in PTC. These findings suggested that miR-451 targeted MIF to inhibit PTC growth and metastasis. It could be investigated as targeted therapies for PTC. Future work is needed to address whether the potential of miR-451 may be fully realized in PTC therapeutic treatments.

Disclosure of conflict of interest

None.

Address correspondence to: Gang Ding, Department of General Surgery, Yidu Central Hospital of Weifang, Weifang Medical University, No. 4138, Linglong Shan Road, Qingzhou 262500, Shandong, China. E-mail: dinggang138@126.com

References

- [1] Erler P, Keutgen XM, Crowley MJ, Zetoune T, Kundel A, Kleiman D, Beninato T, Scognamiglio T, Elemento O, Zarnegar R and Fahey TJ 3rd. Dicer expression and microRNA dysregulation associate with aggressive features in thyroid cancer. *Surgery* 2014; 156: 1342-50; discussion 1350.
- [2] Vriens MR, Weng J, Suh I, Huynh N, Guerrero MA, Shen WT, Duh QY, Clark OH and Kebebew E. MicroRNA expression profiling is a potential diagnostic tool for thyroid cancer. *Cancer* 2012; 118: 3426-3432.
- [3] Siegel RL, Miller KD and Jemal A. Cancer statistics, 2015. *CA Cancer J Clin* 2015; 65: 5-29.
- [4] Hartmann C, Mueller W and von Deimling A. Pathology and molecular genetics of oligodendroglial tumors. *J Mol Med (Berl)* 2004; 82: 638-655.
- [5] Geraldo MV, Fuziwara CS, Friguglietti CU, Costa RB, Kulcsar MA, Yamashita AS and Kimura ET. MicroRNAs miR-146-5p and let-7f as prognostic tools for aggressive papillary thyroid carcinoma: a case report. *Arq Bras Endocrinol Metabol* 2012; 56: 552-557.
- [6] Pacifico F and Leonardi A. Role of NF-kappaB in thyroid cancer. *Mol Cell Endocrinol* 2010; 321: 29-35.
- [7] Silver CE, Owen RP, Rodrigo JP, Rinaldo A, Devaney KO and Ferlito A. Aggressive variants of papillary thyroid carcinoma. *Head Neck* 2011; 33: 1052-1059.
- [8] Vasko VV and Saji M. Molecular mechanisms involved in differentiated thyroid cancer invasion and metastasis. *Curr Opin Oncol* 2007; 19: 11-17.
- [9] Yang Q, Ji M, Guan H, Shi B and Hou P. Shikonin inhibits thyroid cancer cell growth and invasiveness through targeting major signaling pathways. *J Clin Endocrinol Metab* 2013; 98: E1909-1917.
- [10] Hunt JP, Buchmann LO, Wang L and Abraham D. An analysis of factors predicting lateral cervical nodal metastases in papillary carcinoma of the thyroid. *Arch Otolaryngol Head Neck Surg* 2011; 137: 1141-1145.
- [11] Gu L and Sun W. MiR-539 inhibits thyroid cancer cell migration and invasion by directly targeting CARMA1. *Biochem Biophys Res Commun* 2015; 464: 1128-1133.
- [12] Bartel DP. MicroRNAs: target recognition and regulatory functions. *Cell* 2009; 136: 215-233.
- [13] Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell* 2004; 116: 281-297.
- [14] Hwang HW and Mendell JT. MicroRNAs in cell proliferation, cell death, and tumorigenesis. *Br J Cancer* 2007; 96 Suppl: R40-44.
- [15] Ryan BM, Robles AI and Harris CC. Genetic variation in microRNA networks: the implications for cancer research. *Nat Rev Cancer* 2010; 10: 389-402.
- [16] Pallante P, Visone R, Ferracin M, Ferraro A, Berlingieri MT, Troncone G, Chiappetta G, Liu CG, Santoro M, Negrini M, Croce CM and Fusco A. MicroRNA deregulation in human thyroid papillary carcinomas. *Endocr Relat Cancer* 2006; 13: 497-508.
- [17] Agretti P, Ferrarini E, Rago T, Candelieri A, De Marco G, Dimida A, Niccolai F, Molinaro A, Di Coscio G, Pinchera A, Vitti P and Tonacchera M. MicroRNA expression profile helps to distinguish benign nodules from papillary thyroid carcinomas starting from cells of fine-needle aspiration. *Eur J Endocrinol* 2012; 167: 393-400.
- [18] Mutallip M, Nohata N, Hanazawa T, Kikkawa N, Horiguchi S, Fujimura L, Kawakami K, Chiyo-

