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MicroRNA-486 inhibits cell proliferation, invasion and migration *via* down-regulating the TENM1 expressions and affecting ERK and Akt signaling pathways and epithelial-to-mesenchymal transition in papillary thyroid carcinoma

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Abstract. – OBJECTIVE: Papillary thyroid carcinoma (PTC) is one of the general thyroid malignancies. Recently, microRNAs (miRNAs) have identified as pivotal gene regulators in PTC tumorigenesis. The aim of this study was to investigate the role of miR-486 in PTC and its underlying mechanism.

PATIENTS AND METHODS: Fifty-six pairs of PTC tissue and matched normal tissue samples were collected from PTC patients who underwent surgery at our hospital from March 2015 to September 2017. Human thyroid epithelial cell line Nthy-ori3-1and PTC cell lines (BCPAP, K1, HTH83, and TPC-1) were cultured. The mRNA and protein expression level were detected by quantitative Real Time-Polymerase Chain Reaction (qRT-PCR) and Western blot, respectively. Additionally, the proliferation and migration abilities were checked by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) method and transwell assay, respectively. Furthermore, dual-luciferase reporter assay was performed to confirm the combination of miR-486 and TENM1. Xenograft Model experiments were performed to assess the effects of miR-486 on tumor growth *in vivo*.

RESULTS: MiR-486 expression was significantly reduced in PTC, which was associated with the poorer clinicopathologic characteristics and overall survival (OS) of PTC patients. Moreover, miR-486 restoration in PTC cells was confirmed to markedly inhibit proliferation, invasion, and migration *via* the regulation of extracellular-signal-regulated kinase (ERK) and protein kinase B (Akt) signaling pathways and epithelial-mesenchymal transition (EMT). In the meantime, teneurin transmembrane protein 1 (TENM1) was identified as a direct functional target for miR-486 in PTC cells on the basis of bioinformatic analysis and luciferase reporter assays. Additionally, we also verified that miR-486 restoration could prominently repress the PTC growth *in vivo*.

CONCLUSIONS: MiR-486 exerted anti-tumor functions in PTC progression and served as promising biomarkers for the PTC treatment.

Key Words:
Papillary thyroid carcinoma, MicroRNA-486, TENM1, Proliferation, Invasion, Migration.

Introduction

Papillary thyroid carcinoma (PTC), the most prevalent histotype of thyroid carcinoma (TC), has an increasing incidence in recent years[1]. Thanks to the surgical therapies combined with treatments of radioiodine and levothyroxine, the prognosis for most PTC patients is favorable. However, increasing studies[2-4] have demonstrated that, due to the malignant biological features of PTC such as high incidence of lymph nodes metastases, strong capabilities of invasion, and abnormal proliferation with apoptosis frustration, the high recurrence and poor prognosis in patients with PTC occurred occasionally. Therefore, investigating the mechanism of PTC tumorigenesis and progression is emergent.

MicroRNAs (miRNAs) have been proven to be implicated in a range of biological processes

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of multiple tumors through the regulation of target genes[5]. Increasing evidence has indicated that miRNAs are involved in tumor progression and metastasis, functioning as tumor promoters or suppressors[6]. For example, Liu et al[7] found that MiR-96-5p promoted the PTC proliferation, invasion, and metastasis *via* down-regulating CCDC67 serving as an oncogene. Fan et al[8] reported that miR-376a functioned as tumor suppressor by targeting SGK3 in renal cell carcinoma. Li et al[9] indicated that miR-377 suppressed esophageal cancer initiation and progression by suppressing CD133 and vascular endothelial growth factor (VEGF). Considering the modulation in tumorigenesis, miRNAs are currently considered as novel biomarkers for diagnosis and therapies for a variety of tumors. However, the biofunction of miR-486 in PTC needs to be further elucidated.

