

## Original Article

# Effects of microRNA-381-3p and microRNA-193a on the growth and apoptosis of human papillary thyroid carcinoma B-CPAP cells

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**Abstract:** Objective: To investigate the effects of microRNA-381-3p (miR-381-3p) and microRNA-193a (miR-193a) on the growth and apoptosis of human papillary thyroid carcinoma B-CPAP cells. Method: Sixty-two samples of thyroid carcinoma tissues and sixty-two samples of corresponding adjacent tissues were removed and collected from the selected patients with thyroid cancer receiving surgical resection. The expression levels of miR-381-3p and miR-193a in carcinoma tissues and adjacent tissues were detected using quantitative real-time reverse transcription polymerase chain reaction. Expression vectors of miR-381-3p and miR-193a were established and transfected by human papillary thyroid carcinoma B-CPAP cells, followed by cell culture. Cell proliferation, invasion and apoptosis were detected by cell counting kit-8 assay, transwell assay, and flow cytometric analysis. Results: The expression levels of both miR-381-3p and miR-193a in the thyroid carcinoma tissues were significantly lower than those in the adjacent tissues (both  $P < 0.001$ ). MiR-381-3p mimic group and miR-193a mimic group were both significantly lower than blank group or NC group in cell proliferation activity at 48 h and 72 h as well as in number of invaded cells (all  $P < 0.001$ ). The apoptosis rates of miR-381-3p mimic group and miR-193a mimic group were both significantly higher than those of NC group or blank group (all  $P < 0.001$ ). Conclusion: MiR-381-3p and miR-193a present low expression levels in thyroid carcinoma tissues. Overexpression of miR-381-3p and miR-193a inhibited proliferation and invasion of human papillary thyroid carcinoma B-CPAP cells and promote apoptosis of the cells, suggesting that miR-381-3p and miR-193a may be used as diagnostic markers and therapeutic targets for thyroid cancer.

**Keywords:** MicroRNA-381-3p, microRNA-193a, human papillary thyroid carcinoma cells, apoptosis

## Introduction

Thyroid cancer is a common endocrine malignant tumor with increasing incidence owing to various factors like lifestyle and environment [1-3]. Surgical treatment, endocrine therapy, radionuclide therapy, external radiation therapy and physical or thermal ablation methods such as microwave, laser and radiofrequency ablations are currently the main choices for clinical treatment of thyroid cancer [4-7]. Relevant reports reveal that radical thyroidectomy can prolong disease-free survival in most thyroid cancer cases with ten or more years of survival time after the surgery, but there are still a large number of patients presenting with poor prog-

nosis such as recurrence or metastasis [8]. Current research on thyroid cancer prominently focuses on the potential mechanism of the occurrence, development and progression of human thyroid cancer [9].

MicroRNAs (miRNAs) are a class of endogenous, single-stranded non-coding RNAs with cancer-promoting and inhibiting effects [10, 11]. With the in-depth study on miRNAs pertaining to the pathogenesis of thyroid cancer, miR-381-3p and miR-193a may present with aberrant expressions in thyroid cancer [12]. Current research has demonstrated that miR-381-3p possesses cancer-promoting and inhibiting effects in various solid tumors. A stu-

**Table 1.** Primer sequences of miR-381-3p, miR-193a and internal control U6

	Forward primers	Reverse primers
miR-381-3p	5'-TAATCTGACTATACAAGGGCAAGCT-3'	5'-TATGGTTGTCTGCTCTCTGTCTC-3'
miR-193a	5'-GTCGTATCCAGTGCAGGGTCCGAGGTGCACTGGATACGACTCATCTC-3'	5'-TGCCTGGGCUCAAAG CGG GC-3'
U6	5'-CTCGCTTCGGCAGCAC-3'	5'-AACGCTTCACGAATTTGCGT-3'

dy, for example, showed that evident reduction in miR-381-3p expression level could promote the proliferation of cervical cancer cells [13]. Some researchers have confirmed that the miR-193a expression in liver cancer tissues and thyroid cancer tissues is far lower than that in normal tissues [14, 15]. In order to provide a new theoretical basis for the diagnosis and treatment of thyroid cancer in molecular biology, this paper aimed to experimentally explore the expression characteristics of miR-381-3p and miR-193a in thyroid cancer and their effects on the biological characteristics of human papillary thyroid carcinoma B-CPAP cells.

