Research Article

MiR-195 Inhibits Tumor Growth and Metastasis in Papillary Thyroid Carcinoma Cell Lines by Targeting CCND1 and FGF2

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Background. MicroRNA (miRNA) dysregulation was commonly seen in papillary thyroid carcinoma (PTC), and miR-195 was verified to be downregulated in PTC by the large data set analysis from The Cancer Genome Atlas (TCGA). Our study aimed to explore the biological functions and the underlying molecular mechanisms of miR-195 in PTC.

Methods. The relative expression of miR-195 and its target genes were assessed by quantitative RT-PCR assay in 38 pairs of PTC and the adjacent thyroid tissues. Assays were performed to evaluate the effect of miR-195 on the proliferation, migration, and invasion in PTC cell lines. Moreover, we searched for targets of miR-195 and explored the possible molecular pathway of miR-195 in PTC.

Results. We found that miR-195 was downregulated in PTC cell lines and tissues. Overexpression of miR-195 significantly inhibited cell proliferation, migration, and invasion in K1 and BCPAP cell lines. CCND1 and FGF2, which had inverse correlations with miR-195 in clinical specimens, were found to be the direct targets of miR-195. Furthermore, miR-195 might be involved in PTC tumorigenesis by suppressing the Wnt/β-catenin signaling pathway.

Conclusions. These results highlight an important role of miR-195 in the initiation and progression of PTC and implicate the potential application of miR-195 in PTC target therapy.

1. Introduction

Thyroid carcinoma, whose incidence has been dramatically rising all over the world in recent decades, represents the most prevalent endocrine malignancy [1]. Papillary thyroid carcinoma (PTC) accounts for about 80 percent of all thyroid cancer cases [2]. PTC is one of the largest incidence-increasing cancers among Chinese women [3]. By 2019, PTC is predicted to be the third most common cancer in women at a cost of $18–$21 billion dollars in the United States [4]. Comprehensive therapy including thyroidectomy, radioactive iodine (RAI), and TSH suppression therapy affords curable treatment with five-year survival rate over 95% before tumor cell dissemination. However, distant metastasis and recurrence still occurred in some subtype of PTC, and the five-year survival rate of advanced PTC is about 59% [5]. Thus, exploring the underlying molecular mechanism is of great importance for improving the prognosis of PTC patients.

MicroRNAs (miRNAs) are a conserved class of endogenous, small noncoding RNAs. These lead to the silencing of their target genes by either degrading mRNA molecules or inhibiting their translation [6]. miRNAs have been involved in various biological events including tumorigenesis and metastasis, implying their crucial role in the pathogenesis of diverse human malignancies. The classic upregulated miRNAs, such as miR-146b, miR-222, miR-221, and miR-151, were thought to be involved in the development, especially the metastasis of PTC [7, 8]. Circulating miR-222 and miR-146b levels were found to be associated with PTC recurrence and indicate bad clinical survival [9]. Our recent research suggested that miR-20b can modulate MAPK/ERK cascade...
by suppressing SOS1 and ERK2 and repress cell viability, migration, and invasion in PTC [10]. Taken together, it indicates that dysregulated miRNAs play a crucial role in the pathogenesis of PTC.

The miR-15 family members, miR-195 included, are known as tumor suppressors in breast cancer [11], prostate cancer [12], and hepatocellular carcinoma (HCC) [13]. Recently, Cong et al. [14] analyzed the expression of miRNAs and genes in 499 PTC samples and 58 normal thyroid tissues obtained from The Cancer Genome Atlas (TCGA) database and found that miR-195 was downregulated in PTC compared with normal thyroid tissues. However, the role of miR-195 in PTC has not been confirmed, and the molecular mechanism of miR-195 regulation in thyroid carcinoma remains unclear.

In this study, we aim to clarify the biological effects of miR-195 and to explore the possible targets and the regulatory pathway that miR-195 might be involved in.

