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Circular RNA circ_0103552 promotes the invasion and migration of thyroid carcinoma cells by sponging miR-127

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Abstract. – OBJECTIVE: The aim of this study was to examine the regulatory role of circ-0103552 in the procession of thyroid carcinoma (TC) and the related underlying mechanisms.

PATIENTS AND METHODS: The tissues were obtained from 56 patients diagnosed with TC in our hospital. Nthy-ori3-1 cell line and TC cell lines (TPC-1, SW579, 8505C) were purchased from American Type Culture Collection (ATCC). Quantitative Real Time-Polymerase Chain Reaction (qRT-PCR) was conducted to analyze the expression of circ-0103552 in TC tissues and cell lines. Inhibition of circ-0103552 was achieved by circ-0103552 siRNA. Dual-Luciferase report assay was performed to confirm the potential target miRNA of circ-0103552. The transwell assay and wound-healing assay were designed to examine the invasion and migration ability of TC cells, respectively.

RESULTS: Circ-0103552 was detected to be upregulated in TC tissues, as well as in TC cell lines, including TPC-1, SW579, and 8505C. The knockdown of circ-0103552 significantly reduced the invasion and migration ability in TC cells. It was predicted using the circular RNA database that microRNA-127 (miR-127) was a target miRNA of circ-0103552, which was confirmed by the Dual-Luciferase assay. Further studies revealed that circ-0103552 was involved in the invasion and migration of TC by sponging miR-127.

CONCLUSIONS: The present study demonstrated that circ-0103552 acts as a regulator in the invasion and migration of TC by sponging miR-127.

Key Words:
Thyroid carcinoma (TC), Circ-0103552, MicroRNA-127 (miR-127).

Introduction

Thyroid carcinoma (TC) is one of the common malignant tumors in the endocrine system, whose morbidity rate has been increasing in the past few decades[1]. Due to its higher recurrence rate and poor prognosis, TC seriously affects the quality of life of human[2]. With the rapid development of epigenetics and molecular biology in recent years, the molecular targeted therapy has provided a new strategy and method for the clinical diagnosis and treatment of TC, which has a good application prospect[3,4]. Therefore, exploring the molecular biological mechanism in the occurrence and development of TC and searching for new potential intervention targets on this basis have important clinical significance for increasing the cure rate, reducing the recurrence rate of disease, and improving the prognosis of patients.

Circular ribonucleic acids (circRNAs) are the latest research hotspot in the field of RNA, which are promising molecules in the RNA family following miRNAs and lncRNAs[5]. Unlike traditional linear RNAs, circRNA molecules have a closed-loop structure, not affected by RNA exonuclease, so they are expressed more stably[6,7]. CircRNAs are widely involved in tumorigenesis and cancer progression. In particular, Gao et al[8] reported that circRNA has-circ-0007059 restrains proliferation and epithelial-mesenchymal transition in lung cancer cells *via* inhibiting microRNA-378. Chen et al[9] reported that has-circ-101555 functions as a competing endogenous RNA of miR-597-5p to promote colorectal cancer progression. Recently, it has been reported that circRNA circ-0103552 is able to predict the poor prognosis and promote proliferation and invasion of breast cancer cells by sponging miR-1236[10]. However, whether circ-0103552 is involved in the TC progression remains unknown.

Herein, the present study investigated circ-0103552 expression in clinical patients diagnosed with TC and several TC cell lines, and explored the malignant biological behaviors, including cell migration and invasion. In addition, the underlying

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mechanism was also illustrated and may provide a new theoretical basis for the treatment of TC.

Patients and Methods

Patients and Tissue Samples

All TC tissues and adjacent normal tissues were collected from patients diagnosed with TC at Qingdao Municipal Hospital. All patients underwent no endocrine therapy, radiotherapy, or chemotherapy before the operation. The investigation on tissue specimens was approved by the Local Medical Ethics Committee, and the informed consent form was signed by each patient.