## Materials and methods

### *Patients and tissue samples*

The study was approved by the Medical Ethics Committee of The Second Clinical Medical College of Yangtze University. From March 2014 to March 2015, 62 samples of thyroid carcinoma tissues and 62 samples of corresponding adjacent tissues were removed and collected from the selected patients with thyroid cancer receiving surgical resection. Written informed consents were obtained from the patients [16] and their relatives in advance before the study. The included patients had normal liver and kidney functions with no other malignant tumors. All specimens were stored in liquid nitrogen tanks immediately after surgical resection. The samples of the included patients with thyroid cancer were confirmed by postoperative pathology. Patients who received chemotherapy, immunotherapy and radiotherapy before surgery were excluded.

### *Materials and instruments*

Human papillary thyroid carcinoma B-CPAP cells were purchased from Shanghai Enzyme Research Biotechnology Co., Ltd., China. The primer sequences and miRNA negative controls of miR-381-3p, miR-193a and internal control

U6 were designed and synthesized by Shanghai GenePharma Co., Ltd., China. Invitrogen™ TRIzol® reagent was purchased from Thermo Fisher Scientific, USA, while the quantitative real-time polymerase chain reaction (qRT-PCR) kit and minScript® reverse transcription kit were purchased from Dalian TaKaRa Co., Ltd., China. Plus, the following were used in this study: HBS-1096A microplate reader (DeTie Laboratory Equipment, Nanjing, China), CFX96™ real-time PCR detection machine (BioRad, USA), Dulbecco's Modified Eagle's Medium (DMEM; Gibco, USA), fetal bovine serum and trypsin (Hyclone, USA), CCK8 kit (Beijing ThinkFar Technology Co., Ltd., China), Transwell inserts (BD Biosciences, USA), and CyFlow Cube 8 Flow Cytometer (Partec, Germany). See **Table 1.**

### *Detection on expressions of miR-381-3p and miR-193a*

The expressions of miR-381-3p and miR-193a in thyroid cancer tissues and adjacent tissues were detected using qRT-PCR. All samples were taken out of the liquid nitrogen tank 30 min before using, and total RNA from the tissues was extracted according to the instructions on TRIzol® reagent and then dissolved in 20 µL diethyl pyrocarbonate (DEPC)-treated water. Subsequently, the total RNA was reversely transcribed into cDNA using minScript® reverse transcription kit. The volume of reverse transcription system was 15 µL, and the reaction system included 1 µL M-MLV, 1 µL Olig (dT), 0.5 µL RNA enzyme inhibitor, 1 µL dNTPs, and 11.5 µL RNase-free water for supplementation. The reverse transcription reaction was carried out at 37 °C for 30 min, and its inactivation reaction was done at 85 °C for 5 s. The synthesized cDNA was used as a template for amplification using qRT-PCR. The qRT-PCR system was set as 25 µL, containing 1 µL cDNA, 2.5 µL 10× PCR buffer, 1 µL dNTPs, 1 µL upstream primer and 1 µL downstream primer, 0.25 µL Taq DNA polymerase, and 18.25 µL ddH<sub>2</sub>O. The reaction con-

ditions were as follows: pre-denaturation at 95°C for 15 min, denaturation at 95°C for 15 s, annealing at 60°C for 30 s, with 35 cycles; and lastly extension at 72°C for 15 min. Three parallel wells for each sample was prepared for three independent repeats. U6 served as the internal control for miR-381-3p and miR-193a. After the reaction, the real time PCR amplification curve and melting curve were acquired and confirmed, and calculation of the relative quantification ratio for target genes was conducted by  $2^{-\Delta\Delta Ct}$  method.