2. Materials and Methods

2.1. Cell Culture and Clinical Specimens. Human thyroid normal cell line Nthy-ori 3-1 and PTC cell line BCPAP were kindly given by Dr. Haixia Guan (The First Affiliated Hospital of China Medical University, Shenyang, China). K1, another PTC cell line, was purchased from the European Collection of Cell Cultures (ECACC, Salisbury, UK). HEK 293T cell was purchased from the American Type Culture Collection (ATCC, Manassas, USA). Cells were maintained at 37°C in Dulbecco’s modified Eagle’s medium (DMEM, Invitrogen Technologies, USA) with 10% fetal bovine serum (FBS).

All clinical tissues including PTC and the matched normal thyroid tissues were collected at the First Affiliated Hospital and the Cancer Center of Sun Yat-Sen University (Guangzhou, China) from December 2014 to December 2016. The surgical procedure was performed on all patients, and final diagnoses were based upon pathological examination. All subjects provided informed consent, and the study was approved by the ethics committee of Sun Yat-Sen University.

2.2. RNA Extraction and Real-Time Quantitative RT-PCR (RT-qPCR). Total RNA was isolated from cell lines and patient tissues using TRIzol (Life Technologies, USA) and was reverse-transcribed by Prime Script RT reagent kit (Takara, Dalian, China). RT-qPCR was performed to evaluate the expression of miR-195 using a Light Cycler 480II real-time PCR system (Roche Diagnostics, Switzerland) using SYBR Premix Ex Taq™ (Takara, Dalian, China), with U6 and GAPDH as endogenous control. The sequences of primers used in this study were as follows: miR-195 forward primer: 5′-TGAGCAACAGAAATTTGCC-3′; reverse primer: Uni-miR qPCR primer (Takara, Dalian, China), and U6 forward primer: 5′-ACGCAAATTCGTGAAGCCT-3′; reverse primer: Uni-miR qPCR primer (Takara, Dalian, China), and Cyclin D1 (CCND1) forward primer: 5′-TCCTACTACC GCCTACA-3′; reverse primer: 5′-ACCTCCTCCTCTCCTCT CT-3′, and fibroblast growth factor 2 (FGF2) forward primer: 5′-TCAAGCAGAAGAGAGAGGAG-3′; reverse primer: 5′-CCGTAACACATTAGAAGCC-3′, and GAPDH forward primer: 5′-GCACCGTGCAAGGTGAGAAC-3′; reverse primer: 5′-TGTGTAAGAGCAGCAGTTGA-3′. Relative expression quantification was calculated using the comparative cycle threshold (CT) method (2^-ΔΔCT).

2.3. Western Blot Assay. Total cellular protein was prepared as follows: cells were lysed in RIPA buffer. Nuclear and cytoplasmic extractions were collected using the Nuclear and Cytoplasmic Extraction Reagents kit (Thermo Fisher Scientific, Rockford, USA). Then, obtained proteins were subjected to 10% SDS-PAGE and transferred onto PVDF membranes (Roche Diagnostics, Switzerland). After blocking with 5% skimmed milk, the membranes were incubated overnight with the following primary antibodies: anti-cyclin D1 (Lab Vision, #RB010P0), anti-FGF2 (Cell Signaling Technology, #3196S), anti-c-Myc (Cell Signaling Technology, #5605), anti-MMP-13 (Abcam, ab51072), anti-β-catenin (Abcam, ab32572), anti-phospho-β-cateninSer33/37/Thr41 (Cell Signaling Technology, #9561), and anti-GAPDH (Santa Cruz, #sc-25778). This was followed by incubation with HRP-conjugated secondary antibodies from CST. Antigen-antibody complexes were visualized using the ECL solution (Thermo Fisher Scientific, Rockford, USA).

2.4. Immunohistochemistry (IHC). Paraffin-embedded tissue sections were deparaffinized and hydrated using xylene and graded alcohol to water. Antigen retrieval was performed by incubation of the tissue sections with boiled sodium citrate buffer (pH 6.0) for 3 min. Endogenous peroxidase activity was quenched with 3% H2O2. Slides were blocked with 5% BSA to reduce nonspecific binding and then incubated with CCND1 (Lab Vision, #RB010P0) or FGF2 (Cell Signaling Technology, #3196S) primary antibody diluted to a concentration of 1:100 overnight at 4°C. After incubation with the secondary antibody (Gene Tech) for 30 min at room temperature, slides were washed with 3% H2O2, and then slides were detected with the DAB Enhancer solution (Gene Tech) and counterstained with hematoxylin (MX Biotechnologies). Images were taken by a light microscope.

