

## Changes of serum miR-221 and miR-145 levels with papillary thyroid carcinoma and their relationship with invasive activity

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### ABSTRACT

This study aimed to investigate the expression of miR-221 and miR-145 in papillary thyroid carcinoma, and the effect of miR-221 and miR-145 on the invasion ability of thyroid cancer cells and its mechanism. For this purpose, 120 patients with thyroid nodules were divided into the observation group (PTC) of 43 cases and the control group (benign nodules) of 42 cases according to postoperative pathological diagnosis. Total RNA was extracted from serum samples of all patients, and the expression levels of miRNA-145 and miR-221 were detected by fluorescence quantitative PCR. The expression of two kinds of miRNAs in the groups was compared, and their correlation was analyzed. The results showed that the expression of miRNA-145 in thyroid cancer tissues was lower than that in paired adjacent normal tissues ( $P < 0.001$ ). The expression of miRNA-221 in thyroid cancer tissues was higher than that in paired adjacent normal tissues ( $P < 0.001$ ). The proliferation and migration ability of miRNA-145 cells were significantly decreased ( $P < 0.01$ ). The expression of miRNA-221 was up-regulated, and the proliferation and migration ability of cells was significantly enhanced ( $P < 0.05$ ). High expression of miRNA-145 can inhibit cell proliferation and migration and promote apoptosis, while high expression of miRNA-221 will promote cell proliferation and migration and enhance the invasion ability of cancer cells. In general, the expression of miRNA-221 in serum of PTC patients is significantly up-regulated, while the expression level of miR-145 is down-regulated, which can be used as effective indicators to judge the biological activity of the tumor, and the combined detection of the two can significantly enhance the diagnostic value of PTC. Upregulation of miR-145 inhibits PTC cell proliferation; arrests cell cycle and promotes apoptosis miR-145 may play an important role as a tumor suppressor gene.

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### Introduction

Thyroid cancer (TC) is the most common malignant tumor in the endocrine system (1). Thyroid cancer accounts for about 1% of all malignancies worldwide (2). Although thyroid cancer has a good prognosis, the 5-year survival rate can reach 95%, some patients may have lymph node metastasis or distant metastasis after surgery (3). However, the 5-year survival rate of patients with distant metastasis is only about 15% (4). At present, the diagnosis of PTC in China mainly relies on histological examination, but it is still difficult to distinguish some subtypes only by histological examination (5). Therefore, actively looking for molecular markers of thyroid cancer invasion and metastasis, improving early diagnosis and clarifying treatment mechanisms are effective ways to improve the overall treatment level of thyroid cancer (6).

Recent studies have found that microRNA (miRNA) is involved in the whole process of regulation of the occurrence and development of a variety of tumors (7). MiRNA is a kind of endogenous, highly conserved non-coding small RNA composed of 18-25 nucleotides (8). It has the function of post-transcriptional regulation genes and is widely involved in cell differentiation, proliferation, development and apoptosis (9, 10). It is a hotspot in the field of tumor research at present. Through the detection of miRNA expression levels in tumor tissues, miR-221 and miRNA-145 were found to be involved in the occurrence and progression of many malignant tumors (such as prostate cancer, gastric cancer, cervical cancer, colorectal cancer, breast cancer, thyroid cancer, etc.) (11, 12).

Papillary thyroid carcinoma (PTC) is the most common type of thyroid cancer, accounting for 80%

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to 90% of thyroid cancers. In recent years, studies on miRNA and PTC have confirmed that the abnormal expression of miRNA is closely related to the occurrence and development of PTC. Detection of abnormal miRNA expression in PTC tissues and peripheral blood is helpful to determine tumor size, extra-glandular invasion and lymph node metastasis. The most common miRNAs associated with PTC include miRNA-145 and miRNA-221. Deep sequencing technology and in vitro studies confirmed that miRNA-145 and miRNA-221, which play a preferential regulatory role, are involved in the pathogenesis of PTC (13, 14). Relevant studies have shown that mir-221 and mir-145 play a role in activating the Nuclear factor-kappa B (NF- $\kappa$ B) signaling pathway (15, 16). Therefore, the regulation of immune-inflammatory response by activating the NF- $\kappa$ B signaling pathway may be the mechanism by which miRNA-145 and miRNA-221 affect the progression of PTC.

However, there are no reports on the function of miRNA-145 and miRNA-221 in thyroid cancer. Therefore, this study examined the expression of miRNA-145 and miRNA-221 to explore their relationship with invasion and metastasis of thyroid cancer.

