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miR-199a-5p inhibits the progression of papillary thyroid carcinoma by targeting SNAI1

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ABSTRACT

Background: Increasing evidence has emphasized the important roles of differentially expressed miRNAs in papillary thyroid cancer (PTC) development. miR-199a-5p was previously documented to be downregulated in PTCs compared with normal thyroids. However, the role of miR-199a-5p in the progression of PTC and the underlying mechanism remain to be further addressed.
Methods: miR-199a-5p and snail family zinc finger 1 (SNAI1) mRNA expressions in PTC tissues and cells were detected by qRT-PCR. The effects of miR-199a-5p and SNAI1 on cell migration, invasion and epithelial-mesenchymal transition (EMT) were evaluated by cell migration and invasion assays, and western blot, respectively. The relationship between miR-199a-5p and SNAI1 was investigated by luciferase reporter assay and western blot. Xenograft tumor assay was performed to verify the role of miR-199a-5p and molecular mechanism in PTC.
Results: miR-199a-5p expression was significantly downregulated and SNAI1 was markedly upregulated in PTC tissues and cells. miR-199a-5p overexpression and SNAI1 knockdown suppressed the progression of PTC cells *in vitro*, as evidenced by the reduced cell migration, invasion and EMT. Of note, SNAI1 was identified as a target of miR-199a-5p and miR-199a-5p suppressed SNAI1 expression in PTC cells. Xenograft tumor assay proved that miR-199a-5p overexpression suppressed tumor growth in PTC *in vivo* by downregulating SNAI1.
Conclusion: miR-199a-5p inhibited the progression of PTC by downregulating SNAI1, offering new insight into the molecular mechanism underlying PTC progression.

1. Introduction

Thyroid cancer is the most common endocrine malignancy of the thyroid in adults, with a dramatically increasing incidence rate worldwide over the past decades [1]. As the most prevalent subtype among thyroid malignancies, papillary thyroid cancer (PTC) comprises up to more than 90% of all thyroid cancer cases and is usually associated with an excellent prognosis and therapeutic response [2]. However, about 10%–15% of PTC patients frequently manifest distant metastasis and recurrence, which lead to a poor response to standard treatments and a poor clinical outcome [3,4]. Therefore, it is imperative to elucidate the molecular mechanisms underlying the formation and progression of PTC, which contribute to identifying novel diagnostic biomarkers and therapeutic targets.

miRNAs play a critical role in the pathogenesis and progression of various tumors, where they function as either oncogenes or tumor suppressors according to the roles of their target genes [5]. Increasing evidence has emphasized the important roles of differentially expressed miRNAs in PTC development [6]. Previous studies have demonstrated that miR-199a-5p expression was downregulated in certain tumors, including breast cancer [7], colorectal cancer [8]and hepatocellular carcinoma [9], but upregulated in other types of malignancies, such as gastric cancer [10] and osteosarcoma [11], indicating the tumor-type-specific regulatory mechanisms. Interestingly, miR-199a-5p was previously documented to be downregulated in PTCs compared with normal thyroids [12]. However, the role of miR-199a-5p in the progression of PTC and the underlying mechanism remain to be further addressed.

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Snail family zinc finger 1 (SNAI1), a member of zinc-finger transcription factors, has been well acknowledged to play a crucial role in the induction of epithelial-mesenchymal transition (EMT) during tumor progression [13]. More notably, SNAI1 expression was reported to be higher in PTC tissues and significantly correlated with lymph node metastasis in PTC [14]. However, the biological function of SNAI1 in PTC remains undefined.

In our study, bioinformatics analysis predicted that miR-199a-5p contained the binding sites complementary to the 3′UTR of SNAI1. Hence, our study aimed to investigate the role of miR-199a-5p and SNAI1 in the progression of PTC, and the interaction between them.

2. Materials and methods

2.1. Patients and tissue samples

The study was approved by the Research Ethics Committee of the Sixth People's Hospital of Ji'nan and informed written consent was obtained from each participant. A total of 24 pairs of primary PTC tissue specimens and adjacent normal tissue specimens were obtained from PTC patients (age range, 40–62 years; eleven males and thirteen females) undergoing standard surgical procedures at the Sixth People's Hospital of Ji'nan between 2015 and 2016. Specimens were immediately snap-frozen in liquid nitrogen and stored at −80 °C prior to use. The patient samples were histologically diagnosed based upon pathological examination. None of the PTC patients had received any preoperative treatment including chemotherapy or radiotherapy prior to surgery.

2.2. Cell culture and transfection

Two human papillary thyroid carcinoma cell lines (TPC-1and K1) and HEK 293T cells were purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA) and human thyroid follicular epithelial cells Nthy-ori3-1were obtained from JENNIO Biological Technology (Guangzhou, China). These cells were maintained in Dulbecco's Modified Eagle Medium (DMEM) medium (Gibco, Grand Island, NY, USA) supplemented with 10% fetal bovine serum (FBS; HyClone, Logan, UT, USA) and antibiotics (100 U/mL penicillin and 100 μg/mL streptomycin) (Sigma-Aldrich, St. Louis, MO, USA) in a 5% CO_2 humidified incubator at 37 °C. All the miRNA, siRNA and vectors used in this research were purchased from Gene Pharma Co. Ltd. (Shanghai, China) and transfected into TPC-1and K1 cells when grown to 80% confluence using Lipofectamine3000 (Invitrogen, Carlsbad, CA, USA).