Cell Culture and Transfection

Nthy-ori3-1 cell lines and TC cell lines (TPC-1, SW579, 8505C) were cultured in the Roswell Park Memorial Institute-1640 (RPMI-1640; Gibco, Rockville, MD, USA) containing 10% fetal bovine serum (FBS; HyClone, South Logan, UT, USA), 100 U/mL penicillin, and 100 μg/mL streptomycin in an incubator with 5% CO_2 at 37°C. When 90% of cells were fused, they were gently washed with phosphate-buffered saline (PBS) 3 times and digested with 0.25% trypsin. After standing for about 1 min, the fresh Dulbecco's Modified Eagle's Medium (DMEM; Gibco, Rockville, MD, USA) containing 10% FBS was added to terminate the digestion, and the cell suspension continued to be cultured on the dish.

When 50% of SW579 cells were fused, they were transfected with 50 nmol/L circ-0103552-siRNA plasmid, miR-127 mimic, and miR-127 inhibitor according to the instructions of Lipofectamine 3000 (Invitrogen, Carlsbad, CA, USA). After 48 h, the cells were digested with trypsin for subsequent experiments. Thus, the cells were divided into the NC group (untreated SW579 cells), siRNA group (SW579 cells transfected with circ-0103552-siRNA plasmid), and siRNA+inh-miR-127 group (SW579 cells co-transfected with circ-0103552-siRNA plasmid and miR-127 inhibitor).

Transwell Assay

The Matrigel prepared was added into the transwell chamber (60 μL/well), and plated in the incubator at 37°C for 30 min. Then, the cells in the logarithmic growth phase were digested with trypsin and resuspended with the serum-free medium. 200 μL of cell suspension containing 1×10^5 cells were added into the upper chamber, while 600 μL of RPMI-1640 medium containing 10% FBS was added into the lower chamber. After incubation in the incubator with 5% CO_2 at 37°C for 24 h, the cells were washed with PBS, fixed with 4% paraformaldehyde at room temperature, and stained with 0.1% crystal violet dye for 10 min. Finally, the chambers were taken out, the cells adhering to the filter membrane were wiped off using the cotton swab, and the number of cells passing through the membrane was counted in 5 randomly selected fields of view under an inverted phase-contrast microscope.

Wound Healing Assay

After transfection for 48 h, the SW579 cells were seeded in 6-well plates, and then incubated at 37°C, 5% CO_2 incubator. 24 h later, PBS was used to wash the dead cells, while a 10 μL tip was used for scratching. Then, SW579 cells were cultured with complete medium. 48 h later, the plate was observed and photographed under a microscope according to the variation of the width and width of the scratches in each group.

Dual-Luciferase Report Assay

The circRNA database (https://circinteractome.nia.nih.gov/) was used to predict the potential miRNAs target to circ-0103552. HEK293T cells were co-transfected with plasmids containing circ_0103552-wild type (WT) + miR-NC, circ_0103552-WT + miR-127 mimic, circ_0103552-mutant type (MUT) + miR-NC, circ_0103552-MUT + miR-124 mimic, respectively. 48 h after cultivation, the cells in each group were collected, and the Luciferase activity was analyzed according to the manufacturer's instructions using the Dual-Luciferase assay system (Promega, Madison, WI, USA).

Quantitative Real Time-Polymerase Chain Reaction (qRT-PCR) Assay

The total RNA in the cells and tissues was extracted using the TRIzol agent. The concentration and purity of extracted RNA were determined using a microplate reader. Then, RNA was synthesized into complementary deoxyribose nucleic acid (cDNA) according to the reverse transcription kit's instructions. For the quantification of circular RNA and miRNA, β-actin and U6 were used as internal parameters, respectively. The reaction conditions were: pre-denaturation at 95°C for 10 min; 95°C for 10 s, 60°C for 60 s, repeated for 40 cycles. Three replicate wells were set for each gene, and the gene expression was calculated by the $2^{-\Delta\Delta Ct}$ method, and the experiment was repeated three times. The prim-

ers used in this study were presented as follows: circ-0103552 (F: 5'-TGCCCTCTTTGCAAATCTCT-3', R: 5'-TCAGGAGCTTTTTGAAGCTGT-3'), miR-127 (F: 5'-GCGGCTCGGATCCGTCTGAGCT-3', R: 5'-GTGCAGGGTCCGAGGT-3'), β-actin (F: 5'-CCTTTGTGGGCTGGTTCCTT-3', R: 5'-CTGCTCTGCTCGCTCTAAGG-3') and U6 (F: 5'-TGCGGGTGCTCGCTTCGGCAGC-3', R: 5'-CCAGTGCAGGGTCCGAGGT-3').