## Materials and methods

### Subjects

A total of 120 patients admitted to our hospital from January 2018 to June 2021 who received subtotal thyroidectomy and were diagnosed with thyroid cancer by surgical pathological sections were selected as the study subjects.

According to the postoperative pathological diagnosis, the patients were divided into observation group (PTC patients) and control group (benign nodule patients). The observation group consisted of 60 patients, 35 males and 25 females, aged from 35 to 63 ( $49.21 \pm 6.92$ ) years. The control group consisted of 60 patients, 37 males and 23 females, aged from 37 to 66 ( $48.91 \pm 7.01$ ) years. All samples in this study were collected and used with the consent and informed consent of thyroid cancer patients, which had been approved by the medical ethics committee of the hospital before implementation.

### Inclusion and exclusion criteria

Inclusion criteria: All patients were diagnosed with thyroid cancer for the first time, and the interval between examination and operation was  $< 1$  week; No chemotherapy or local or systemic radiotherapy was performed before surgery, and the lesions with desirable pathological tissue were confirmed by pathological biopsy; There were no obvious lesions in cardiopulmonary function, liver and kidney function and blood system. Complete clinical data; Informed consent was signed by patients or their families.

Exclusion criteria: Multiple tumors and non-primary lesions; Patients without comprehensive treatment; Patients with poor physical condition, unable to tolerate relevant examinations or pregnant or lactating women with cognitive dysfunction or mental illness; Patients who fell off, dropped out or lost to follow-up in clinical trials.

### Reagent

All used materials and reagents included TaqMan miRNA Assay Kit (Applied Biosystems), TaqMan miRNA Reverse transcription Kit (Applied Biosystems), 10mirVana miRNA Isolation Kit (Thermo Scientific), MiR-221, miR-145 (Sangong Bioengineering (Shanghai) Co., LTD.), Penicillin-streptomycin solution (double antibody,  $100 \times$ ), PBS buffer trypsin (Wuhan Punosai Life Technology Co., LTD.), Embryo bovine serum, DMEM High Sugar medium, Matrigel Base glue (Wuhan Punosai Life Technology Co., LTD.), Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (Sangong Bioengineering (Shanghai) Co., LTD.), Cell counting kit 8, CCK-8 (biyuntian (Shanghai) biotechnology co., LTD.), Real-time PCR Kit (Bao Bioengineering (Dalian) Co., LTD.), NF- $\kappa$ B antibody, HRP labeled Goat Anti-Rabbit IgG (Tianjin Biosno Biotechnology Co., LTD.), RNAiso Plus Total RNA Extraction Reagent (Bao Bioengineering (Dalian) Co., LTD.).

### Experimental Instruments

The used experimental instruments were Micropipette Gilson Inc, Real-time Fluorescence quantitative PCR instrument CFX96 Touch Bio-rad Inc, High-speed low-temperature centrifuge Eppendorf Company,  $-80^{\circ}\text{C}$  ultra-low temperature refrigerator  $-20^{\circ}\text{C}$  refrigerator of Haier Company,

Mili-q Ultra-Pure Water Manufacturing Systems Millipore Inc, Tissue Lyser - ii Qiagen.

### Tissue collection and cell culture

After thyroid cancer was diagnosed, thyroid cancer tissue samples and corresponding paracancer normal thyroid tissue (> 5cm from tumor edge) were taken from all selected patients. Tissue samples were labeled as patient group (PTC group) and operation control group (NTT group) and properly stored in liquid nitrogen. In the morning of the second day, 5 ml of fasting venous blood was taken and placed in an EDTA anticoagulant tube for labeling and collection. The supernatant was centrifuged at 3000 r/min for 15 min in a low-temperature centrifuge. The supernatant was placed in an EP tube and stored in a refrigerator at -80°C.

Thyroid cell line TPC-1 cells were purchased from Shanghai Hongshun Biotechnology Co., LTD., and cultured in DMEM medium containing 10% fetal bovine serum, 100 U/mL penicillin and 100 µg/mL streptomycin at 37°C and 5% CO<sub>2</sub>. The cells were inoculated at a density of 0.5×10<sup>6</sup> live cells per 100mm plate, and the medium was changed every other day.