Epithelial-mesenchymal transition (EMT) has been implicated in a variety of physiological and pathological processes such as embryonic development, organ fibrosis, and cancer progression[10]. It is an important biological process which has significant morphological changes between the epithelial cell-like properties and mesenchymal states[11,12]. Moreover, extracellular-signal-regulated kinase (ERK) and protein kinase B (Akt) are important signaling pathways which are implicated in cell differentiation, survival, growth, and proliferation in different cell types[13-15]. In the current study, we hypothesized that miR-486 regulated PTC progression *via* modulating ERK and Akt pathways and EMT. Therefore, the correlation between PTC and ERK/Akt/EMT signaling pathways was investigated to better understand the potential molecular mechanisms.

TENM1 (teneurin transmembrane protein 1), a member of the teneurin subfamily and tenascin family, has been the research focus for decades attributed to the vital roles in multiple biological progress. For example, teneurin-4 was a novel regulator in suppressing chondrogenic differentiation[16]. Moreover, existing evidence supports the abnormal expressions and involvement of teneurins in cancer-related regulatory networks of various tumors[17]. There is already established scientific evidence linking the overexpression of TENM1 in PTC[18], but the functional role of TENM1 in the development of PTC has not been well studied. Here, we aimed to investigate its functions in PTC progression for the development of the novel diagnostic biomarkers.

Patients and Methods

Clinical Tissues

56 pairs of PTC tissue and matched normal tissue samples were collected from PTC patients who underwent surgery at the Yantai Affiliated Hospital of Binzhou Medical University from March 2015 to September 2017. None of the patients had received any preoperative treatment. The collected tissue samples were frozen immediately in liquid nitrogen and stored at –80°C for further assays. Written informed consent for tissue donation (for research purposes) was obtained from the patients. This investigation was approved by the Ethics Committee of the Yantai Affiliated Hospital of Binzhou Medical University.

Cell Culture

Human thyroid epithelial cell line Nthy-ori3-1and PTC cell lines (BCPAP, K1, HTH83, and TPC-1) were obtained from the cell bank of the Chinese Academy of Sciences (Shanghai, China). All cells were maintained in Roswell Park Memorial Institute-1640 (RPMI-1640; Invitrogen, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (FBS; Gibco, Rockville, MD, USA) in a humidified incubator at 37°C and 5% CO_2.

Cell Transfections

MiR-486 mimics, inhibitor, and corresponding negative control (NC) were obtained from GenePharma Co., Ltd. (Shanghai, China). Then, Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) was utilized to transfect these miRNAs into PTC cells (TPC-1 and HTH83). 48 h after transfection, cells were harvested and subjected for further analysis.

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

The total RNA was isolated from tissue samples or cultured cells with TRIzol reagent (Invitrogen, Carlsbad, CA, USA). Then, Prime-Script RT reagent Kit (TaKaRa, Otsu, Shiga, Japan) was used to reverse transcribe RNA into complementary deoxyribose nucleic acid (cDNA) following the manufacturer's instructions. Amplification and detection were performed by the SYBR Premix Ex Taq (TaKaRa, Otsu, Shiga, Japan) on the ABI PRISM 7900 Sequence Detection System (Applied Biosystems, Foster City, CA, USA). Relative expressions were examined using the $2^{-\Delta\Delta CT}$

method. Expressions of U6 were used as an endogenous control for miR-486 while glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was an internal control for TENM1. The sequences of the primers were described in Table I.

Cell Proliferation Assays

Cell proliferation was determined by the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) method (Sigma-Aldrich, St. Louis, MO, USA). 24 h post-transfection, PTC cells were plated into the 96-well plates and cultivated at 37°C. After the cultivation for different times (0, 24, 48, and 72 h), MTT reagent (20 μL, 5 mg/mL) was added into each well according to the manufacturer's protocols. After incubating for another 4 h at 37°C, 150 μL dimethyl sulfoxide (DMSO; Sigma-Aldrich, St. Louis, MO, USA) was added into each well to dissolve the precipitated formazan. Then, optical density $(OD)_{490}$ was detected using a microplate reader (Thermo Fisher Scientific, Inc., Waltham, MA, USA).