Statistical Analysis

All the data were expressed as mean standard deviation. One-way analysis of variance (ANOVA) followed by Post Hoc Test (Least Significant Difference) method was used to analyze whether there was a difference between the groups based on the statistical software Statistical Product and Service Solutions (SPSS) 19.0 (SPSS Inc., Chicago, IL, USA). $p<0.05$ was considered statistically significant.

Results

Expression of Circ-0103552 in TC Tissues and Cells

We used RT-qPCR to detect the circ-0103552 expression in TC tissues and related adjacent normal tissues, as well as Nthy-ori3-1 cell lines and TC cell lines (TPC-1, SW579, 8505C). The results showed that circ-0103552 was significantly higher in TC tissues than adjacent normal tissues (Figure 1A). Also, compared to Nthy-ori3-1 cell lines, the expression of circ-0103552 showed a different level of elevation in TPC-1, SW579, and 8505C cell lines, respectively (Figure 1B).

MiR-127 Was the Potential Target MiRNA of Circ-0103552

Given the firm evidence that putative miRNA acted as a regulatory function of circRNAs[11], we searched the CircInteractome database (https://circinteractome.nia.nih.gov/) to predict the interaction between circ-0103552 and potential miRNA targets and selected miR-127 as the prioritized one (Figure 2A). We, then, determined the miR-127 expression levels in TC tissues, the adjacent normal tissues, Nthy-ori3-1 cell lines, and TC cell lines (TPC-1, SW579, 8505C) by RT-qPCR, respectively. Notably, miR-127 was found downregulated in TC tissues compared to that in normal tissues (Figure 2B). Besides, a decreased expression of miR-127 was observed in TPC-1, SW579, and 8505C cell lines than that in the Nthy-ori3-1 cell lines (Figure 2C).

To further confirm the database prediction, we performed Dual-Luciferase report assay, and the results showed that the overexpression of miR-127 could remarkably reduce the activity of the Luciferase expression in circ-0103552-WT, while the Luciferase activity of circ-0103552-MUT had no change, indicating that miR-127 directly interacted with circ-0103552 (Figure 2D).