### Extraction of miRNA

MiRNA was extracted from tissues using the mirVana miRNA extraction kit. For the paraffin-embedded specimens, the tissues were scraped and put into the centrifuge tube, and an appropriate volume of paraffin-removed solution was added. After intense spiral socket oscillation for 10 s, the samples were immediately centrifuged and incubated at 56°C for 3 min before cooling for later use. For fresh tissue specimens or tissue blocks, they were stored at -80°C, taken an appropriate amount of tissue and quickly frozen in liquid nitrogen, then ground into powder in a mortar. The tissue was placed into the EP tube and then 500µl Lysis/Binding Buffer was added to shake violently. 1/10 volume of miRNA Homogenate was incubated on ice for 10 min. Phenolic chloroform and Lysis/Binding Buffer were added and were centrifuged at 12000 g for 20 min. The watery phase was moved into a new EP tube and 1/3 volume of anhydrous ethanol was added, and then it mixed totally. The liquid was transferred to the separation column, and it was centrifuged at 10000 g for 15 s and

the waste liquid was discarded. 700µL miRNA Wash Solution 1 was added for instantaneous centrifugation and the waste liquid was removed. 500µL miRNA Wash Solution 2/3 was added for instantaneous centrifugation, then the waste liquid was removed and it was centrifuged at 10000 g for 1 min. 80µL preheated DEPC water was added and it was centrifuged by 10,000 g for 30 s. Finally, it was stored at -80°C.

### Lentivirus transfection

Lentiviruses were transfected into thyroid cancer cell lines using Polybrene. Thyroid cancer cells were transfected with Mir-145-inhibitor and Mir-221-inhibitor lentivirus, and the experiment was divided into three groups: control group (NC group), Mir-145 group and Mir-221 group. After transfection, Mir-145 expression was determined by QRT-PCR, and mRNA and protein expression were determined by QRT-PCR and western blot, respectively.

### Detection of miRNA

The expression levels of Mir-221 and Mir-145 in the two groups were detected by real-time immunofluorescence quantitative polymerase chain reaction (RT-PCR). Total RNA was extracted from the serum using the mirVana miRNA extraction kit, and the reverse transcription reaction was performed after the detection by UV spectrophotometer. Reverse transcription and RT-PCR were performed using the Taqman miRNA reverse transcription kit and the RT-PCR detection kit according to the relevant instructions. The reverse transcription reaction conditions were 95°C for 30 s, 95°C for 5 s and 60°C for 34 s, with a total of 40 cycles. PCR reaction was preheated at 95°C for 10 min, followed by 40 cycles of denaturation at 95°C for 10 s each, annealing at 60°C for 20 s, and extension at 72°C for 10 s. 7500 System SDS software was used to check the amplification curve and dissolution curve, and Ct values were automatically analyzed. Three multiple Wells were performed for each sample and each sample was repeated 3 times. GAPDH was used as an internal reference, and the relative contents of Mir-221 and Mir-145 were calculated by 2<sup>-δ δ Ct</sup> method. 2<sup>-δ δ Ct</sup> < 0.5 was defined as low expression, 0.5~2.0 was defined as normal expression, > 2.0 was defined as high expression.

The primers sequences were as the following:

Upstream primer of Mir-221:

5'-TTGAGAAGTTTGTGCAGTCG-3'

Downstream primer of Mir-221:

5'-TGCGTATACTTTGGCAT-3'

Upstream primer of miR-145:

5'GTCCAGTTTTCCCAGGAAT3';

Downstream primer miR-145:

5' TGGTGTCGTGGAGTCG 3'.

### Western blotting experiment

RIPA lysate was added to thyroid tissue and ground to homogenate on ice. The lysate was placed at 4°C for 30 min and centrifuged at 13 000 r/min for 10 min. Part of the supernatant was taken and the protein concentration was detected by BCA kit. The other part of the supernatant is added with 4× sample loading buffer solution, after boiling and denaturation, SDS-PAGE gel electrophoresis is carried out. The primary antibody is incubated at 4°C, the secondary antibody is incubated at room temperature, ECL luminescent solution is developed, X medical film is developed and fixed, and finally, photo analysis is carried out.

### Detection of cell migration ability

Tpc-1 cells were inoculated on a six-well plate, and transfection reagent was added after cell adherence, and cell growth density reached more than 90%. Use 200μL sterile spear tip to scratch "1" shape in 6-well plate, then rinse suspension cells with PBS immediately, add to medium for further culture. Cell healing was observed under an inverted fluorescence microscope at 0 h, 24 h, 48 h and 72h after scratches, respectively. Images were processed with ImagePro Plus 6.0 and the relative migration distance was recorded.

### CCK-8 was used to measure cell proliferation

Follow instructions for the CCK-8 kit. Cells from different transfection groups (5×10<sup>3</sup>) were cultured in 96-well plates for 24h. In the beginning, 10μL CCK-8 solution was added to each well and incubated at 37°C for 2h. The absorbance A value of each well was measured at 450nm with an absorbance meter. Cell viability was the absorbance fraction compared with control. Three groups were recorded as control group (NC group), Mir-145 group and Mir-221 group to study the effects of Mir-145 and Mir-221 on thyroid cancer cells.