Invasion and Migration Assays

Invasion and migration assays were conducted with transwell chamber (8-μm pore size; Corning Co., Corning, NY, USA) to measure the invasion and migration abilities of PTC cells with different transfections. For invasion assays, PTC cells with different transfections in serum-free medium were seeded into the top chambers which were precoated with Matrigel (BD Biosciences, Franklin Lakes, NJ, USA), medium containing 10% FBS was added to the lower chambers as a chemoattractant. Once incubated for 48 h at 37°C, the cells remained on the upper surface were wiped off completely and the invasive cells were fixed and stained with methanol and crystal violet, respectively. Then, five random fields for each chamber were photographed and counted under a microscope (Olympus, Tokyo, Japan). For migration assays, the chamber was not precoated with Matrigel.

Dual Luciferase Reporter Assay

The wild-type (WT) or mutant (MUT) 3′-untranslated region (3′-UTR) of TENM1 designed and prepared by GenePharma Co., Ltd, (Shanghai, China) were inserted into the pmirGLO vectors (Promega, Madison, WI, USA). For the luciferase assay, PTC cells were co-transfected with miR-486 mimics and WT or MUT TENM1 3'-UTR reporter plasmid by Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA). The luciferase activity was measured with the Dual-Luciferase Reporter Assay System (Promega, Madison, WI, USA) after 48 h of transfection. Firefly luciferase activity was normalized against that of *Renilla* luciferase.

Western Blots

PTC cells were lysed in the radioimmunoprecipitation assay (RIPA) lysis buffer (Beyotime, Shanghai, China) with protease inhibitors (Roche, Basel, Switzerland) on ice. A bicinchoninic acid (BCA) protein assay kit (Thermo Fisher Scientific, Waltham, MA, USA) was utilized to quantify the protein concentration. Then, equal amounts of protein were separated with 10% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE), followed by transferring to polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA, USA). The membranes were blocked in 5% non-fat milk diluted in Tris-Buffered Saline and Tween-20 (TBST) and subsequently incubated at 4°C overnight with the indicated primary antibodies against TENM1 (1:200; sc-398018, Santa Cruz Biotechnology, Santa Cruz, CA, USA), E-cadherin (1:1000, ab15148, Abcam, Cambridge,

Table I. Primer sequences for qRT-PCR.

Primer	Sequence
miR-486 forward	5'- TCATACTGTGGGAAACGCTT-3'
miR-486 reverse	5'- GACACTCAGGGCAGGCAAA -3'
U6 forward	5'- CTCGCTTCGGCAGCACA-3'
U6 reverse	5'- AACGCTTCACGAATTTGCGT-3'
TENM1 forward	5'- TTTCCCGACCTGCCTTTACC-3'
TENM1 reverse	5'- ATGCACTGCAATCACATAGGC -3'
GAPDH forward	5'- CAGCCTCAAGATCATCAGCA-3'
GAPDH reverse	5'- TGTGGTCATGAGTCCTTCCA-3'

U6: small nuclear RNA, snRNA; TENM1: teneurin transmembrane protein 1 GAPDH: glyceraldehyde-3-phosphate dehydrogenase.

MA, USA), Vimentin (1:1000, ab137321, Abcam, Cambridge, MA, USA), ERK (1:1000, ab17942, Abcam, Cambridge, MA, USA), Akt (1:1000, sc-56878, Santa Cruz Biotechnology, Santa Cruz, CA, USA), p-ERK (1:2000, ab192591, Abcam, Cambridge, MA, USA), p-Akt (1:1000, sc-81433, Santa Cruz Biotechnology, Santa Cruz, CA, USA), and GAPDH (1:1000, ab181603, Abcam, Cambridge, MA, USA). After washed, the membranes were incubated with the corresponding horseradish peroxidase (HRP)-conjugated secondary antibodies (1:2000, ab6721, Abcam, Cambridge, MA, USA) at room temperature for 2 h. Protein bands signals were visualized using an enhanced chemiluminescence kit (ECL; Pierce, Minneapolis, MN, USA). GAPDH was used as an internal control.