- maru T, Enokida H, Nakagawa M, Okamoto Y and Seki N. Glutathione S-transferase P1 (GSTP1) suppresses cell apoptosis and its regulation by miR-133alpha in head and neck squamous cell carcinoma (HNSCC). *Int J Mol Med* 2011; 27: 345-352.
- [19] Fang Y, Xue JL, Shen Q, Chen J and Tian L. MicroRNA-7 inhibits tumor growth and metastasis by targeting the phosphoinositide 3-kinase/Akt pathway in hepatocellular carcinoma. *Hepatology* 2012; 55: 1852-1862.
- [20] Song YX, Yue ZY, Wang ZN, Xu YY, Luo Y, Xu HM, Zhang X, Jiang L, Xing CZ and Zhang Y. MicroRNA-148b is frequently down-regulated in gastric cancer and acts as a tumor suppressor by inhibiting cell proliferation. *Mol Cancer* 2011; 10: 1.
- [21] Gu Y, Li D, Luo Q, Wei C, Song H, Hua K, Song J, Luo Y, Li X and Fang L. MicroRNA-145 inhibits human papillary cancer TPC1 cell proliferation by targeting DUSP6. *Int J Clin Exp Med* 2015; 8: 8590-8598.
- [22] Xiong Y, Kotian S, Zeiger MA, Zhang L and Kebebew E. miR-126-3p Inhibits Thyroid Cancer Cell Growth and Metastasis, and Is Associated with Aggressive Thyroid Cancer. *PLoS One* 2015; 10: e0130496.
- [23] Pan X, Wang R and Wang ZX. The potential role of miR-451 in cancer diagnosis, prognosis, and therapy. *Mol Cancer Ther* 2013; 12: 1153-1162.
- [24] Zeng T, Peng L, Chao C, Fu B, Wang G, Wang Y and Zhu X. miR-451 inhibits invasion and proliferation of bladder cancer by regulating EMT. *Int J Clin Exp Pathol* 2014; 7: 7653-7662.
- [25] Huang JY, Zhang K, Chen DQ, Chen J, Feng B, Song H, Chen Y, Zhu Z, Lu L, De W, Wang R and Chen LB. MicroRNA-451: epithelial-mesenchymal transition inhibitor and prognostic biomarker of hepatocellular carcinoma. *Oncotarget* 2015; 6: 18613-18630.
- [26] Yin P, Peng R, Peng H, Yao L, Sun Y, Wen L, Wu T, Zhou J and Zhang Z. MiR-451 suppresses cell proliferation and metastasis in A549 lung cancer cells. *Mol Biotechnol* 2015; 57: 1-11.
- [27] Zhang F, Huang W, Sheng M and Liu T. MiR-451 inhibits cell growth and invasion by targeting CXCL16 and is associated with prognosis of osteosarcoma patients. *Tumour Biol* 2015; 36: 2041-2048.
- [28] Liu N, Jiang N, Guo R, Jiang W, He QM, Xu YF, Li YQ, Tang LL, Mao YP, Sun Y and Ma J. MiR-451 inhibits cell growth and invasion by targeting MIF and is associated with survival in nasopharyngeal carcinoma. *Mol Cancer* 2013; 12: 123.
- [29] Wang T, Zang WQ, Li M, Wang N, Zheng YL and Zhao GQ. Effect of miR-451 on the biological behavior of the esophageal carcinoma cell line EC9706. *Dig Dis Sci* 2013; 58: 706-714.
- [30] Nan Y, Han L, Zhang A, Wang G, Jia Z, Yang Y, Yue X, Pu P, Zhong Y and Kang C. MiRNA-451 plays a role as tumor suppressor in human glioma cells. *Brain Res* 2010; 1359: 14-21.
- [31] Li HY, Zhang Y, Cai JH and Bian HL. MicroRNA-451 inhibits growth of human colorectal carcinoma cells via downregulation of Pi3k/Akt pathway. *Asian Pac J Cancer Prev* 2013; 14: 3631-3634.
- [32] Lv G, Hu Z, Tie Y, Du J, Fu H, Gao X and Zheng X. MicroRNA-451 regulates activating transcription factor 2 expression and inhibits liver cancer cell migration. *Oncol Rep* 2014; 32: 1021-1028.
- [33] Liu X, Zhang X, Xiang J, Lv Y and Shi J. miR-451: potential role as tumor suppressor of human hepatoma cell growth and invasion. *Int J Oncol* 2014; 45: 739-745.
- [34] Li HP, Zeng XC, Zhang B, Long JT, Zhou B, Tan GS, Zeng WX, Chen W and Yang JY. miR-451 inhibits cell proliferation in human hepatocellular carcinoma through direct suppression of IKK-beta. *Carcinogenesis* 2013; 34: 2443-2451.
- [35] Xu H, Mei Q, Shi L, Lu J, Zhao J and Fu Q. Tumor-suppressing effects of miR451 in human osteosarcoma. *Cell Biochem Biophys* 2014; 69: 163-168.
- [36] Yuan J, Lang J, Liu C, Zhou K, Chen L and Liu Y. The expression and function of miRNA-451 in osteosarcoma. *Med Oncol* 2015; 32: 324.
- [37] Li Z, Wu S, Lv S, Wang H, Wang Y and Guo Q. Suppression of liver receptor homolog-1 by microRNA-451 represses the proliferation of osteosarcoma cells. *Biochem Biophys Res Commun* 2015; 461: 450-455.
- [38] Calandra T and Roger T. Macrophage migration inhibitory factor: a regulator of innate immunity. *Nat Rev Immunol* 2003; 3: 791-800.
- [39] Bernhagen J, Mitchell RA, Calandra T, Voelter W, Cerami A and Bucala R. Purification, bioactivity, and secondary structure analysis of mouse and human macrophage migration inhibitory factor (MIF). *Biochemistry* 1994; 33: 14144-14155.
- [40] Calandra T, Bernhagen J, Mitchell RA and Bucala R. The macrophage is an important and previously unrecognized source of macrophage migration inhibitory factor. *J Exp Med* 1994; 179: 1895-1902.
- [41] Bacher M, Metz CN, Calandra T, Mayer K, Chesney J, Lohoff M, Gems D, Donnelly T and Bucala R. An essential regulatory role for macrophage migration inhibitory factor in T-cell activation. *Proc Natl Acad Sci U S A* 1996; 93: 7849-7854.

