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MiR-202-3p functions as a tumor suppressor and reduces cell migration and invasion in papillary thyroid carcinoma

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Abstract. – OBJECTIVE: MicroRNAs (miRNAs) are recently identified as key regulators of tumor development and progression. MiR-202-3p functions as tumor suppressor in some cancer types. The aim of the study is to determine its expression pattern and explore the functions underlying the mechanism of miR-202-3p in papillary thyroid carcinoma (PTC).

PATIENTS AND METHODS: By using quantitative RT-PCR (QRT-PCR) analyses, we detected miR-202-3p expression in PTC tissues and cell lines. Transwell migration and invasion assays were performed to measure the migration and invasion ability of tumor cells transfected with miR-202-3p mimic. Western blot analysis was used to detect the protein expression.

RESULTS: Our results showed that miR-202-3p expression was frequently downregulated in 96 cases PTC tissues compared to adjacent normal tissues. Lower expression of miR-202-3p associated with lymph node metastasis of patients with PTC. Overexpression of miR-202-3p inhibited cell migration and invasion in TPC-1 and BCPAP cells. Furthermore, enforced expression of miR-202-3p inhibited WNT signaling by downregulating β-catenin expression in TPC-1 and BCPAP cells.

CONCLUSIONS: Our findings indicated that miR-202-3p may represent a novel therapeutic target of in PTC.

Key Words

MicroRNAs, Papillary thyroid carcinoma, miR-202-3p, Cell migration, Cell invasion.

Introduction

Thyroid cancer is the most common malignancy of the endocrine system[1]. Papillary thyroid carcinoma (PTC) is known to have a favorable prognosis with a cancer-related mortality rate <10%[2]. Surgical removal is an effective therapeutic strategy, and the overall five-year survival rate for patients who are diagnosed at early stage thyroid cancer is over 95%. However, patients with advanced thyroid cancer only have a five-year survival rate of about 60%[3,4]. Thus, it is urgent to explore potential therapeutic targets for thyroid cancer. MicroRNAs (miRNAs) are identified as small non-coding RNAs that exert post-transcriptional regulation of gene expression via targeting mRNA to induce mRNA degradation[5]. MiRNAs are involved in participating in tumorigenesis of thyroid cancer. Such as, microRNA-139 targets fibronectin 1 to inhibit papillary thyroid carcinoma progression[6]. MicroRNA-335 is downregulated in papillary thyroid cancer and suppresses cancer cell growth, migration and invasion by directly targeting ZEB2[7]. MiR-577 inhibits papillary thyroid carcinoma cell proliferation, migration and invasion by targeting SphK2[8]. MicroRNA-202-3p has been reported to act as tumor suppressor in some cancer[9]; however, the role of miR-202-3p in PTC is little known. In the study, our results showed that miR-202-3p expression was frequently downregulated in PTC tissues compared to adjacent normal tissues. Overexpression of miR-202-3p reduced cell migration and invasion ability in PTC. Furthermore, enforced expression of miR-202-3p inhibited WNT signaling in PTC. Thus, these results indicated that miR-202-3p may represent a novel therapeutic target of thyroid cancer.

Patients and Methods

Tissue Sample Collection

A total 96 pairs of PTC and adjacent normal tissues were obtained from patients undergoing surgery at the Department of General Surgery,

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the Haian Hospital Affiliated to Nantong University between February 2014 and March 2017. All samples were immediately snap-frozen in liquid nitrogen and then stored at -80°C until later RNA analysis. Tissue samples consisted of PTC tissues and adjacent normal tissues, which were all confirmed by histopathological examination. The collected clinicopathological characteristics included age, sex, lymph node metastasis, TNM stage and so on. None of the patients had received preoperative local or systemic treatment. The present study was approved by the Ethics Committee of the Haian Hospital Affiliated to Nantong University and written informed consent was obtained from all study participants.

Total RNA Extraction and Quantitative Real Time Polymerase Chain Reaction (qRT-PCR)

Total RNA was isolated from the tissue specimens or cells using TRIzol (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. The complementary DNA (cDNA) was reversed transcripted from RNA sample using a TaqMan MicroRNA Reverse Transcription Kit (Applied Biosystems, Carlsbad, CA, USA). The reaction was performed with TaqMan MicroRNA PCR Kit (Applied Biosystems, Foster City, CA, USA) with SYBR Premix Ex Taq™ (TaKaRa Biotechnology Co., Ltd., Dalian, China) on an ABI Prism 7500 Sequence Detection System (Applied Biosystems, Foster City, CA, USA). U6 was used to normalize the expression of miR-202-3p. The data were analyzed using the $2^{-\Delta\Delta Ct}$ methods.