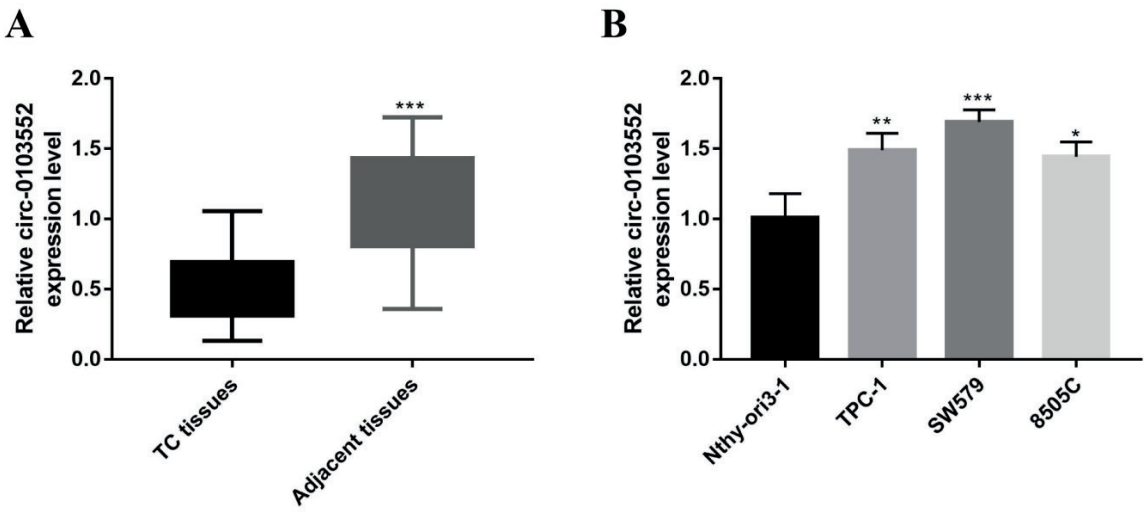

Figure 1. RT-qPCR detection of circ-0103552 expression in TC tissues and cell lines. A, circ-0103552 expression level was upregulated in TC tissues compared with adjacent normal tissues. B, Circ-0103552 expression level was upregulated in TC cell lines (TPC-1, SW579, 8505C) compared with Nthy-ori3-1 cell lines. (*p<0.05, **p<0.01, ***p<0.001 vs. TC tissues and Nthy-ori3-1 cell lines, respectively).

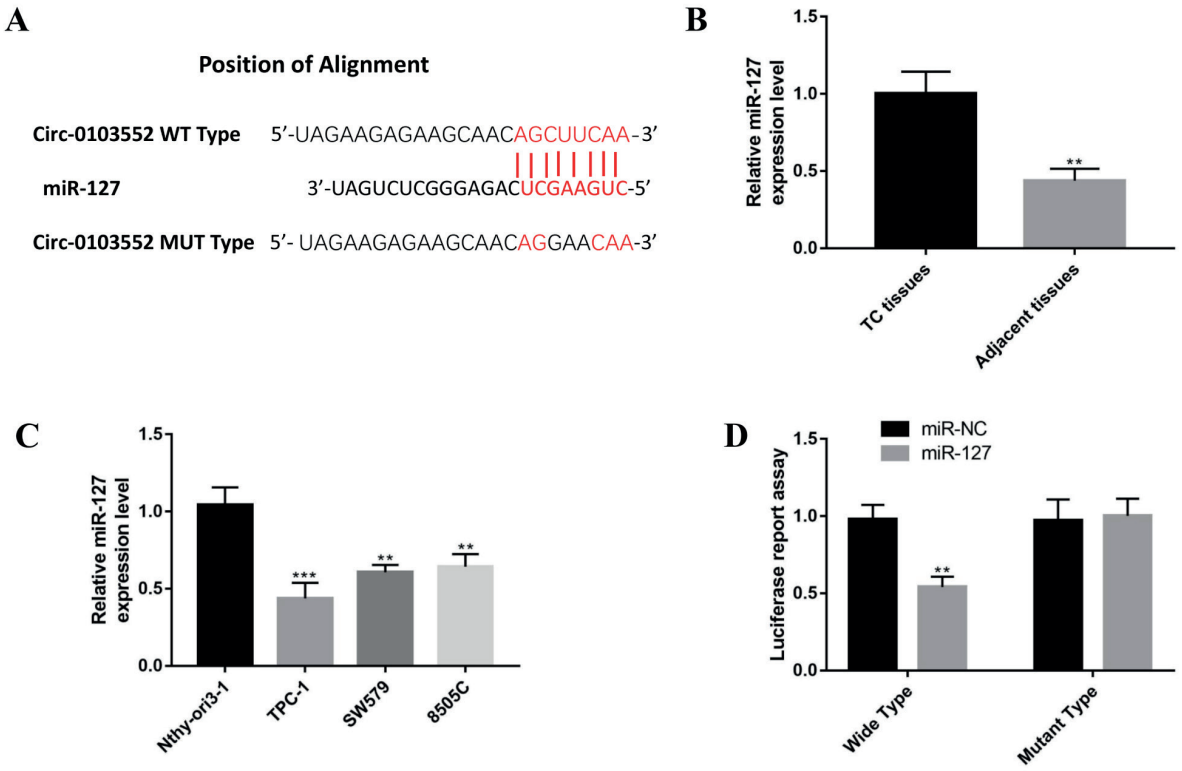

Figure 2. MiR127 is a target miRNA of circ-0103552. **A,** The position of alignment between circ-0103552 and miR-127. **B,** MiR-127 expression level was downregulated in TC tissues compared with adjacent normal tissues. **C,** MiR-127 expression level was downregulated in TC cell lines (TPC-1, SW579, 8505C) compared with Nthy-ori3-1 cell lines. D, Dual-Luciferase report assay showed that miR-127 significantly inhibited the activity luciferase expression in circ-0103552-WT. (**p<0.01, ***p<0.001 *vs.* NC group).