- [42] Daryadel A, Grifone RF, Simon HU and Yousefi S. Apoptotic neutrophils release macrophage migration inhibitory factor upon stimulation with tumor necrosis factor-alpha. *J Biol Chem* 2006; 281: 27653-27661.
- [43] Rossi AG, Haslett C, Hirani N, Greening AP, Rahman I, Metz CN, Bucala R and Donnelly SC. Human circulating eosinophils secrete macrophage migration inhibitory factor (MIF). Potential role in asthma. *J Clin Invest* 1998; 101: 2869-2874.
- [44] Conroy H, Mawhinney L and Donnelly SC. Inflammation and cancer: macrophage migration inhibitory factor (MIF)–the potential missing link. *QJM* 2010; 103: 831-836.
- [45] Bando H, Matsumoto G, Bando M, Muta M, Ogawa T, Funata N, Nishihira J, Koike M and Toi M. Expression of macrophage migration inhibitory factor in human breast cancer: association with nodal spread. *Jpn J Cancer Res* 2002; 93: 389-396.
- [46] Tomiyasu M, Yoshino I, Suemitsu R, Okamoto T and Sugimachi K. Quantification of macrophage migration inhibitory factor mRNA expression in non-small cell lung cancer tissues and its clinical significance. *Clin Cancer Res* 2002; 8: 3755-3760.
- [47] He XX, Yang J, Ding YW, Liu W, Shen QY and Xia HH. Increased epithelial and serum expression of macrophage migration inhibitory factor (MIF) in gastric cancer: potential role of MIF in gastric carcinogenesis. *Gut* 2006; 55: 797-802.
- [48] Dumitru CA, Gholaman H, Trellakis S, Bruderek K, Dominas N, Gu X, Bankfalvi A, Whiteside TL, Lang S and Brandau S. Tumor-derived macrophage migration inhibitory factor modulates the biology of head and neck cancer cells via neutrophil activation. *Int J Cancer* 2011; 129: 859-869.
- [49] Cheng SP, Liu CL, Chen MJ, Chien MN, Leung CH, Lin CH, Hsu YC and Lee JJ. CD74 expression and its therapeutic potential in thyroid carcinoma. *Endocr Relat Cancer* 2015; 22: 179-190.