### *Cell culturing and transfection*

Human papillary thyroid carcinoma B-CPAP cells cultured in DMEM supplemented with 10% FBS were placed at 37°C in an incubator with 5% CO<sub>2</sub> for cell transfection. When the cells reached approximately 80% confluency during transfection, 0.25% trypsin was added for digestion. After digestion, the cells were placed in the culture medium to complete passage. B-CPAP cells in logarithmic phase were selected for transfection and grouped before transfection. Cells without transfection were divided into blank group, negative RNA control group (NC group), miR-381-3p mimic group, and miR-193a mimic group. The Lipofectamine<sup>®</sup> 2000 transfection reagent (11668-019; Shanghai Kemin Biotechnology Co., Ltd., China) was diluted and mixed with DNA according to the manufacturer's instructions. NC, miR-193a mimic and miR-381-3p mimic were transfected into B-CPAP cells by Lipofectamine<sup>®</sup> 2000 respectively, followed by incubation for 5 min at room temperature. Then the mixed solution was further mixed with the transfected B-CPAP cells, followed by further transfection at 37°C in an incubator with CO<sub>2</sub>. The expression of miR-381-3p in B-CPAP cells transfected with miR-381-3p and miR-381-3p-NC, miR-193a, and miR-193a-NC was detected by qRT-PCR at 48 h after transfection.

### *Cell counting kit-8 (CCK8) assay for proliferation detection*

The transfected B-CPAP cells were seeded in 96-well plates with 100  $\mu$ L per well in each group, and then diluted to a density of 4,000 cells per mL after trypsinization procedure, followed by incubation with the plate in a cell incubator for 24 h. After incubation, the culture solution was discarded, and NC, miR-381-3p mimic and miR-193a mimic were added into the wells separately for transfection. At 48 h

after transfection, the cells were collected and diluted to a density of 20,000 cells per mL, then seeded into 96-well plates with 100  $\mu$ L per well in each group and cultured at 37°C in an incubator with 5% CO<sub>2</sub>. Each well was added with 10  $\mu$ L CCK8 solution at 24 h, 48 h and 72 h after the growth of the cells adherent to the wall. Then the cells were continuously cultured in the incubator. After one-hour incubation, the absorbance was measured at 450 nm by HBS-1096A microplate reader (Nanjing Detie Laboratory Equipment Co., Ltd., China) to detect the cell proliferation. Each experiment was repeated three times.

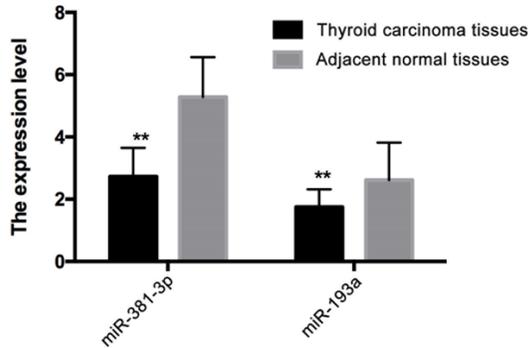
### *Transwell assay for invasion ability determination*

The cells were digested by trypsin, and then centrifuged for 5 min, followed by discarding the culture solution and twice washings with PBS. Subsequently, the cells were resuspended in serum-free culture medium supplemented with BSA to adjust the cell density to  $5 \times 10^4$  cells per mL. The lower compartment of the 6-well plate was added with 1 mL DMEM supplemented with fetal bovine serum. The top of the filter membrane in a transwell insert was plated with 2 mL of cell suspension. After 48 h culturing, the supernatant was removed with cotton swabs, and the transwell insert was taken out and washed with PBS. Cells on the underside of the filter were fixed with 70% ethanol solution for 30 min, and washed again with PBS after taking out, and then were stained with 0.1% crystal violet (Shanghai Baoman Biotechnology Co., Ltd., China). After staining, numbers of invaded cells were counted in six randomly selected fields underneath a microscope, and the average value was calculated. Each experiment was repeated three times.