Immunohistochemical (IHC) Staining Analyses

Tissue samples fixed with 10% buffered formalin and embedded with paraffin were utilized for IHC analysis. Briefly, tissue sections were dewaxed and rehydrated with graded alcohols. An endogenous antigen-retrieval reaction was conducted with citrate buffer in a microwave oven and the endogenous peroxidase activity was inhibited by 3% hydrogen peroxide (H_2O_2) in ethanol. Next, 1% normal goat serum in 0.01 M phosphate-buffered saline (PBS) was used to block nonspecific binding. Subsequently, slides were incubated with primary anti-TENM1 antibody (1:200; sc-398018, Santa Cruz Biotechnology, Santa Cruz, CA, USA) at 4°C in a humidified chamber overnight. Then, the slides were incubated with biotinylated HRP-conjugated secondary antibody (1:1000, ab6721, Abcam, Cambridge, MA, USA), followed by stained with diaminobenzidine (DAB) as the chromogen and counterstained with hematoxylin.

Xenograft Model Experiments

MiR-486 stably-expressed TPC-1 cells (lenti-miR-486) or the negative miR-control (lenti-control) were subcutaneously transplanted into the right flank of male BALB/c nude mice (4-6 weeks of age). Two weeks after the transplantation, the width and length of the tumor were assessed every 3 days. Tumor volumes were detected using the following formula: tumor volume=length×width^2/2. 26 days after injection, the mice were killed and their tumors were dissected and trimmed. This work was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of Yantai Affiliated Hospital of Binzhou Medical University. This study was approved by the Animal Ethics Committee of Yantai Affiliated Hospital of Binzhou Medical University Animal Center.

Statistical Analysis

All experiments were repeated at least 3 times. All data are shown as the mean ± standard deviation (SD). Statistical Product and Service Solutions (SPSS) software version 17.0 (SPSS Inc., Chicago, IL, USA) was applied for the statistical analysis. Differences between the two groups were analyzed by the Student's *t*-test. Comparison between groups was done using one-way analysis of variance (ANOVA) test followed by Post-Hoc Test (Least Significant Difference). The Kaplan-Meier method and log-rank test were applied to estimate the survival rates and compare the survival curves respectively. $p<0.05$ was considered statistically significant.

Results

MiR-486 Expressions were Significantly Decreased in PTC Tissues

To investigate the functions and mechanisms of miR-486 in PTC, we first measured the miR-486 expressions in PTC tissues. QRT-PCR results demonstrated that, compared to the adjacent normal tissues, the miR-486 expressions in PTC tissues were notably reduced (Figure 1A). Then, the PTC patients were divided into two groups with the mean miR-486 expression level as the cut-off. We determined the prognostic functions of miR-486 by using the Kaplan-Meier analysis. Statistical analysis showed that the overall survival of PTC patients who had high miR-486 expressions was higher than that with low miR-486 expressions (Figure 1B). Furthermore, we determined the correlation between miR-486 expressions and clinicopathological characteristics of PTC patients. As presented in Table II, we found that decreased miR-486 expressions were significantly correlated with the malignant clinicopathologic characteristics. These results suggest that miR-486 downregulation may contribute to the progression and development of PTC.