2.5. RNA Oligoribonucleotides and Plasmid/Lentivirus Constructs. MiR-195 mimics and negative control (NC) were purchased from GenePharma (Shanghai, China). The specific sequences are as follows: miR-195 sense: 5′-UAGCAGC ACAGAAUAUUGGC-3′; antisense: 5′-CAAAUUUUUC UGCUGCUAUU-3′; NC sense: 5′-UUUCUGGCAAGCGUG UCAGUTT-3′; antisense: 5′-AGUCGACAGUUGCGAGAA TT-3′, pcDNA 3.1-CCND1 and pcDNA 3.1-FGF2 were purchased from GeneRay (Shanghai, China). The 3′-untranslated regions (3′-UTRs) of CCND1 and FGF2 were cloned into the pGL3-basic vector (Promega, USA) with XbaI and PciI at the downstream of the luciferase gene. To mutate the binding sequence of miR-195 in the 3′-UTRs, a QuikChange, Site-Directed, Mutagenesis kit (Promega, USA) was used following the instruction. The mature sequence of miR-195 was amplified and cloned into the lentiviral vector LV3-GFP-puro (GenePharma, Shanghai, China) to generate LV3-miR-195 cell, and negative control LV3-NC was conducted as the same way.
2.6. Cell Transfection. RNA oligoribonucleotides or plasmids were transfected using Lipofectamine 3000 (Invitrogen, USA) following the manufacturer’s protocol. A total of 200 nM of miRNA mimics or 1000 ng plasmid were used for each 6-well plate transfection. BCPAP and K1 cells were infected with recombinant LV3-miR-195/LV3-NC lentivirus-transducing units plus polybrene (GenePharma, Shanghai, China). After 48 h of transfection, puromycin was added into the culture cells constantly. Stable transfected cells were obtained after 2–4 weeks.

2.7. Luciferase Reporter Assay. HEK 293T cells seeded in 24-well plates were cotransfected with 400 ng of firefly luciferase reporter containing the 3′-UTR (wild-type or mutant) of CCND1 or FGF2, 10 ng of pRL-TK, and 20 pmol of miRNA mimics. Luciferase activities were measured 48 h after transfection using the dual-luciferase reporter assay system (Promega, Madison, USA).

2.8. Cell Proliferation and Colony Formation Assays. EdU assay was performed to assess the cell proliferative ability using the EdU kit (Ribobio, Guangzhou, China) following the manufacturer’s manuals. For the colony formation assay, after two days of transfection, indicated cells were seeded at 1000/well into each 6-well plate and cultured for two weeks. Cell colonies were stained in a dye solution containing 1% crystal violet.

2.9. Scratch Wound-Healing Assay. After 48 h of transfection, cells were about 90–95% confluence in 6-well plate. Streaks were carefully scratched with sterile pipette tips. Then, cells were cultured in medium with no serum overnight. The widths of wound were observed and photographed using an inverted microscope (Leica, Germany).

2.10. Cell Migration and Invasion Assays. Cells resuspended in 100 μL serum-free medium were plated in the top chamber of each insert (Corning, USA) with a non-Matrigel-coated membrane for the Transwell migration assay and a Matrigel-coated membrane (BD Bioscience, MA, USA) for the invasion assay. Lower chambers of the inserts were filled with 600 μL medium with 10% FBS. After several hours of incubation, cells that invaded to the lower surface of the insert were fixed, stained, and imaged using an DMI4000B inverted microscope (Leica, Germany).

2.11. Xenograft Tumor Formation. K1 cells stably infected with the LV3-miR-195 or LV3-NC were harvested and washed by phosphate-buffer saline. Then, cells (5 × 10⁶) were subcutaneously injected into the right flank of BALB/c nude mice (8 per group). The width and length of tumors were measured every 5 days. Tumor volumes were calculated by the formula: \( V = \text{width}^2 \times \text{length}/2 \). On day 23 after implantation, mice were sacrificed and the tumor weights were assessed. The animal study was approved by the Animal Ethical Committee of Sun Yat-Sen University.