### *Flow cytometry assay for apoptosis detection*

After digestion with trypsin, cells at 48 h after transfecting with miR-381-3p, miR-193a and NC were collected respectively, fixed for 24 h with 75% ethanol solution at 20°C, and then centrifuged at 3,000 rpm for 5 min at constant temperature of 4°C. After discarding the ethanol solution, the cells were rinsed once with PBS, and again centrifuged at 3,000 rpm for 5 min at constant temperature of 4°C, followed by discarding the supernatant. The samples were all added with 500  $\mu$ L DNA stabilizing solution with full mixing. Finally, the prepared

## Thyroid cancer with microRNA-381-3p and microRNA-193a



**Figure 1.** Expression of miR-381-3p and miR-193a in thyroid carcinoma and adjacent normal tissues. The expressions of miR-381-3p and miR-193a in thyroid cancer tissues and adjacent tissues were detected using quantitative reverse transcriptase-PCR. Three parallel wells for each sample was prepared for three independent repeats. \*\* $P < 0.001$  vs. the miRNA expression level in the adjacent tissue.

solution was transferred to flow tubes. After incubation on ice in dark for 30 min, the samples were detected by CyFlow® Cube 8 flow cytometer.

### Statistical analysis

The data obtained in this study were analyzed using the SPSS software version 17.0 (IBM Corp, Armonk, NY, USA). Enumeration data were expressed as the number of cases/percentage (n, %). Between-group comparison of enumeration data was conducted by chi-square test. Measurement data were expressed as mean  $\pm$  standard deviation ( $\bar{x} \pm sd$ ). Between-group comparison of measurement data was carried out by t-test. Multi-group comparison was conducted by analysis of variance expressed by ANOVA, and the pairwise post hoc comparison of analysis of variance was conducted by Fisher's LSD test. For all analyses,  $P$  values  $< 0.05$  were considered statistically significant.

## Results

### Expressions of miR-381-3p and miR-193a in thyroid carcinoma and adjacent tissues

The expression levels of miR-381-3p in thyroid carcinoma tissues and adjacent tissues were  $2.73 \pm 0.92$  and  $5.28 \pm 1.28$ , and the expression levels of miR-193a in thyroid carcinoma tissues and adjacent tissues were  $1.75 \pm 0.57$  and

$2.62 \pm 1.20$ , respectively. By comparison, the results indicate that the expression levels of both miR-381-3p and miR-193a in thyroid carcinoma tissues were significantly lower than those in adjacent tissues (both  $P < 0.001$ ). See **Figure 1**.

### Relative expressions of miR-381-3p and miR-193a in cells after transfection

The expression levels of miR-381-3p in miR-381-3p mimic group, NC group and blank group were  $5.01 \pm 1.32$ ,  $2.69 \pm 0.92$  and  $2.72 \pm 0.89$ , respectively. The expression level of miR-381-3p in miR-381-3p mimic group was significantly higher than that in NC group or in blank group (both  $P < 0.001$ ). The expression levels of miR-193a in miR-193a mimic group, NC group and blank group were  $2.63 \pm 1.21$ ,  $1.74 \pm 0.58$  and  $1.75 \pm 0.54$ , respectively. The expression level of miR-193a in miR-193a mimic group was significantly higher than that in NC group or in blank group (both  $P < 0.001$ ). While the expression levels of miR-193a and miR-381-3p showed no significant differences between NC group and blank group (both  $P > 0.05$ ). See **Figure 2**.