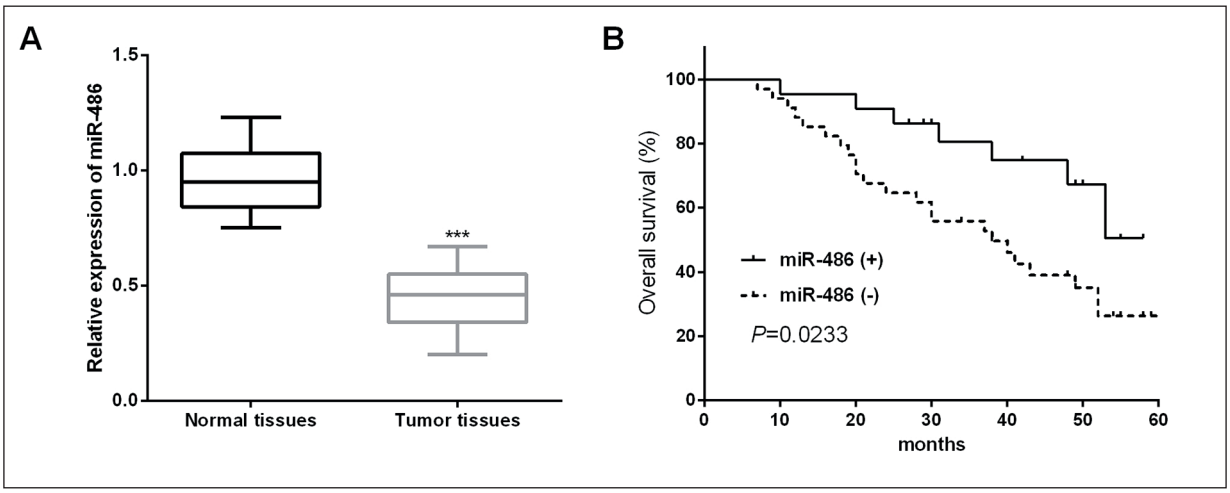

Figure 1. MiR-486 was notably downregulated in PTC tissues. **A,** Expressions of miR-486 were measured by qRT-PCR. **B,** Kaplan-Meier analysis presented shorter overall survival rate of PTC patients with low miR-486 expressions. ***$p<0.001$.

MiR-486 Overexpression Repressed PTC Cell Proliferation

To further evaluate the functions of miR-486 in PTC progression, we then determined its expressions in different PTC cells. The qRT-PCR results demonstrated a significant decrease of miR-486 expressions in PTC cell lines (BCPAP, K1, HTH83, and TPC-1) in comparison to the normal thyroid epithelial cell Nthy-ori3-1 (Figure 2A). Subsequently, miR-486 mimics or inhibitor was transfected into TPC-1 or HTH83 cells, the endogenous miR-486 expression of which was relatively lower or higher, to restore or inhibit the miR-486 expressions. QRT-PCR assays indicated that miR-486 was successfully overexpressed in TPC-1 cells (Figure 2B) and suppressed in HTH83 cells (Figure 2C). The MTT assay verified that miR-486 upregulation significantly sup-

Table II. Correlation of miR-486 expression with the clinicopathological characteristics of the PTC patients.

Clinicopathological features	Cases (n = 56)	miR-486# expression		*p*-value
		High (n = 21)	Low (n = 35)	
Age (years)				0.5743
> 60	31	12	19	
≤ 60	25	9	16	
Gender				0.3656
Male	27	8	19	
Female	29	13	16	
Tumor size (cm)				0.2984
≥ 5.0	28	7	21	
< 5.0	28	14	14	
Lymph node metastasis				0.0019*
Yes	21	16	5	
No	35	5	30	
TNM stage				0.0024*
I +II	24	16	8	
III+IV	32	5	27	
Distant metastasis				0.0021*
Yes	35	6	29	
No	21	15	6	

PTC: papillary thyroid carcinoma; TNM: tumor-node-metastasis. #The mean expression level of miR-486 was used as the cutoff
*Statistically significant.