### Cell invasion ability was detected by the Transwell method

After 48 h cultures, the cells were digested into single-cell suspension (5 × 10<sup>4</sup> cells/mL). The upper chamber of the Transwell chamber was coated with Matrigel, and a 1 mL DMEM cell culture medium containing 10% FBS was added to the lower chamber. After 24 hours of culture with crystal violet staining, the cells on the upper ventricle surface were swabbed with cotton swabs and fixed with 95% ethanol solution for 30min. 5 fields were randomly selected under a 200× microscope for photographing and counting. The experiment was repeated 3 times. The number of invaded cells was expressed as the average number of cells per field of vision.

### Statistical Analysis

SPSS 20.0 was used as statistical analysis software. The measurement data conforming to normal distribution were presented in the form of mean ± standard deviation, and the Shapiro-Wilk method was used to test the normal distribution of quantitative data. If the variance of the data was homogeneity, the paired sample T-test was used, and the data not conforming to normal distribution was presented in the form of mean (value range). Mann-Whitney U test was used for comparison. Analysis of variance and  $\chi^2$  test were used to measure the cell viability and the mass and volume of the tumor. Test level  $\alpha=0.05$ .  $P < 0.05$  was considered as a statistically significant difference.

### Results and discussion

#### RT-QPCR detection results

Rt-PCR results showed that the relative expression level of Mir-145 in PTC tissues (0.369±0.082) was significantly lower than that in adjacent tissues (1.029±0.365), with statistical significance ( $P < 0.05$ ). The relative expression of Mir-221 (0.912±0.071) was significantly higher than that of adjacent tissues (0.411±0.037), with statistical significance ( $P < 0.05$ ). The relative expression level of Mir-145 in patients with the largest tumor diameter  $\geq 1$  cm was significantly lower than that in patients with the largest tumor diameter  $< 1$  cm, and the relative expression level of Mir-145 in patients with multiple tumors was significantly lower than that in patients with single tumors. The expression level of Mir-221 was significantly higher than that of patients with the largest tumor diameter  $< 1$  cm and



the relative expression level of Mir-221 was significantly higher in patients with multiple tumors than in patients with a single tumor, with statistical significance (ALL  $P < 0.05$ ), as shown in Table 1.

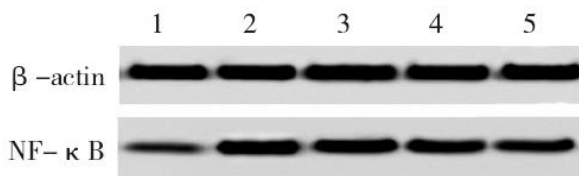
**Table 1.** Expression levels of miR-145 and miRNA-221 in thyroid cancer tissues and adjacent normal tissues

Group	Thyroid cancer (n=60)	Benign lesions (n=60)	Normal adjacent tissue (n=60)
miR-145	0.369±0.082	0.973±0.351*	1.029±0.365*
miR-221	0.912±0.071	0.431±0.032*	0.411±0.037*

Note \* indicates comparison with the patient's cancerous area, \* $P < 0.05$ .

**Results of NF-κB expression in each group**

Western blot analysis showed that NF-κB protein level in the PTC group and miRNA-221 group (Figure 1) was higher than that in the NTT group and NC group, and the level in the PTC group was higher than that in the miRNA-221 group. The level of NF-κB in the Mir-145 group was higher than that in the NTT group and NC group, but lower than that in the PTC group and miRNA-221 group ( $P < 0.05$ ), suggesting that miRNA can regulate immune-inflammatory response by activating NF-κB signaling pathway and thus affect PCT cells.