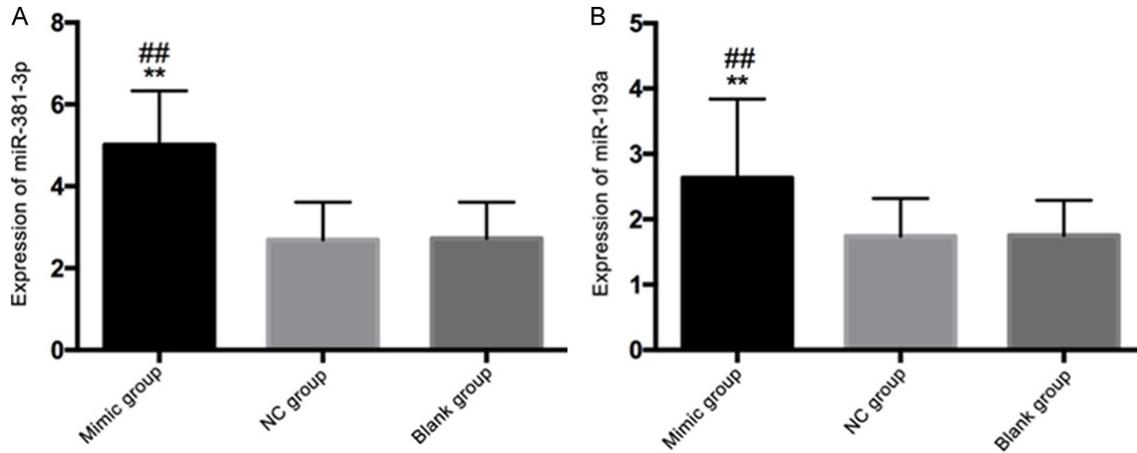
### Comparison on proliferation ability of B-CPAP cells after transfection

On intra-group comparison, the results showed that the cell proliferation activity in both miR-381-3p mimic group and miR-193a mimic group decreased gradually from 24 h to 72 h, and the differences were statistically significant when compared between any two different time points in the group (all  $P < 0.001$ ). There were no significant differences on cell proliferation activity at 24 h among miR-381-3p mimic group, NC group and blank group (all  $P > 0.05$ ). At 48 h and 72 h, the cell proliferation activity of miR-381-3p mimic group and miR-193a mimic group were both significantly lower than that of NC group or blank group (all  $P < 0.001$ ). No significant differences were shown between NC group and blank group on cell proliferation activity at 48 h and 72 h (both  $P > 0.05$ ). See **Figure 3**.

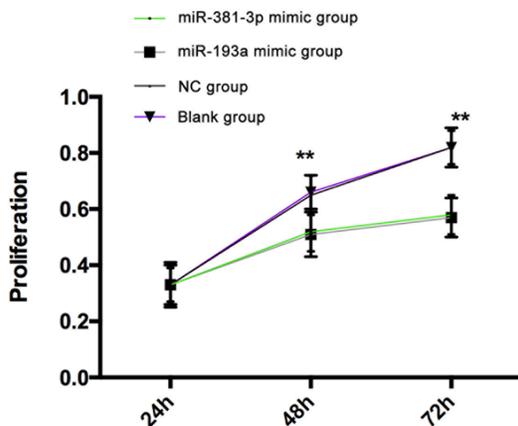
### Comparison on invasion ability of B-CPAP cells after transfection

The numbers of invaded cells in miR-381-3p mimic group, miR-193a mimic group, NC

## Thyroid cancer with microRNA-381-3p and microRNA-193a



**Figure 2.** Relative expressions of miR-381-3p (A) and miR-193a (B) in cells after transfection in different groups. The relative expressions of miR-381-3p and miR-193a in cells after transfection were detected using quantitative reverse transcriptase-PCR. Three parallel wells for each sample was prepared for three independent repeats. \*\* $P < 0.001$  vs. NC group or blank group; \*\*\* $P < 0.001$  vs. blank group.



**Figure 3.** Comparison on proliferation ability of B-CPAP cells after transfection among different groups. Cell counting kit-8 assay was used for identifying proliferation ability of B-CPAP cells after transfection. Each experiment was repeated three times. The cell proliferation activities at 48 h and 72 h of miR-381-3p mimic group and miR-193a mimic group were significantly lower than that of NC group or blank group, \*\* $P < 0.001$  vs. miR-381-3p mimic group or miR-193a mimic group.

group and blank group were  $120.40 \pm 61.37$ ,  $122.21 \pm 57.30$ ,  $383.74 \pm 53.26$ , and  $389.56 \pm 50.10$ , respectively. The number of invaded cells in miR-381-3p mimic group and miR-193a mimic group were both significantly lower than that in blank group or NC group (all  $P < 0.001$ ). However, there were no significant differences in the number of invaded cells between miR-381-3p mimic group and miR-