Cell Lines and Cell Culture

The four human PTC cell lines (TPC-1, SW1736, BCPAP and K1) and a human thyroid epithelial cell line Nthy-ori3-1 were purchased from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China). Cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM, Gibco, Grand Island, NY, USA) and supplemented with 10% fetal bovine serum (FBS) (Gibco, Grand Island, NY, USA). All of cell lines were incubated at 37°C in humidified atmosphere consisting of 5% CO_2.

Cell Migration and Invasion Assays

Cell migration and invasion assays were performed using transwell chambers (Corning Incorporated, Corning, NY, USA) precoated without or with Matrigel (BD Biosciences, Franklin Lakes, NJ, USA). The lower chamber was added containing Dulbecco's Modified Eagle Medium (DMEM) with 10% fetal bovine serum (FBS) (Gibco, Grand Island, NY, USA). For the upper chambers, 5×10^4 transfected cells resuspended in 200 µl Dulbecco's Modified Eagle Medium (DMEM) without fetal bovine serum (FBS) were plated in the top chamber. After cell transfection at 48 h, cells on the upper chamber were removed by a cotton swab, while cells in the lower surface of the membrane were fixed with 100% methanol at room temperature for 20 min and stained with 0.1% crystal violet at room temperature. Then, cells were counted at a magnification of ×200 by inverted microscopy (LI-COR Biosciences, Lincoln, NE, USA) in five random fields.

Western Blot Analysis

Proteins were extracted from PTC cells using a Total Protein Extraction kit (Nanjing KeyGen Biotech Co., Ltd., Nanjing, China). Protein extracts were quantified by a BCA protein assay (Beyotime Institute of Biotechnology, Haimen, China). A total 20 µg protein sample was separated on 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and then electrophoretically transferred to polyvinylidene difluoride membranes (EMD Millipore, Billerica, MA, USA). The membranes were blocked with 5% non-fat milk for 2 h at room temperature, and then incubated with primary antibodies against β-catenin (1:1000, Protein Tech Group, Inc., Chicago, IL, USA) and GAPDH (1:1000, Protein Tech Group, Inc., Chicago, IL, USA) overnight at 4°C. After that, the membranes were incubated with a horseradish peroxidase(HRP)-conjugated goat anti-rabbit secondary antibody (1:1000, Protein Tech Group, Inc., Chicago, IL, USA) for 1 h; the protein blots were visualized by chemiluminescence (Super Signal@ West Pico Chemiluminescent Substrate, Thermo Fisher Scientific, Inc., Waltham, MA, USA). The GAPDH expression was used as an internal control.

Statistical Analysis

Statistical analyses were evaluated using SPSS 18.0 (SPSS Inc., Chicago, IL, USA). All data are presented as the mean ± standard deviation from three independent experiments. Differences were assessed by two-tailed Student's *t*-test or one-way analysis of variance (ANOVA) followed by Student-Newman-Keuls post-hoc test. A $p<0.05$ was considered to indicate a statistically significant difference.

Results

The Expression of miR-202-3p in PTC Tissues and Cell Lines

In order to investigate miR-202-3p expression in PTC tissues and cells, we performed qRT-PCR analyses. As shown in Figure 1A, our results indicated that miR-202-3p expression was significantly downregulated in 96 cases of PTC tissues compared to adjacent normal tissues ($p<0.05$). Furthermore, we revealed that the expression of miR-202-3p in PTC cells was also markedly increased in TPC-1, SW1736, BCPAP and K1 cells compared to human thyroid epithelial cell line Nthy-ori3-1 (Figure 1B, $p<0.05$).

MiR-202-3p Expression Significantly Associates With Lymph Node Metastasis of PTC

The expression of miR-202-3p in papillary thyroid carcinoma tissues was classified into two groups according to the median expression. The correlations of miR-202-3p with patients' age, sex, tumor size, TNM stage or other clinical features, were analyzed by Chi-square test. The relative expression of miR-202-3p in PTC patients showed a significant correlation with lymph node metastasis in patients with PTC ($p<0.05$). However, the relative expression did not correlate with age, sex, tumor size, clinical stage or other clinical features ($p>0.05$).