Effects of Circ-0103552 and MiR-127 on the Invasion and Migration on TC Cells

To investigate whether circ-0103552 involved in the invasion and migration of TC cells by sponging miR-127, we used circ-0103552 siRNA and miR-127 inhibitor to interfere with the expression of circ-0103552 and miR-127, respectively (as shown in Figure 3A, 3B). Then, the transwell assay and wound-healing assay were performed. We observed that the TC cells underwent circ-0103552 siRNA transfection showed a significant decrease in the migration ability, with a number of 94.86 ± 4.491migrated cells in the siRNA group compared to 148.1 ± 6.726 migrated cells in the NC group. However, when the expression of miR-127 was inhibited at the same time, the migration ability of TC cells was partially restored, with a total number of 134.4 ± 4.815 migrated cells in siRNA + inh-miR-127 group (Figure 3C, 3D). Similarity, the results of the invasion ability in TC cells with different treatments were consistent with the results of the migration (Figure 4A-4D). Collectively, these findings suggested that circ-0103552 was closely involved in the progression of TC by sponging miR-127.

Discussion

In recent years, the morbidity rate of TC has been constantly increasing, and it has become one of the fastest-growing malignant tumors in many countries[12]. Currently, the treatment methods for TC include surgery[2], postoperative endocrine therapy[13], radioactive iodine-131[14], and chemo-radiotherapy[15]. Although the prognosis is satisfactory after these treatments, and the long-term survival rate of patients is greatly improved, however, many patients have a recurrence *in situ* or distant metastasis after treatment due to the strong invasion and migration ability of TC, causing great pain and burden to patients[16].

CircRNAs are a kind of non-coding RNA with regulatory functions widely existing in organ-

isms[17], which generally form a cyclic structure through head-to-tail connection, with stable expression[18]. The first circRNA ciRS-7 was discovered by Hansen et al[19], which could be stably expressed in the brain as a miR-7 sponge and play an important regulatory role in the genes. Increasingly more studies have found that circRNAs can regulate the progression of a variety of tumors in humans. Of note, Xu et al[20] reported that circular RNA hsa_circ_0003221 promoted the proliferation and migration of bladder cancer cells. Yuan et al[21] uncovered that circRNA circ_0026344, as a prognostic biomarker, suppressed colorectal cancer progression *via* microRNA-21 and microRNA-31. Besides, Liu et al[22] found that circRNA hsa_circ_0008039 enhanced breast cancer cell proliferation and migration by regulating the miR-432-5p/E2F3 axis.

The previous paper showed that circular RNA circ_0103552 played an important role in the proliferation and invasion of breast cancer cells[10,] but whether it was associated with TC has never been reported. In the present study, we firstly detected the circ-0103552 expression level in TC tissues and their adjacent tissues by RT-qPCR and found that it was significantly upregulated in the former. Later, we indicated the expression of circ-0103552 in several TC cell lines, including TPC-1, SW579, 8505C, not surprisingly, each of them showed a different level of upregulation compared to Nthy-ori3-1 cell lines. In view of this, we speculated that circ-0103552 may positively mediate the progress of TC. Later, we performed transwell assay and wound-healing assay to examine the effect of circ-0103552 on the migration and invasion of TC cells. The results showed that inhibition of