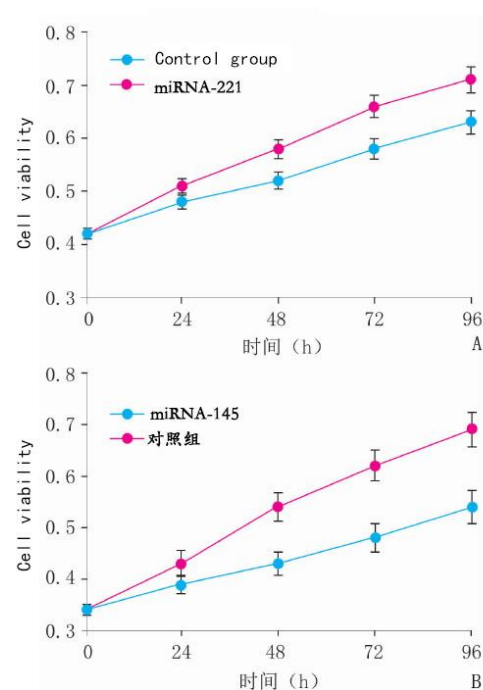
**Figure 1.** NF-κB expression level in each group. Note: 1: NTT group. 2: PTC group. 3: miRNA-211 group. 4: miRNA-145 group 5: NC group

**Effects of miRNA-221 and miRNA-145 on tPC-1 cell activity of thyroid cancer cells**

The proliferation test results of CCK-8 were shown in Figure 2. The proliferation rate of TPC-1 cells increased gradually with the prolongation of miRNA-221, and the miRNA-221 group was higher than the control group ( $F=13.22, P=0.005$ ). With the extension of the action time of miRNA-145. The proliferation rate of TPC-1 cells increased gradually, and the miRNA-145 group was lower than the control group ( $F=15.31, P=0.014$ ).

**Effects of miRNA-221 and miRNA-145 expression levels on tPC-1 cell migration**

Scratch test showed that over time, the migration ability of the control group was significantly enhanced compared with the miRNA-221 group at 72 h migration distance, suggesting that up-regulation of miRNA-221 expression could promote cell migration. Compared with the migration and expansion distance of the miRNA-145 group, there was no significant difference in the migration ability between the miRNA-145 group and the control group at 48h, and the migration ability of the miRNA-145 group was significantly reduced at 72 h, indicating that up-regulation of miRNA-145 expression could inhibit cell migration, as shown in table 2 and Figure 3.



**Figure 2.** Effect of miRNA-221 and miRNA-145 on the activity of TPC-1 cells

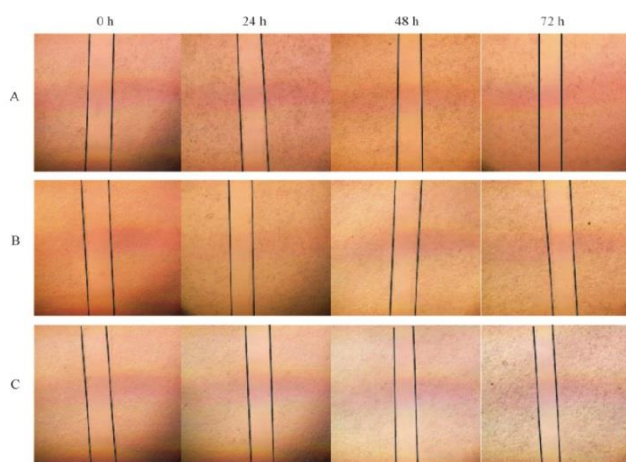
**Table 2.** Effect of miRNA-221 and miRNA-145 on the migration of nasopharyngeal carcinoma cell line TCP-1 cells

Group	Control group	miRNA-221 group	miRNA-145 group
24h migration expansion distance	13.214±1.311	16.119±1.214	16.139±1.225
48h migration expansion distance	28.917±3.112	37.198±3.456*#	25.198±2.836*
72h migration expansion distance	37.556±3.566	40.611±3.540*#	28.611±3.661*#

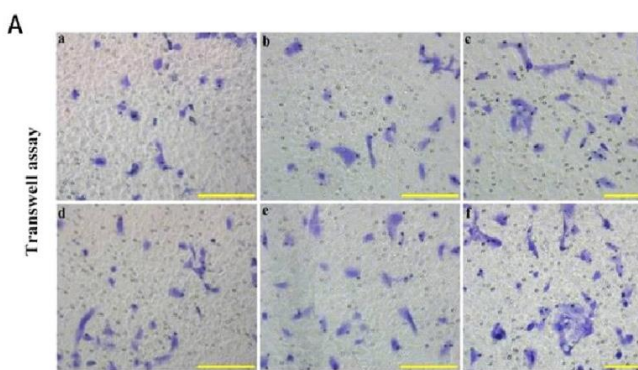
Note: \* represents the comparison between before and after groups, \* $P < 0.05$ . # represents the comparison with the control group, # $P < 0.05$ .

### Detection of cell invasion ability

The invasion ability test showed that compared with the control group and miRNA-221 group, the invasion ability of miRNA-221 group was significantly increased (Figure 4) ( $P < 0.01$ ). Cells in the miRNA-145 group were less invasive than those in the control group (Figure 4A). These results suggest that miRNA-221 can enhance the invasion ability of TPC-1 cells, and miRNA-145 can reduce the invasion ability of TPC-1 cells.