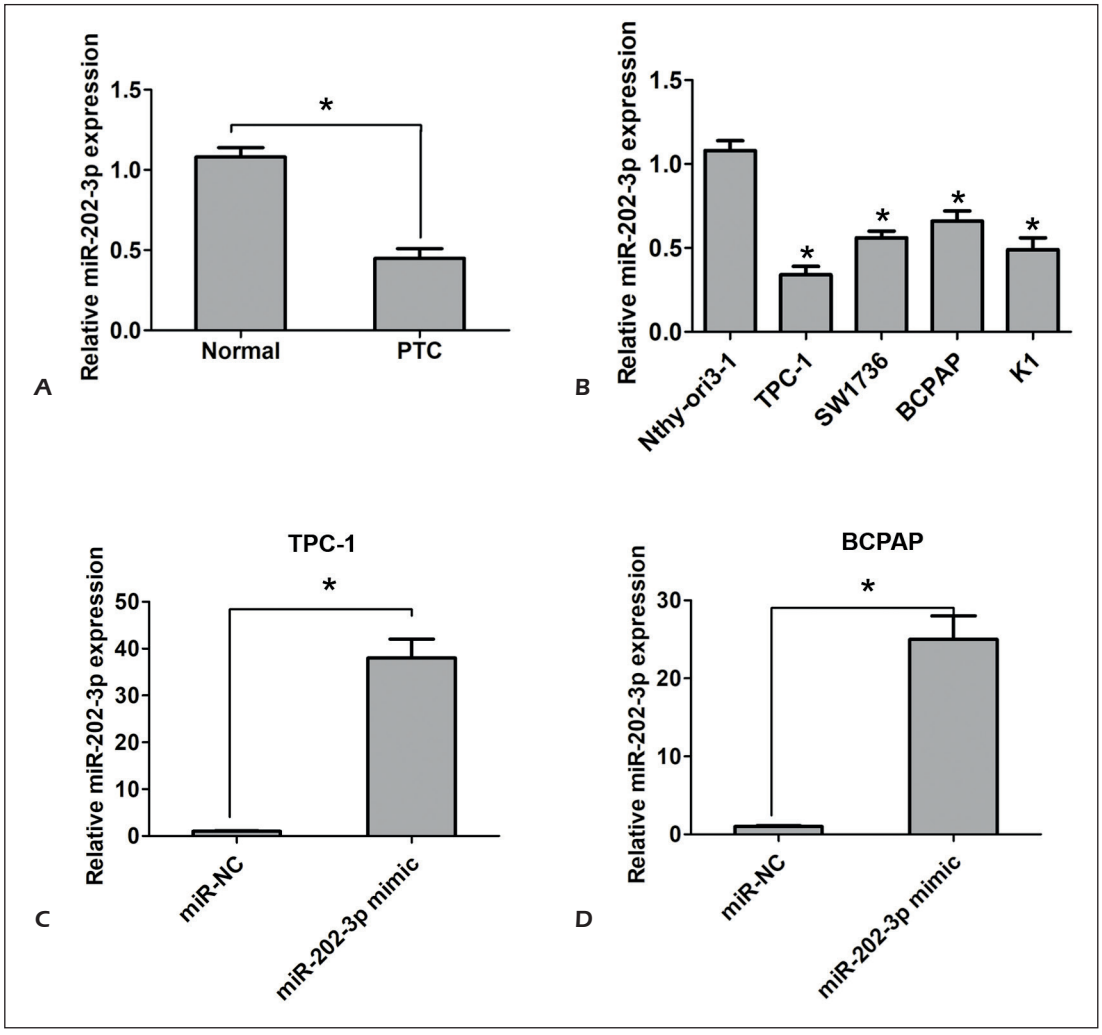

Figure 1. Downregulation of miR-202-3p in PTC tissues and cells. **A**, Measurement of miR-202-3p expression in 96 PTC tissues and adjacent normal tissues. **B**, Measurement of miR-202-3p expression in PTC cell lines (TPC-1, SW1736, BCPAP, K1) and non-malignant human thyroid epithelial cell line Nthy-ori3-1. **C**, Measurement of miR-202-3p expression after TPC-1 or BCPAP cells were transfected with miR-202-3p mimic or miR-NC. *$p<0.05$.