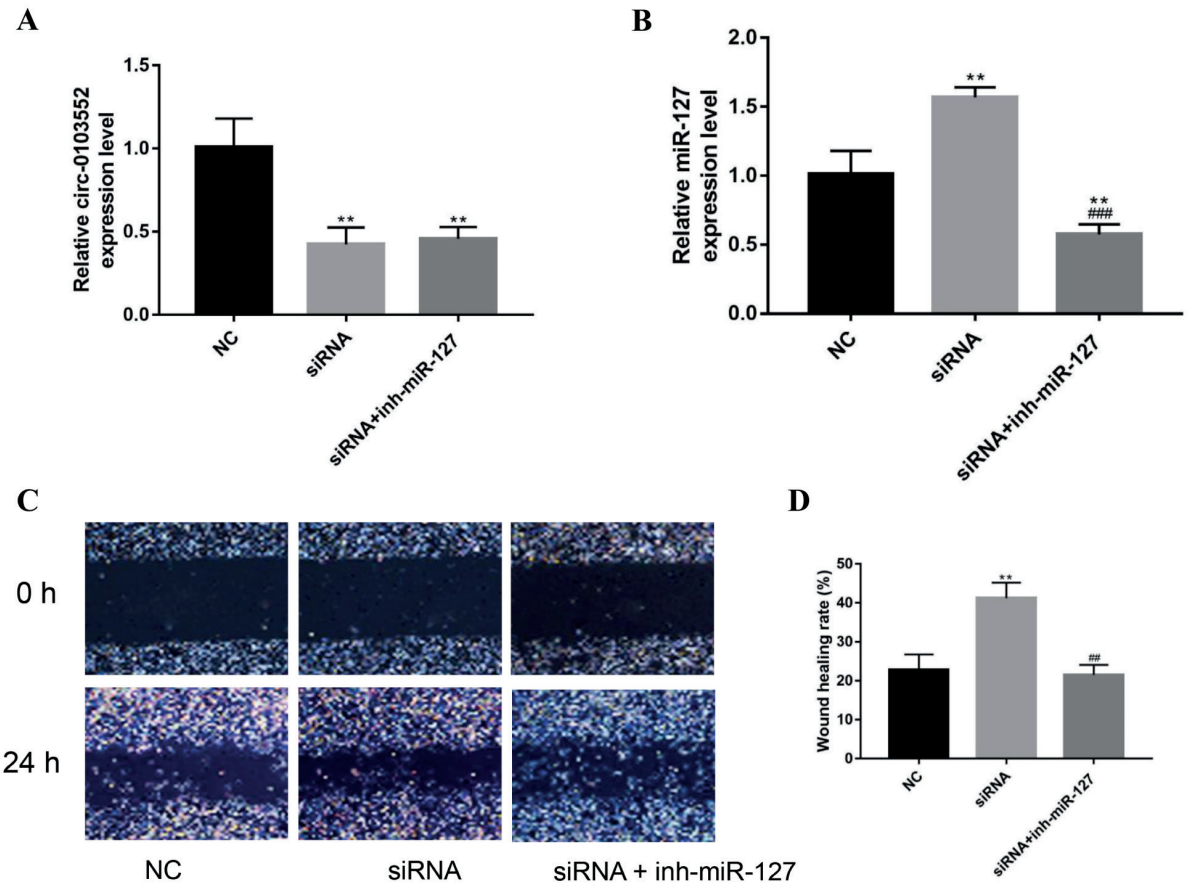

Figure 3. Effects of circ-0103552 and miR-127 on the migration ability of TC cells detected by wound-healing assay. **A,** Expression of circ-0103552 was significantly inhibited in siRNA group and siRNA group + inh-miR-127 group compared to NC group. **B,** Expression of miR-127 was significantly upregulated in siRNA group compared to NC group and siRNA group + inh-miR-127 group. **C,** The results of wound-healing assay in NC group, siRNA group, and siRNA group + inh-miR-127 group (magnification: 4x). **D,** The wound healing rate in NC group, siRNA group, and siRNA group + inh-miR-127 group (**p<0.01 *vs.* NC group, ##p<0.01 *vs.* siRNA group)