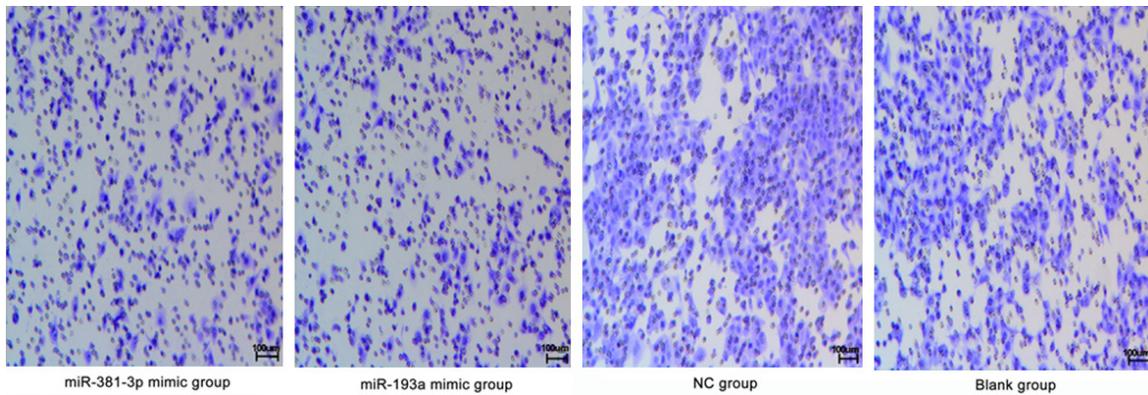
193a mimic group, and between blank group and NC group (both  $P > 0.05$ ). See **Figures 4** and **5**.

### Comparison on apoptosis ability of B-CPAP cells after transfection

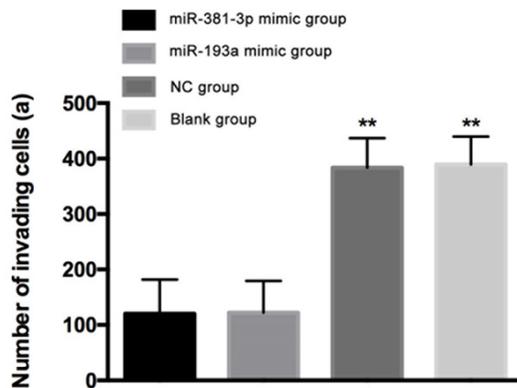
The apoptosis rates of miR-381-3p mimic group and miR-193a mimic group were  $21.39\% \pm 2.57\%$  and  $20.95\% \pm 3.73\%$ , respectively. There was no significant difference in the apoptosis rate between miR-381-3p mimic group and miR-193a mimic group ( $P > 0.05$ ). However, both miR-381-3p mimic group and miR-193a mimic group were significantly higher than that of NC group ( $3.82\% \pm 0.21\%$ ) or blank group ( $3.51\% \pm 0.46\%$ ) in apoptosis rate (all  $P < 0.001$ ). No significant difference was shown between NC group and blank group in apoptosis rate ( $P > 0.05$ ). See **Figures 6** and **7**.

### Discussion

Changes in biological functions including growth and apoptosis of carcinoma cells are closely linked with the occurrence and progression of the tumor [17]. In recent years, risk factors causing thyroid cancer have received extensive concern from clinical research as the increase on morbidity and mortality of thyroid cancer worldwide [18, 19]. MiRNAs degrade or inhibit the transcription or translation of mRNAs with cancerpromoting and inhibiting effects in thyroid carcinoma cells mainly by binding to the 3'



**Figure 4.** The numbers of invaded cells in miR-381-3p mimic group, in miR-193a mimic group, in NC group and in blank group. The picture scale is 100:1.