2.12. Statistical Analysis. We used SPSS software (version 20.0) for all statistical analyses. The significance of different groups of data was calculated with two-tailed Student’s t test or with one-way ANOVA analysis. All data are presented as the mean ± standard deviation (SD) from at least triple replicates. Spearman’s correlation analysis was performed between miR-195 and its target genes. \( P < 0.05 \) was considered statistically significant.

3. Results

3.1. MiR-195 Is Downregulated in PTC Clinical Specimens and Cell Lines. To determine the potential role of miR-195 in PTC, we analyzed the relative expression of miR-195 in 38 pairs of PTC tissues and two cell lines. The average expression of miR-195 was downregulated in PTC tissues compared with the matched normal thyroid tissues (\( P < 0.05 \), Figure 1(a)). Furthermore, compared with normal thyroid cell line Nthy-ori 3–1, miR-195 was significantly decreased in K1 and BCPAP cell lines (\( P < 0.01 \), Figure 1(b)).

We assessed the correlation between miR-195 and clinicopathologic status of PTC patients. The results showed that
the level of miR-195 was almost significantly associated with cervical LN metastasis ($P = 0.07$, Supplementary Table 1 available online at https://doi.org/10.1155/2017/6180425). Possible associations between the level of miR-195 and extra-thyroidal invasion and TNM stage were analyzed; the data showed a trend that more invasive and advanced stage cancers have lower expression level of miR-195. However, no significant $P$ value was found (Supplementary Table 1). This was thought to be caused by the small number of clinical samples.

3.2. MiR-195 Inhibits PTC Cell Growth In Vivo and In Vitro. MiR-195 mimics was transfected into K1 and BCPAP cell lines. The relative expression of miR-195 was significantly higher in the mimic group than the control group after transfection (Supplementary Figure 1). To identify the role of miR-195 in cell growth, EdU assay and colony formation assay were performed.

The EdU assay showed that the number of EdU positive cells was significantly lower in miR-195 overexpressing cells than control cells (Figures 2(a), 2(b), 2(c), and 2(d)). The colony formation assay confirmed the decrease rate of growth in miR-195 overexpressing cells. It showed that miR-195 overexpressing PTC cells generated a significantly lower number of colonies as compared with control cells (Figures 2(e) and 2(f)).
Additionally, the xenograft tumor formation assay was performed to assess the growth-inhibitory effect of miR-195 in vivo. Intriguingly, we found miR-195 significantly reduced tumor growth (Figure 3(a)). Both the average tumor volume and the tumor weight were obviously lower in LV3-miR-195 group mice compared with those in the LV3-NC group (Figures 3(b) and 3(c)). These results indicate that miR-195 suppressed thyroid tumor growth in vitro and in vivo.

3.3. MiR-195 Suppresses PTC Cell Migration and Invasion.
To elucidate the effects of miR-195 on the migration and invasion of PTC cells, Matrigel-coated or Matrigel-uncoated Transwell assays were analyzed. Both the invasive and migratory activities in K1 and BCPAP cells were suppressed by miR-195 (Figures 4(a), 4(b), 4(c), and 4(d)). Also, the wound healing assay illustrated that miR-195 overexpression impaired the wound closure ability of K1 and BCPAP cells (Figures 4(e) and 4(f)). Collectively, these data suggest that miR-195 inhibited PTC cell migration and invasion.