**Figure 3.** Effect of miRNA-221 and miRNA-145 expression levels on TPC-1 cells migration ( $\times 40$ ) (72 h) Note: A: NC group. B: miRNA-221 group. C: miRNA-145 group



**Figure 4.** Effect of miRNA-221 and miRNA-145 expression levels on TPC-1 cells invasion ability; Note: a, d: NC group; b, e: miRNA-145 group; c, f: miRNA-221 group

In recent years, the research on miRNAs and tumor occurrence and development has exploded, and it has been found that miRNAs are involved in the regulation of cancer cell proliferation, apoptosis, differentiation, invasion, metastasis, angiogenesis and tumor microenvironment, which are closely related to

the occurrence, development and drug resistance of cancer cells (17-19). In recent years, a large number of studies have focused on the possibility of miRNA as a new molecular marker for preoperative screening, early diagnosis and prognosis assessment of tumors. In addition, the carcinogenic or tumor-suppressive effects of miRNA may inhibit the occurrence and development of tumors and may also lead to malignant transformation of cells (20).

A number of experiments have proved that the abnormal expression of miRNA is closely related to the occurrence and development, degree of malignancy and sensitivity to treatment of thyroid cancer. Rt-PCR was used to detect the expression of miRNA in PTC and non-tumor tissues, and it was found that the expression of miRNA-221 in PTC tissues was significantly higher than that in nodular goitosis and normal para-cancer thyroid tissues. It is also correlated with tumor TNM staging, capsule invasion and lymph node metastasis (21, 22). The expression of miRNA-221 and interleukin-17 in PTC tissues is significantly correlated, suggesting that miRNA-221 may regulate the expression of interleukin-17 through some mechanism during the occurrence and development of PTC, and has the potential as a tumor-related marker (23, 24). Current clinical status indicates that thyroid cancer is a malignant tumor with a relatively good prognosis, but if metastasis occurs, the survival rate is low (25).

In this study, the expressions of Mir-221 and Mir-145 in 120 patients with PTC with high clinical characteristics were analyzed. The expression levels of miRNA-221 and miRNA-145 in thyroid cancer patients were analyzed by RT-QPCR and chi-square test. To investigate the effects of miRNA-221 and miRNA-145 on the biological behavior of thyroid cancer cell tPC-1 by transfection of miRNA-221 and miRNA-145 in vitro. Rt-PCR results showed that the relative expression level of Mir-145 in PTC tissues ( $0.369 \pm 0.082$ ) was significantly lower than that in para-cancer tissues ( $1.029 \pm 0.365$ ), with statistical significance ( $P < 0.05$ ). The relative expression of Mir-221 ( $0.912 \pm 0.071$ ) was significantly higher than that of adjacent tissues ( $0.411 \pm 0.037$ ), with statistical significance ( $P < 0.05$ ). Western blot analysis showed that NF- $\kappa$ B protein level in the PTC group and miRNA-221 group was higher than that in the NTT group and NC group. The level of NF- $\kappa$ B in the PTC

group was higher than that in the miRNA-221 group, and the level of NF- $\kappa$ B in the Mir-145 group was higher than that in the NTT and NC groups. However, it was lower than that in the PTC group and miRNA-221 group ( $P < 0.05$ ). These results indicate that miRNA can regulate the immune-inflammatory response by activating the NF- $\kappa$ B signaling pathway, thereby affecting PCT cells. Cck-8 and scratch experiments showed that the proliferation rate of TPC-1 cells was gradually increased with the prolongation of miRNA-221, and that of the miRNA-221 group was higher than that of the control group. The proliferation rate of TPC-1 cells was gradually increased with the prolongation of mirNA-145, and that of the miRNA-145 group was lower than that of the control group. Over time, the migration ability of the mirNA-221 group was significantly enhanced.

The migration ability of the miRNA-145 group was significantly decreased, suggesting that up-regulation of miRNA-221 could promote cell migration, while up-regulation of miRNA-145 could inhibit cell migration. Invasive ability results show

The number of tPC-1 cells overexpressing miRNA-221 crossing matrix gum was significantly increased, and that of TPC-1 cells overexpressing miRNA-145 crossing matrix gum was significantly decreased, suggesting that miRNA-221 can promote the proliferation and migration of tPC-1 thyroid cancer cells. However, miRNA-145 can inhibit the growth of miRNA-145. High expression of miRNA-145 can inhibit cell proliferation and migration and promote cell apoptosis. Clinical case analysis found that miRNA-221 levels were different in different ages, lymph node metastasis, extra glandular invasion, tumor size and TNM stage, indicating that miRNA-221 expression was associated with higher clinical stage, lymph node metastasis and other factors, which was consistent with the results of other studies (26, 27).