Upregulation of miR-202-3p Reduces Cell Migration and Invasion in PTC

We further up-regulated miR-202-3p expression in TPC-1 and BCPAP cells by miR-202-3p mimic transfection to detect the functional effects of miR-202-3p on cell migration and invasion in PTC. QRT-PCR analysis results demonstrated that miR-202-3p mimic evidently increased the expression of miR-202-3p in the TPC-1 and BCPAP cells (Figure 1C-1D, $p<0.05$). We then performed transwell migration assay to examine cell migration capacity. Our findings revealed that miR-202-3p up-regulation obviously suppressed the cell migration ability of TPC-1 and BCPAP cells compared to miR-NC group (Figure 2A-2B, $p<0.05$). The transwell invasion assays were used to examine the invasion capacities of TPC-1 and BCPAP cells after transfection with the miR-202-3p mimic or miR-NC. According to the results, miR-202-3p up-regulation significantly suppressed the cell invasion ability of TPC-1 and BCPAP cells compared to miR-NC group (Figure 3A-3B, $p<0.05$). These results suggest that miR-202-3p served tumor-suppressive functions in PTC cell migration and invasion ability.

Upregulation of miR-202-3p Inhibits WNT Signaling Pathway in PTC

WNT signaling has been reported to be involved in thyroid cancer migration and invasion; for instance, miR-146b-5p induces epithelial-mesenchymal transition process and promotes PTC metastasis through the regulation of Wnt/β-catenin signaling [10]. In our data, we found that miR-202-3p overexpression suppressed the expression of β-catenin compared to the control groups in TPC-1 and BCPAP cells (Figure 4A-4B). Thus, our results indicated enforced expression of miR-202-3p inhibited WNT signaling by downregulating β-catenin expression in PTC.

Discussion

Abnormal expression of miR-202-3p has been observed in different types of cancer and significantly correlated with tumor progression or invasion [11]. Decrease of miR-202-3p expression was found in gastric cancer and markedly suppressed cell proliferation and induced cell apoptosis by inhibiting the expression of γ-catenin and BCL-2[12]. MiR-202-3p was significantly downregulat-