3.4. MiR-195 Directly Targets the 3′-UTRs of CCND1 and FGF2.
We searched for putative targets of miR-195 using miRanda (http://www.microrna.org/microrna/home.do) and TargetScan (http://www.targetscan.org/). Among hundreds of promising targets, CCND1 and FGF2 were chosen because of their well-known importance in cell growth [15] and metastasis [16], respectively. The predicted binding sites of miR-195 seed sequence and 3′-UTRs of its target genes are shown in Figures 5(a) and 5(b). The sequences precisely modified were marked in red. The mRNA expression levels of CCND1 and FGF2 were significantly decreased with miR-195 transfection in K1 and BCPAP cells (Figure 5(c)). In the same vein, the protein levels of these two genes were suppressed in miR-195 overexpressing cells (Figure 5(d)). To confirm the direct relationships between miR-195 and its target genes, a dual-luciferase reporter assay was performed. It revealed that cotransfection of miR-195 inhibited the activity of luciferase reporter with wild-type 3′-UTR of CCND1 and FGF2. However, this effect was abrogated when the target site was mutated (Figure 5(e)). Furthermore, we assessed the relative expression of CCND1 and FGF2 mRNAs by qRT-PCR in the same set of clinical samples shown in Figure 1(a). Obvious inverse correlations between miR-195 and CCND1 as well as FGF2 were confirmed (Figures 5(f) and 5(g)). CCND1 and FGF2 protein expression of the same clinical samples was analyzed by immunohistochemistry staining. As shown in Figure 5(h), intensive CCND1 and FGF2 expression was detected in PTC as compared with normal thyroid tissues.

These data indicate that miR-195 negatively regulates CCND1 and FGF2 expression by directly targeting their 3′-UTRs and this target effect is consistent with the inverse correlations in clinical samples.

3.5. Overexpression of CCND1 or FGF2 Can Rescue the Inhibitory Function of miR-195 in PTC Cells.
Next, we investigated whether CCND1 and FGF2 were functionally related with miR-195. K1 and BCPAP cells were cotransfected with miR-195 and CCND1 or FGF2 plasmids (Figures 6(a) and 6(b)). The results showed reexpressing CCND1 partially abrogated the growth inhibitory effect of miR-195 (Figure 6(c)). Meanwhile, restored expression of FGF2 could antagonize the miR-195 induced inhibition of cell migration and invasion (Figures 6(d) and 6(e)).
Figure 4: MiR-195 suppresses PTC cell migration and invasion. (a) Transwell migration assays of K1 and BCPAP cells overexpressing miR-195 were performed without the Matrigel-coated chamber. Magnification, 200x. (b) Quantification of migrated cells. (c) Transwell invasion assays of K1 and BCPAP cells overexpressing miR-195 were performed with the Matrigel-coated chamber. Magnification, 200x. (d) Diagrams of invasive cells. (e) Wound healing assays of PTC cells after transfection of miR-195. (f) Percentage of wound healing closure assays. **P < 0.01 compared with the control group, ***P < 0.001 compared with the control group.
**Figure 5:** MiR-195 directly targets the 3′-UTRs of CCND1 and FGF2. (a, b) Predicted binding sites of miR-195 seed sequence and 3′-UTRs of CCND1 and FGF2. The sequences precisely modified were marked in red. (c) The relative mRNA expression of CCND1 and FGF2 in K1 and BCPAP cells 24 h after miR-195 transfection. (d) Protein levels of CCND1 and FGF2 48 h after miR-195 transfection. (e) Luciferase assays of CCND1 and FGF2 in 293T cell line. (f, g) Significant inverse correlations were presented between miR-195 and CCND1 as well as FGF2 in human PTC tissues. (h) IHC detection of CCND1 and FGF2 proteins in normal thyroid and PTC tissues. Two representative examples are shown. Magnification, 400x. **P < 0.01 compared with the control group, ***P < 0.001 compared with the control group.
As a whole, these findings point that miR-195 inhibits cell growth by targeting CCND1 and suppresses migration and invasion by targeting FGF2 in PTC cells.