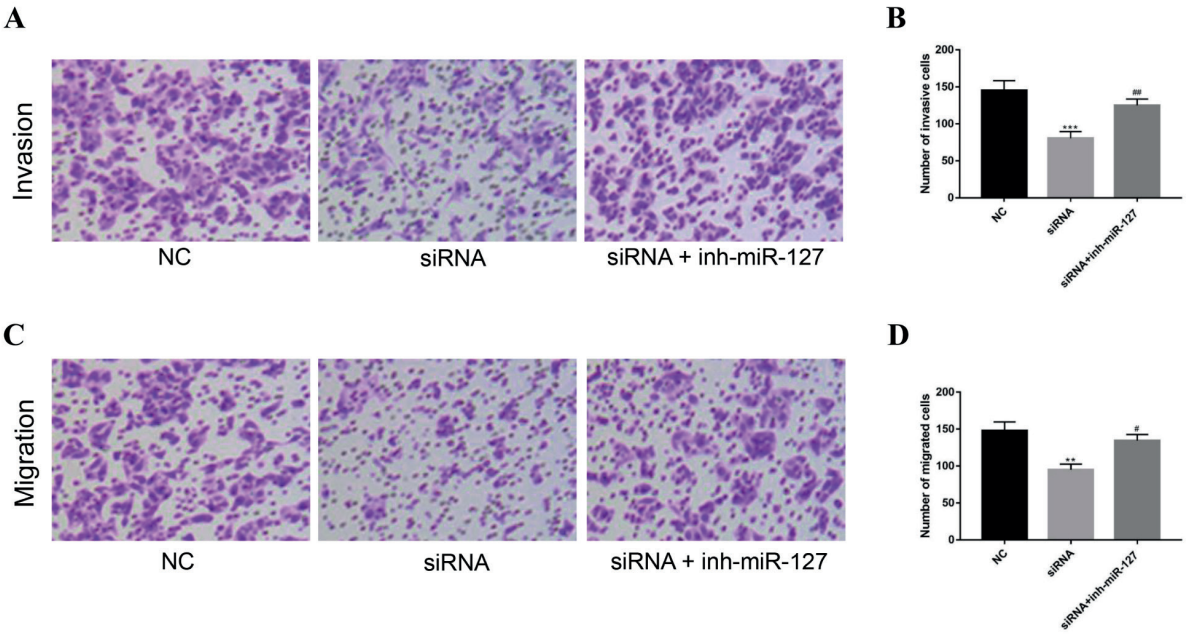

Figure 4. Effects of circ-0103552 and miR-127 on the migration and invasion ability of TC cells detected by transwell assay. **A-B,** Number of invasive cells among NC group, siRNA group, and siRNA group + inh-miR-127 group (magnification: 4x). **C-D,** Number of migrated cells among NC group, siRNA group, and siRNA group + inh-miR-127 group (magnification: 4x). (**p<0.01, ***p<0.001 *vs.* NC group, #p<0.05, ##p<0.01 *vs.* siRNA group).

circ-0103552 in TC cells significantly suppressed their migration and invasion ability, and these provided strong evidence that circ-0103552 was involved in the procession of TC.

Considering that circRNAs play critical roles in regulating the progression of numbers of tumors by sequestering the miRNAs[19,23], we thus aimed to figure out the target miRNA by searching circInteractome database (https://circinteractome.nia.nih.gov/). The results showed that circ-0103552 could serve as a sponge for miR-127 and miR-127 widely participated in human tumors. Guo et al[24] found that miR-127 inhibited cell proliferation, cell cycle progression, cell migration, and invasion in gastric cancer. Liu et al[25] determined that miR-127 inhibited ovarian cancer migration and invasion by upregulating ITGA6. In addition, Shi et al[26] observed that the downregulation of miR-127 markedly reversed the malignant transition, and compromised the stem-like properties and the *in vivo* tumorigenic capability of breast cancer cells. In this study, we found that miR-127 was remarkably downregulated both in TC tissues and TC cell lines (TPC-1, SW579, 8505C). Besides, the inhibition of miR-127 in TC cells could resist the impaired migration and invasion ability caused by circ-0103552 siRNA. Moreover, the Dual-Luciferase report assay indicated that miR-127 directly interacted with circ-0103552. Above all, these results illustrated that circ-0103552 mediated the procession of TC by sponging miR-127.

Conclusions

In summary, circ-0103552 was richly expressed in TC tissues and cell lines. Besides, the inhibition of circ-0103552 impaired the migration and invasion of TC by acting as a miR-127 sponge. These findings provided a better understanding of TC pathogenesis, and circ_0103552 may be considered as a new therapeutic target for TC treatment.

Conflict of Interests

The Authors declare that they have no conflict of interests.

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