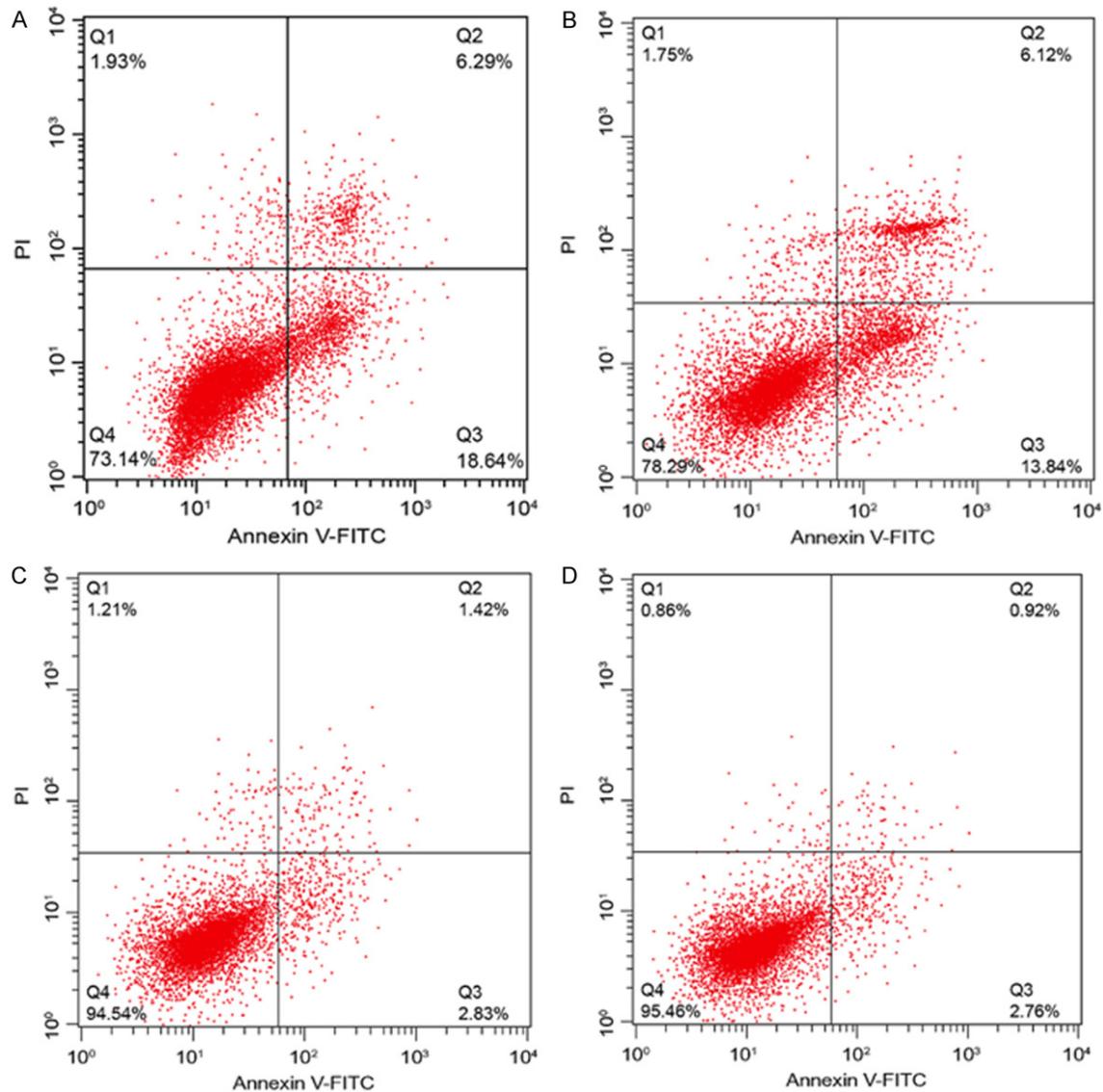
**Figure 5.** Comparison of invasion of B-CPAP cells among different groups. miR-381-3p mimic group. Transwell assay was used for invasion ability determination. Each experiment was repeated three times. \*\*P<0.001 vs. miR-381-3p mimic group or miR-193a mimic group.

untranslated region (3'UTR) of the target gene, thus regulating various functions of the carcinoma cells including cell cycle, differentiation, and cell stress [20].