3.6. MiR-195 Suppresses the Wnt/β-Catenin Pathway in PTC Cells and Xenograft Tumors. As the Wnt/β-catenin cascade is of great importance in PTC pathogenesis, we investigated the effects of miR-195 on this signaling pathway. MiR-195 overexpressing remarkably increased the phosphorylation of β-catenin (Figure 7(a)). To trace the amount of β-catenin translocating into the nucleus, which is the effective factor transcribing various tumor-promoting genes, we separated nuclear proteins from cytoplasmic ones and found upregulation of miR-195 caused a decline of nuclear β-catenin (Figure 7(a)). Moreover, the downstream protein of the Wnt/β-catenin pathway, namely, c-Myc, was obviously suppressed (Figure 7(a)). Besides, as FGF2 is an in vivo modulator of matrix metallopeptidase 13 (MMP-13) expression in malignant tumors [17], a significant decrease in the level of MMP-13 protein was also observed in miR-195 overexpressing cells (Figure 7(a)). We also collected proteins from the xenograft in mice and detected the expression level of targets as well as proteins involved in Wnt/β-catenin pathways. As expected, miR-195 overexpression group demonstrated increased phos-β-catenin and decreased nuclear β-catenin. The expression levels of CCND1, FGF2, and c-Myc proteins were significantly reduced in the miR-195 group compared with the NC group (Figure 7(b)).

Taken together, these data indicate that miR-195 could influence the Wnt/β-catenin signaling pathway and MMP 13 in PTC cells.

4. Discussion

miRNAs and protein-coding RNAs consist of a complicated network, which modulates the initiation and progression of
Figure 7: MiR-195 suppresses the Wnt/β-catenin pathway in PTC cells and xenograft tumors. (a) Western blotting analysis of proteins involved in Wnt/β-catenin pathway and MMP13 in PTC cells. The phosphorylation of β-catenin at Ser33/37/Thr41 sites increased in miR-195 overexpressing K1 and BCPAP cells. CCND1, c-Myc, MMP13 proteins, and nuclear β-catenin declined while cytoplasmic β-catenin almost unchanged after miR-195 transfection. (b) Expression profile changes of CCND1, FGF2, and proteins involved in Wnt/β-catenin pathways in xenograft tumor tissues. The miR-195 overexpression group demonstrated increased phos-β-catenin and decreased nuclear β-catenin. The expression levels of CCND1, FGF2, and c-Myc proteins were significantly reduced in the miR-195 group compared with the NC group. Tumors 1, 2, 3, and 4 are from four different mice. Lamin B and GAPDH presented as control. Nuc: nuclear; Cyto: cytoplasmic.
cancers including PTC. In this network, numbers of miRNAs such as miR-146b [18], miR-222 [19], and miR-20b [10] have been proven to promote or suppress the progression of PTC. However, there are still numerous miRNAs whose biological functions and molecular mechanisms remain unknown. Our current study showed that the relative expression of miR-195 was significantly decreased in PTC tissues and cell lines, and it suppressed proliferation, migration, and invasion in PTC cells. The antiproliferative function of miR-195 in vivo was demonstrated by xenograft tumor formation experiment.

It was found that miR-195 was reduced in HCC and exerted a role in the tumorigenesis of HCC [20]. In prostate cancer, miR-195 inhibited cell epithelial-mesenchymal transition (EMT) [21]. Moreover, miR-195 exerted its tumor suppressive effect by targeting VEGF [22], IKKα, and TAB3 [13] in HCC. MiR-195 overexpressing enhanced the radiosensitivity of breast cancer by targeting BCL-2 [23]. In the present study, we found CCND1 and FGF2 were functional targets of miR-195 in PTC and miR-195 was inversely correlated with these two targets.

CCND1, one of the highly conserved members of the cyclin family, was characterized by a periodicity in protein abundance throughout the cell cycle. The deregulation of CCND1 expression was regarded as a hallmark of cancer by causing continuous abnormal proliferation, thus playing as an oncogene [15, 24]. Overexpression of CCND1 gene was observed in both benign and malignant thyroid tumors [25]. In addition, it was found that CCND1 has been regulated by multitudinous miRNAs. For example, miR-138 inhibited nasopharyngeal carcinoma growth by targeting CCND1 oncogene [26]. Cai et al. found CCND1, CDK2, and CDK6 directly targeted by miR-186 in lung adenocarcinoma [27]. Our results showed that restoration of miR-195 induced PTC cell growth arrest by targeting CCND1 in vivo and in vitro.