2.3. Quantitative real-time PCR (qRT-PCR)

Total RNA was isolated from tissues and cultured cells with TRIzol (Invitrogen) and RNA concentration was quantified using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). For the detection of SNAI1 mRNA expression, first strand complementary DNA was synthesized using M-MLV Reverse Transcriptase (Invitrogen), followed by RT-PCR with the SYBR® Premix Ex Taq™ II (TaKaRa, Dalian, China), with GAPDH as an internal control. For the detection of mature miR-199a-5p expression, miRNA was reverse transcribed using the TaqMan miRNA Reverse Transcription Kit (Applied Biosystems) and miR-199a-5p expression was detected using TaqMan miRNA assays (Applied Biosystems Inc., Foster City, CA, USA), with U6 small nuclear (snRNA) as an endogenous control. RT-PCR was carried out on Step One Plus™ Real-time PCR Systems (Applied Biosystems, Foster City, CA, USA). Relative expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method [15].

2.4. Cell invasion and migration assay

The upper chambers of transwell inserts (8 μm pores; BD Biosciences, San Jose, CA, USA) precoated with matrigel (BD Biosciences) were used for cell invasion assay. Briefly, 2×10^4

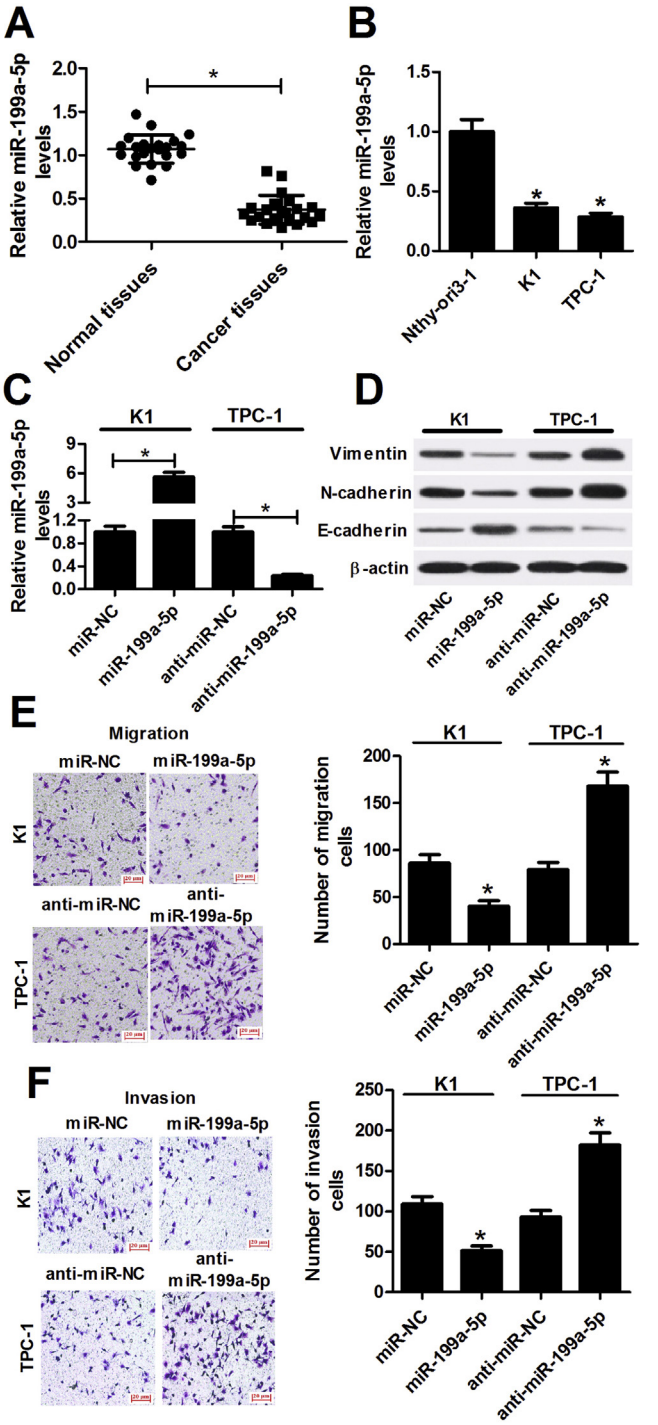

Fig. 1. miR-199a-5p suppressed the progression of PTC cells. (A) The expression of miR-199a-5p in 22 pairs of primary PTC tissues and their adjacent normal tissues was detected by qRT-PCR. (B) The expression of miR-199a-5p in PTC cell lines (K1 and TPC-1) and thyroid follicular epithelial cells Nthy-ori3-1 was detected by qRT-PCR. (C) qRT-PCR analysis of miR-199a-5p expression in the transfected K1 and TPC-1 cells. (D) Western blot analysis of the protein levels of Vimentin, N-cadherin and E-cadherin in treated K1 and TPC-1 cells. (E and F) Cell migration and invasion abilities were evaluated by cell invasion and migration assays in introduced K1 and TPC-1 cells. *P < 0.05.

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