Due to the characteristics of miRNA, tumor suppression by regulating its expression is a new strategy for tumor treatment. Mirna-based gene therapy has entered a new stage and has great application prospects (28). Although the time of miRNA-221 and miRNA-145 acting in different cells is different, the mode of action is also quite different. However, the expression of miRNA can be increased by synthesizing miRNA analogues when miRNA

expression is inhibited or down-regulated clinically. Or when abnormal miRNA expression leads to pathological changes, antisense oligonucleotides can be directly combined with miRNA to block its activity, thus playing a therapeutic role (29, 30).

Due to the limitations of the experimental level, the targets of miRNA-221 and miRNA-145 on thyroid cancer cells have not been explored. Therefore, the clinical use of miRNA-221 and miRNA-145 as markers of thyroid cancer will be further explored and verified.

In conclusion, the abnormal expression of miRNA can become the most accurate and sensitive method for the diagnosis and prognosis of thyroid cancer, and more large-scale and in-depth studies remain to be carried out.

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### Interest conflict

The authors declare no conflict of interest.

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### References

1. Moshtaghioun SM, Fazel-Yazdi N, Mandegari M, Shirinzadeh-Dastgiri A, Vakili M, Fazel-Yazdi H. Evaluation the Presence of SERPINA5 (Exon 3) and FTO rs9939609 Polymorphisms in Papillary Thyroid Cancer Patients. *Asian Pac J Cancer Prev* 2021; 22(11): 3641-3646.
2. Spirina LV, Chizhevskaya SY, Kovaleva IV, Kondakova IV. The Association of the BRAF-V600E Mutation with the Expression of the Molecular Markers in the Primary Tumor and Metastatic Tissue in Papillary Thyroid Cancer. *Asian Pac J Cancer Prev* 2021; 22(7): 2017.
3. Mandegari M, Dastgheib SA, Asadian F et al. A Meta-Analysis for Association of XRCC1, XRCC2 and XRCC3 Polymorphisms with Susceptibility to Thyroid Cancer. *Asian Pac J Cancer Prev* 2021; 22(7): 2221.
4. Nedooshan JJ, Yazdi MF, Neamatzadeh H, Shehneh MZ, Kargar S, Seddighi N. Association between TP53 codon 72 G>C polymorphism and