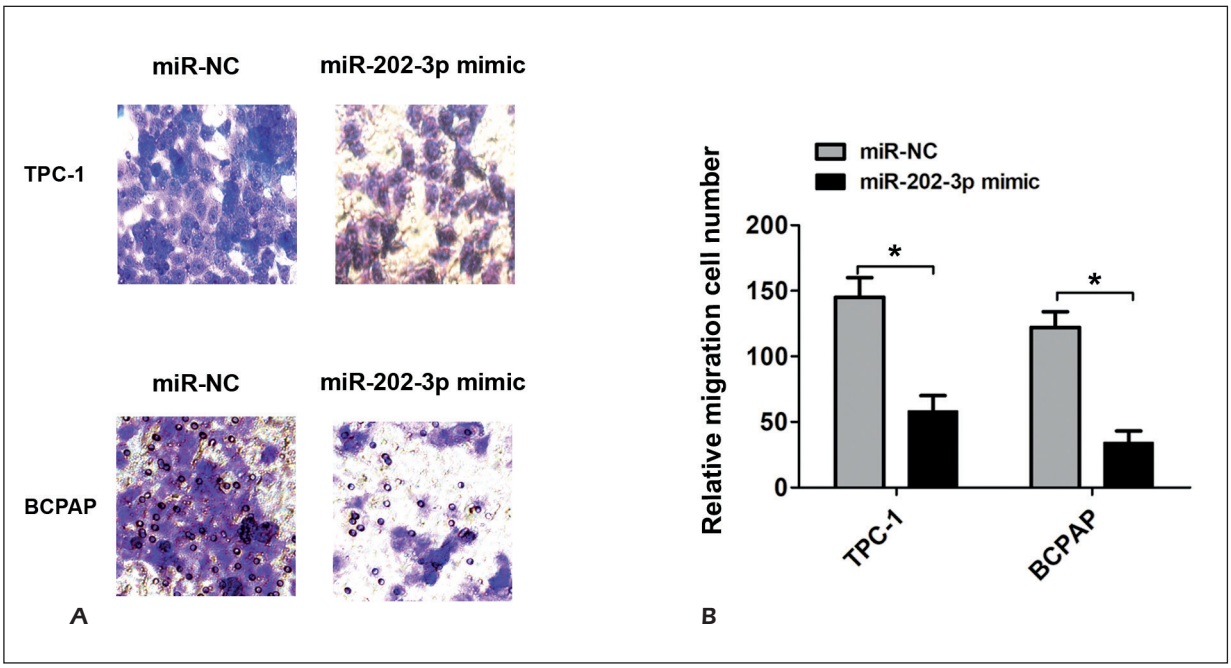

Figure 2. MiR-202-3p mimic inhibits cell migration in PTC. **A**, Transwell migration assays were performed to determine the migration ability in TPC-1 or BCPAP cells. **B**, Transwell migration cell number was shown after TPC-1 or BCPAP cells were transfected with miR-202-3p mimic or miR-NC. *$p<0.05$.

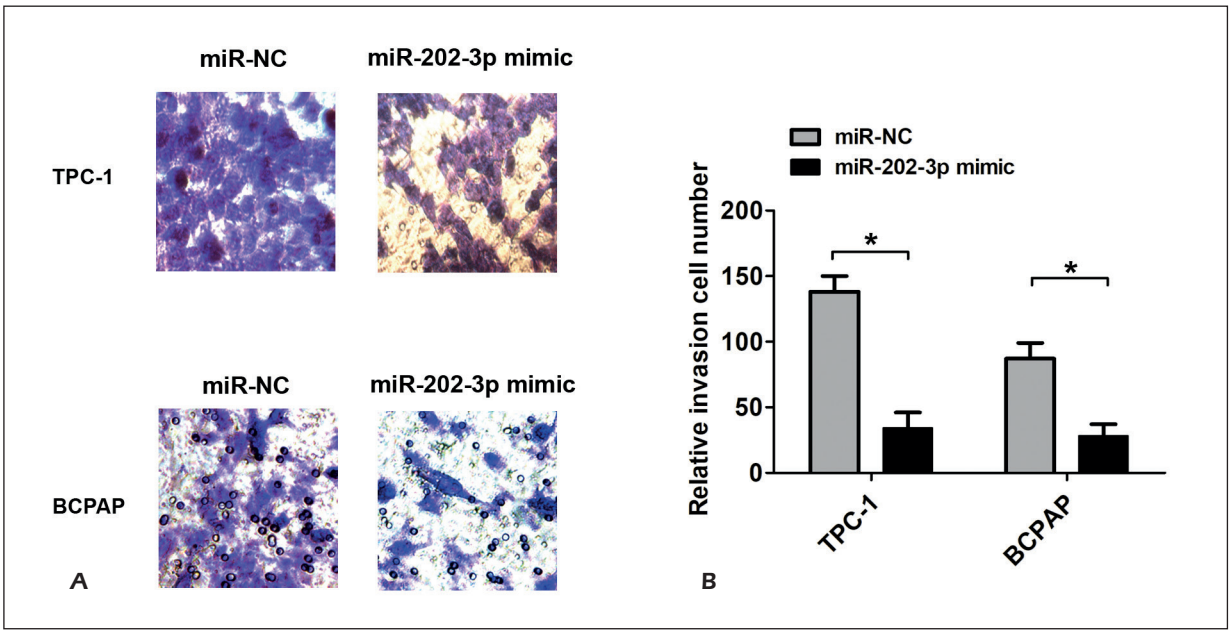

Figure 3. MiR-202-3p mimic inhibits cell invasion in PTC. **A**, Transwell invasion assays were performed to determine the invasion ability in TPC-1 or BCPAP cells. **B**, Transwell invasion cell number was shown after TPC-1 or BCPAP cells were transfected with miR-202-3p mimic or miR-NC. *p<0.05.