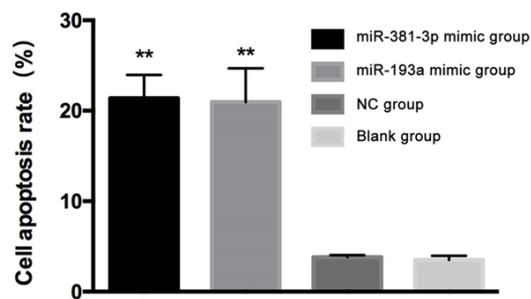
Relevant studies show that miRNAs target proteins of related pathways through the 3'UTR regions of their target genes to affect thyroid tumorigenesis, and these miRNAs present with aberrant expressions in thyroid cancer [21]. To date, some studies suggest that miR-381-3p and miR-193a have predictive effects on the diagnosis and disease changes of thyroid cancer [22]. Relevant studies on targeted regulation of miRNA expression have found that miR-381-3p, although down-regulated in oral cancer, can effectively repress the proliferation of carcinoma cells by overexpressing miR-381-3p

as a carcinoma cell inhibitor [23]. MiR-381-3p is down-regulated in cervical cancer and thyroid cancer, and appropriate overexpression of miR-381-3p can restrain the proliferation of cervical carcinoma cells and thyroid cancer cells [24]. The abnormal down-regulation of miR-193a in colorectal carcinoma cells is the critical cause for proliferation of the carcinoma cells [25]. In this study, we found that the expression levels of both miR-381-3p and miR-193a in thyroid carcinoma tissues were significantly lower than those in adjacent tissues. MiR-381-3p and miR-193a serve as important regulatory factors affecting cancer progression. Meanwhile, we believe that appropriate overexpression of miR-381-3p and miR-193a in human papillary thyroid carcinoma B-CPAP cells can effectively inhibit the proliferation of thyroid carcinoma cells.

Moreover, we found that miR-381-3p and miR-193a can inhibit invasion and promote apoptosis of B-CPAP cells. The main cause of death among cancer patients is often due to invasion and metastasis of carcinoma cells [26, 27]. The condition that the invasion of carcinoma cells in number is controlled and the apoptosis speed is accelerated indicates the alleviation to a certain extent on continuous deterioration of the cancer [28]. Immunohistochemical assay in studies on miRNAs and carcinoma cells confirmed that overexpression of miRNAs effectively inhibits carcinoma cell invasion in number with significantly increased apoptosis on carcinoma cells [29]. Therefore, we believe that appropriate regulation of miRNAs can affect invasion in number and apoptosis rate of B-CPAP cells.



**Figure 6.** Apoptosis abilities of B-CPAP cells after transfection in mir-381-3p mimic group (A), mir-193a mimic group (B), NC group (C) and blank group (D). Flow cytometry assay was used for apoptosis detection. Each experiment was repeated three times.



**Figure 7.** Comparison of apoptosis rate of B-CPAP cells after transfection among different groups. Flow cytometry assay was used for apoptosis detection. Each experiment was repeated three times. \*\*P<0.001 vs. mir-381-3p mimic group or mir-193a mimic group.

In this study, there are still some deficiencies. For instance, the detailed mechanism of effects of miR-381-3p and miR-193a on human papillary thyroid carcinoma B-CPAP cells has not been explored in this study, so more studies are needed for further in-depth research and discussion. This study believes that miR-381-3p and miR-193a both participate in the biological process of B-CPAP cells, or can be used as diagnostic markers and therapeutic targets for thyroid cancer. Therefore, we will carry out miRNA detection on thyroid carcinoma cells of different types in the later research, and continuously perfect the exploration on the relationship between miR-381-3p and miR-193a in

thyroid carcinoma cells of other pathological types.

In conclusion, miR-381-3p and miR-193a present low expression levels in thyroid carcinoma tissues. Overexpression of miR-381-3p and miR-193a can inhibit proliferation and invasion of human papillary thyroid carcinoma B-CPAP cells and promote apoptosis of the cells.

### Disclosure of conflict of interest

None.

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