- thyroid carcinoma risk: An up-to-date meta-analysis. *Asian Pac j cancer biol* 2016; 1(4): 89-95.
5. Liu F-C, Lin H-T, Lin S-F, Kuo C-F, Chung T-T, Yu H-P. Nationwide cohort study on the epidemiology and survival outcomes of thyroid cancer. *Oncotarget* 2017; 8(45): 78429.
  6. Othman NH, Abd Ghani NN, Mohd NZ. Clinico-pathological Characteristics and Survival Analysis of 300 Thyroid Cancer Cases in One Referral Hospital in Kelantan, Malaysia: A 10-year study. *Asian Pac j cance care* 2018; 3(3): 53-53.
  7. Tian X, Chen Z, Shi S et al. Clinical diagnostic implications of body fluid miRNA in oral squamous cell carcinoma: A meta-analysis. *Medicine* 2015; 94(37).
  8. Hou B, Ishinaga H, Midorikawa K et al. Circulating microRNAs as novel prognosis biomarkers for head and neck squamous cell carcinoma. *Canc Biol Ther* 2015; 16(7): 1042-1046.
  9. Ercisli MF, Kahrizi D, Azizaram Z. Environmental factors affecting the risk of breast cancer and the modulating role of vitamin D on this malignancy. 2020.
  10. Azeez SH, Jafar SN, Azizaram Z, Fang L, Mawlood AH, Ercisli MF. Insulin-producing cells from bone marrow stem cells versus injectable insulin for the treatment of rats with type I diabetes. *Cell Mol Biomed Rep* 2021; 1(1): 42-51.
  11. Yu W, Ding J, He M et al. Estrogen receptor  $\beta$  promotes the vasculogenic mimicry (VM) and cell invasion via altering the lncRNA-MALAT1/miR-145-5p/NEDD9 signals in lung cancer. *Oncogene* 2019; 38(8): 1225-1238.
  12. Xu Y, Yu X, Wei C, Nie F, Huang M, Sun M. Over-expression of oncogenic pseudogene DUXAP10 promotes cell proliferation and invasion by regulating LATS1 and  $\beta$ -catenin in gastric cancer. *J Experim Clin Cancer Res* 2018; 37(1): 1-12.
  13. Bilal I, Xie S, Elburki MS, Azizaram Z, Ahmed SM, Jalal Balaky ST. Cytotoxic effect of diferuloylmethane, a derivative of turmeric on different human glioblastoma cell lines. *Cell Mol Biomed Rep* 2021; 1(1): 14-22.
  14. Chruścik A, Lam AK-y. Clinical pathological impacts of microRNAs in papillary thyroid carcinoma: A crucial review. *Exp Mol Pathol* 2015; 99(3): 393-398.
  15. Zhao D, Zhuang N, Ding Y, Kang Y, Shi L. MiR-221 activates the NF- $\kappa$ B pathway by targeting A20. *Biochem Biophys Res Commun* 2016; 472(1): 11-18.
  16. Chen G, Gao Y, Wang G, Dai G, Tong L. MiR-145 inhibits the migration and invasion of papillary thyroid carcinoma cells through NF- $\kappa$ B pathway regulation. *J Cell Biochem* 2020; 121(5-6): 3325-3332.
  17. O'Bryan S, Dong S, Mathis JM, Alahari SK. The roles of oncogenic miRNAs and their therapeutic importance in breast cancer. *Eur J Cancer* 2017; 72: 1-11.
  18. Zhao M, Ang L, Huang J, Wang J. MicroRNAs regulate the epithelial-mesenchymal transition and influence breast cancer invasion and metastasis. *Tumor Biol* 2017; 39(2): 1010428317691682.
  19. Evans-Knowell A, LaRue A, Findlay V. MicroRNAs and their impact on breast cancer, the tumor microenvironment, and disparities. *Adv Cancer Res* 2017; 133: 51-76.
  20. Acunzo M, Romano G, Wernicke D, Croce CM. MicroRNA and cancer—a brief overview. *Adv Biol Regul* 2015; 57: 1-9.
  21. Feng Z, Song Y, Qian J et al. Differential expression of a set of microRNA genes reveals the potential mechanism of papillary thyroid carcinoma. Paper presented at: *Annales d'endocrinologie*, 2019.
  22. Yang L, Zheng R, Wang N et al. Analysis of incidence and mortality of thyroid cancer in China, 2013. *Chin J Oncol* 2017; 39(11): 862-867.
  23. Nassar FJ, Nasr R, Talhouk R. MicroRNAs as biomarkers for early breast cancer diagnosis, prognosis and therapy prediction. *Pharmacol Ther* 2017; 172: 34-49.
  24. Chen W, Zhou S, Mao L et al. Crosstalk between TGF- $\beta$  signaling and miRNAs in breast cancer metastasis. *Tumor Biol* 2016; 37(8): 10011-10019.
  25. Randle RW, Balentine CJ, Levenson GE et al. Trends in the presentation, treatment, and survival of patients with medullary thyroid cancer over the



- past 30 years. *Surgery* 2017; 161(1): 137-146.
26. Wang T, Xu H, Qi M, Yan S, Tian X. miRNA dysregulation and the risk of metastasis and invasion in papillary thyroid cancer: a systematic review and meta-analysis. *Oncotarget* 2018; 9(4): 5473.
27. Rosignolo F, Sponziello M, Giacomelli L et al. Identification of thyroid-associated serum microRNA profiles and their potential use in thyroid cancer follow-up. *J Endocrine Soc* 2017; 1(1): 3-13.
28. Rossignoli F, Spano C, Grisendi G et al. MSC-delivered soluble TRAIL and paclitaxel as novel combinatory treatment for pancreatic adenocarcinoma. *Theranostics* 2019; 9(2): 436.
29. Kazemi E, Zargooshi J, Kaboudi M, Heidari P, Kahrizi D, Mahaki B, Mohammadian Y, Khazaei H, Ahmed K. A genome-wide association study to identify candidate genes for erectile dysfunction. *Brief Bioinforma* 2021;22(4):bbaa338. <https://doi.org/10.1093/bib/bbaa338>.
30. Luo M, Wu C, Guo E et al. FOXO3a knockdown promotes radioresistance in nasopharyngeal carcinoma by inducing epithelial-mesenchymal transition and the Wnt/ $\beta$ -catenin signaling pathway. *Cancer lett* 2019; 455: 26-35.