ed in colorectal cancer and microRNA-202-3p inhibits cell proliferation by targeting ADP-ribosylation factor-like 5A in human colorectal carcinoma [9]. MiR-202-3p is upregulated in type 1 g-NEN lesions and might play important roles in the pathogenesis of type 1 gastric neuroendocrine neoplasms (g-NENs) by targeting DUSP1 [13]. MicroRNA-202 inhibits cell proliferation, migration and invasion of glioma by directly targeting metadherin [14]. These results indicated that miR-202-3p acts as a tumor suppressor in tumors and showed potential value in tumor therapy. In the present work, our findings showed that miR-202-3p expression was frequently downregulated in PTC tissues compared to adjacent normal tissues. Similarly, the miR-202-3p was also downregulated in PTC cell lines. Lower expression of miR-202-3p associated with lymph node metastasis of patients with PTC. Furthermore, we found that overexpression of miR-202-3p reduced cell migration and invasion ability. These results indicated that miR-202-3p was also a tumor suppressor in PTC and further investigation of miR-202-3p provides significant value for PTC treatment. WNT

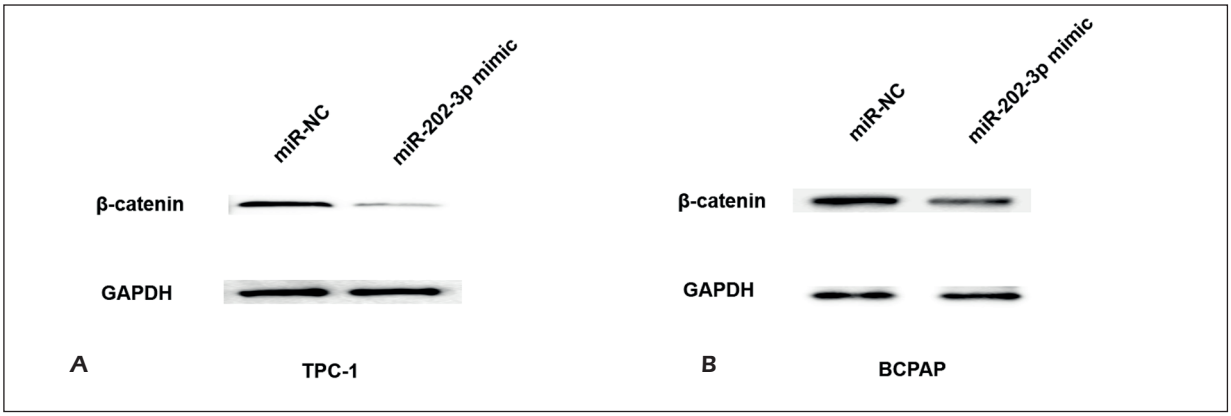

Figure 4. MiR-202-3p mimic inhibits the Wnt/β-catenin signaling pathway. **A**, The protein expression of β-catenin was shown after TPC-1 cells were transfected with miR-202-3p mimic or miR-NC. **B**, The protein expression of β-catenin was shown after BCPAP cells were transfected with miR-202-3p mimic or miR-NC.

signaling has been reported to be involved in PTC development. Such as, knockdown of TRIM44 inhibits the proliferation and invasion in papillary thyroid cancer cells through suppressing the Wnt/β-catenin signaling pathway[15]. Downregulation of CSN6 attenuates papillary thyroid carcinoma progression by reducing Wnt/β-catenin signaling and sensitizes cancer cells to FH535 therapy[16]. Knockdown of IQGAP1 inhibits cell proliferation and epithelial-mesenchymal transition by Wnt/β-catenin pathway in thyroid cancer [17]. In our results, by using Western blot analysis, we found that miR-202-3p overexpression significantly suppressed the expression of β-catenin compared to the control groups in TPC-1 and BCPAP cells, which may indicate that enforced expression of miR-202-3p could inhibit cell migration and invasion by regulating WNT signaling in PTC. However, further investigations are needed.

Conclusions

We showed that miR-202-3p expression was frequently downregulated in PTC tissues and cell liens. Furthermore, enforced expression of miR-202-3p inhibited cell migration, invasion and WNT signaling in PTC. Thus, these results indicated that miR-202-3p may represent a novel therapeutic target of PTC.

Conflict of Interests

The authors declare no conflicts of interest.