Fibroblast growth factor 2 (FGF2), a member of the FGF family, controls various cellular processes in different contexts, including migration and invasion [28]. FGF2 is highly expressed in differentiated thyroid cancer [29], as well as in many other malignancies including breast cancer [30] and HCC [31], implying its important role in tumorigenesis. Afterwards, FGF2 was proven to be associated with lymph node invasion and distant metastasis in differentiated thyroid cancers [32, 33]. It suggests that FGF2 plays an important role in thyroid cancer progression. Furthermore, the molecular mechanisms of FGF2 involving oncogenesis were gradually clarified. MiR-503 inhibited tumor angiogenesis in HCC by targeting FGF2 and VEGFA [34]. Besides, miR-646 was found to suppress osteosarcoma cell metastasis by downregulating FGF2 [35]. Meanwhile, FGF2 has been reported to regulate MMP-13 expression with a time- and dose-dependent relation in chondrosarcoma cells [17], leading to the degradation of extracellular migration inhibitory factor (MIF) to promote cancer metastasis [36]. Upregulation of MMP-13 was significantly related with TNM stage and recurrent disease in PTC [37]. Our current study showed that miR-195 inhibited cell migration and invasion by targeting FGF2 and the con-

transfection of miR-195 and FGF2 plasmid which can abrogate the inhibitory effect. The possible mechanism of anti-invasive effect derives from the decreasing level of the downstream MMP-13 protein. Taken together, miR-195-FGF2-MMP-13 axis may be a new target for thyroid cancer metastasis.

The Wnt pathway, especially the canonical Wnt signaling, is involved in many developmental and physiological processes [38], especially in human cancer [39]. Wnt activation induced β-catenin stabilization and nuclear accumulation, leading DNA-bound transcription factor TCF to complex with β-catenin. Together with some other coactivator, this complex activates target genes such as CCND1 and c-Myc [40]. Whereas Wnt signaling restraining can result in β-catenin phosphorylation at serines 33 and 37 and threonine 41, leading to β-catenin ubiquitination and degradation [41]. Activation of the Wnt/β-catenin signaling is often caused by activating mutations of CTNNB1 (which encodes β-catenin) in thyroid cancer, particularly in poorly differentiated thyroid cancer (PDTC) and anaplastic thyroid carcinoma (ATC) [42, 43]. Delocalization of β-catenin was reported to significantly correlate with upregulation of CCND1 in PTC tissues, suggesting the Wnt/β-catenin signaling involved in PTC tumorigenesis [44]. The miR-195/16 cluster was found to decrease the expression of β-catenin by targeting WNT3A signaling in prostate cancer [45]. Whether miR-195 has a similar molecular mechanism as the same family member does remains unknown. Our data demonstrated that miR-195 overexpression increased β-catenin phosphorylation, decreased nuclear β-catenin amount, and thus, suppressed the Wnt/β-catenin signaling in PTC.

With the crucial functions of the Wnt signaling in cancerous transformation and growth, the need for specific drugs targeting the Wnt pathway is urgent. Vandetanib, selectively targeting RET, VEGFR, and EGFR tyrosine kinases, has been approved for the treatment of medullary thyroid carcinoma (MTC). More than that, it was found to inhibit cell growth and migration in PTC by stabilizing β-catenin and decreasing the downstream target genes c-Myc and CCND1 [46]. Meanwhile, some traditional nonsteroidal anti-inflammatory drugs (NSAIDs), such as aspirin, showed an interesting anticancer effect by inhibiting the Wnt signaling [47]. The current study showed the obvious evidence of miR-195 for suppressing the Wnt/β-catenin signaling, indicating the potential target for thyroid cancer therapy. The prospective clinical utility of miR-195 deserves further study.

5. Conclusions

In summary, downregulation of miR-195 is demonstrated in PTC. MiR-195 exerts its tumor suppressive function by targeting CCND1 and FGF2 and restraining the activity of the Wnt/β-catenin signaling. Our findings suggest an important role of miR-195 in the pathogenesis of PTC, and miR-195-FGF2-MMP-13 axis might be a potential new target for PTC.
Ethical Approval

All procedures performed in this study involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. All procedures performed in this study involving animals were in accordance with the ethical standards of the institution or practice at which the study was conducted.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Authors’ Contributions

Yali Yin and Shubin Hong have contributed equally to this work.

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