References

1) Roh JL, Park JY, Park CI. Total thyroidectomy plus neck dissection in differentiated papillary thyroid carcinoma patients: pattern of nodal metastasis, morbidity, recurrence, and postoperative levels of serum parathyroid hormone. Ann Surg 2007; 245: 604-610.
2) Mao Y, Xing M. Recent incidences and differential trends of thyroid cancer in the USA. Endocr Relat Cancer 2016; 23: 313-322.
3) Hundahl SA, Fleming ID, Fremgen AM, Menck HR. A National Cancer Data Base report on 53,856 cases of thyroid carcinoma treated in the U.S., 1985-1995 [see commetns]. Cancer 1998; 83: 2638-2648.
4) Lee YS, Lim YS, Lee JC, Wang SG, Kim IJ, Lee BJ. Clinical implication of the number of central lymph node metastasis in papillary thyroid carcinoma: preliminary report. World J Surg 2010; 34: 2558-2563.
5) Lu J, Getz G, Miska EA, Alvarez-Saavedra E, Lamb J, Peck D, Sweet-Cordero A, Ebert BL, Mak RH, Ferrando AA, Downing JR, Jacks T, Horvitz HR, Golub TR. MicroRNA expression profiles classify human cancers. Nature 2005; 435: 834-838.
6) Ye Y, Zhuang J, Wang G, He S, Ni J, Xia W. MicroRNA-139 targets fibronectin 1 to inhibit papillary thyroid carcinoma progression. Oncol Lett 2017; 14: 7799-7806.
7) Kan Q, Su Y, Yang H. MicroRNA-335 is downregulated in papillary thyroid cancer and suppresses cancer cell growth, migration and invasion by directly targeting ZEB2. Oncol Lett 2017; 14: 7622-7628.
8) Xue KC, Hu DD, Zhao L, Li N, Shen HY. MiR-577 inhibits papillary thyroid carcinoma cell proliferation, migration and invasion by targeting SphK2. Eur Rev Med Pharmacol Sci 2017; 21: 3794-3800.
9) Wang Q, Huang Z, Guo W, Ni S, Xiao X, Wang L, Huang D, Tan C, Xu Q, Zha R, Zhang J, Sheng W, He X, Du X. microRNA-202-3p inhibits cell proliferation by targeting ADP-ribosylation factor-like 5A in human colorectal carcinoma. Clin Cancer Res 2014; 20: 1146-1157.
10) Deng X, Wu B, Xiao K, Kang J, Xie J, Zhang X, Fan Y. MiR-146b-5p promotes metastasis and induces epithelial-mesenchymal transition in thyroid cancer by targeting ZNRF3. Cell Physiol Biochem 2015; 35: 71-82.
11) Gadducci A, Sergiampietri C, Lanfredini N, Guiggi I. Micro-RNAs and ovarian cancer: the state of art and perspectives of clinical research. Gynecol Endocrinol 2014; 30: 266-271.
12) Zhao Y, Li C, Wang M, Su L, Qu Y, Li J, Yu B, Yan M, Yu Y, Liu B, Zhu Z. Decrease of miR-202-3p expression, a novel tumor suppressor, in gastric cancer. PLoS One 2013; 8: e69756.
13) Dou D, Shi YF, Liu Q, Luo J, Liu JX, Liu M, Liu YY, Li YL, Qiu XD, Tan HY. Hsa-miR-202-3p, up-regulated in type 1 gastric neuroendocrine neoplasms, may target DUSP1. World J Gastroenterol 2018; 24: 573-582.
14) Yang J, Fan B, Zhao Y, Fang J. MicroRNA-202 inhibits cell proliferation, migration and invasion of glioma by directly targeting metadherin. Oncol Rep 2017; 38: 1670-1678.
15) Zhou Z, Liu Y, Ma M, Chang L. Knockdown of TRIM44 inhibits the proliferation and invasion in papillary thyroid cancer cells through suppressing the Wnt/beta-catenin signaling pathway. Biomed Pharmacother 2017; 96: 98-103.
16) Wen D, Liao T, Ma B, Qu N, Shi RL, Lu ZW, Wang YL, Wei WJ, Ji QO. Downregulation of CSN6 attenuates papillary thyroid carcinoma progression by reducing Wnt/beta-catenin signaling and sensitizes cancer cells to FH535 therapy. Cancer Med 2018; 7: 285-296.
17) Su D, Liu Y, Song T. Knockdown of IQGAP1 inhibits proliferation and epithelial-mesenchymal transition by Wnt/beta-catenin pathway in thyroid cancer. Onco Targets Ther 2017; 10: 1549-1559.