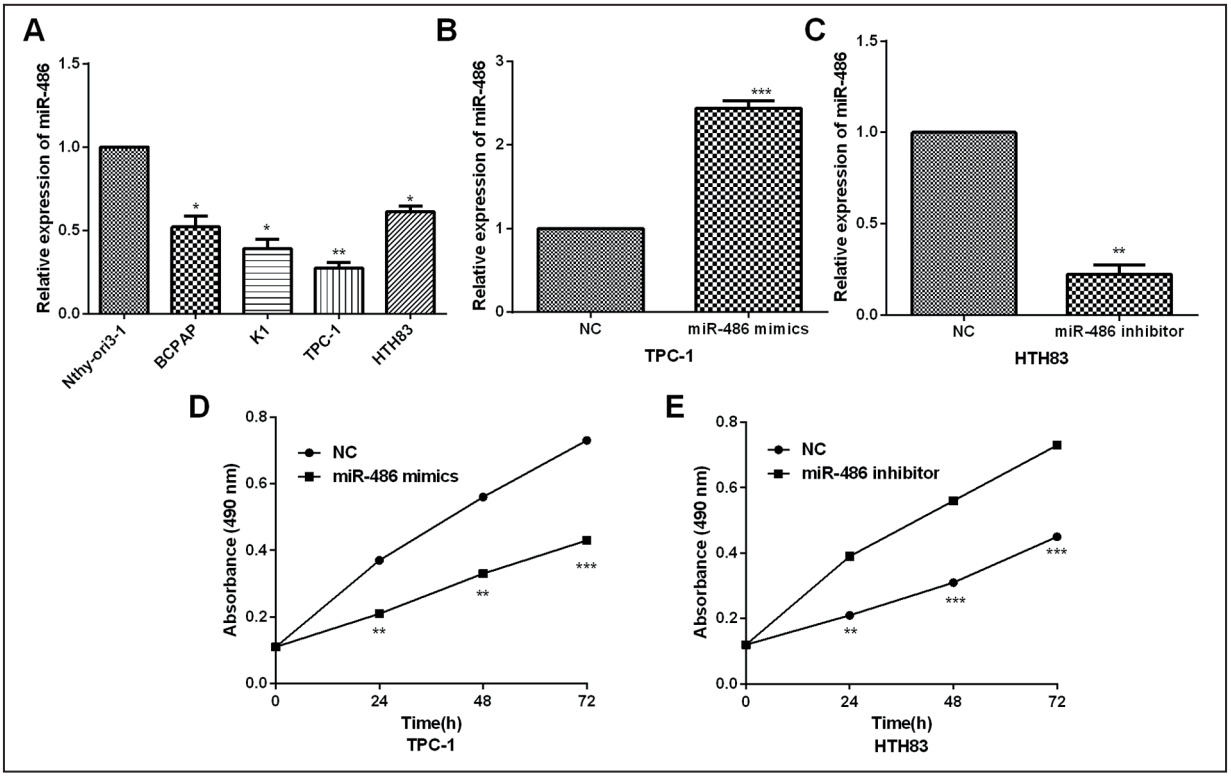

Figure 2. MiR-486 upregulation significantly suppressed the proliferation of PTC cells. **A,** Expressions of miR-486 in PTC cells were measured using qRT-PCR. **B,** Expressions of miR-486 in miR-486 mimics-transfected TPC-1 cells. **C,** MiR-486 expressions in HTH83 treated with miR-486 inhibitor. **D, E,** Proliferation of TPC-1 or HTH83 treated with miR-486 mimics or inhibitor were detected by MTT assays. *p<0.05, **p<0.01, ***p<0.001.

pressed TPC-1 cell proliferation (Figure 2D). On the contrary, we found that the proliferative capacity was enhanced by the miR-486 knockdown in PTC cells (Figure 2E).

MiR-486 Overexpression Suppressed PTC Cell Invasion and Migration

The above results have demonstrated that miR-486 overexpression could inhibit PTC cell proliferation. We next investigated whether miR-486 could affect PTC cell migration and invasion by using transwell assays. Results indicated that miR-486 overexpression could markedly repress the invasion and migration of TPC-1 cells (Figures 3A and 3B), while miR-486 inhibition notably enhanced the invasion and migration capacities (Figures 3C and 3D).

TENM1 was a Functional Target of MiR-486

The potential targets of miR-486 were predicted through TargetScan. TENM1 was selected for further validation since TENM1 mRNA had complimentary miR-486 binding sites within its 3'-UTR (Figure 4A). Then, we carried out luciferase reporter assays to confirm the combination. TENM1 3'-UTR-WT or MUT was transfected into luciferase reporter vectors along with miR-486 mimics into the PTC cells. The luciferase reporter assay indicated that the luciferase activity was significantly decreased by the cotransfection with TENM1 3'-UTR-WT and miR-486 mimics, while on the other hand, the luciferase activity remained unaffected by the cotransfection with TENM1 3'-UTR-MUT and miR-486 mimics (Figure 4B). To further analyze the regulatory effects of miR-486 on the TENM1 expressions, we detected the expressions of TENM1 in TPC-1 or HTH83 cells. Results revealed that miR-486 restoration in TPC-1 cells prominently reduced the TENM1 expressions whereas miR-486 inhibition notably enhanced the expressions of TENM1 (Figures 4C and 4D).

MiR-486 Regulated the ERK and Akt Signaling Pathways and EMT in PTC Cells

The underlying mechanisms of the suppressive effects mediated by miR-486 on PTC pro-

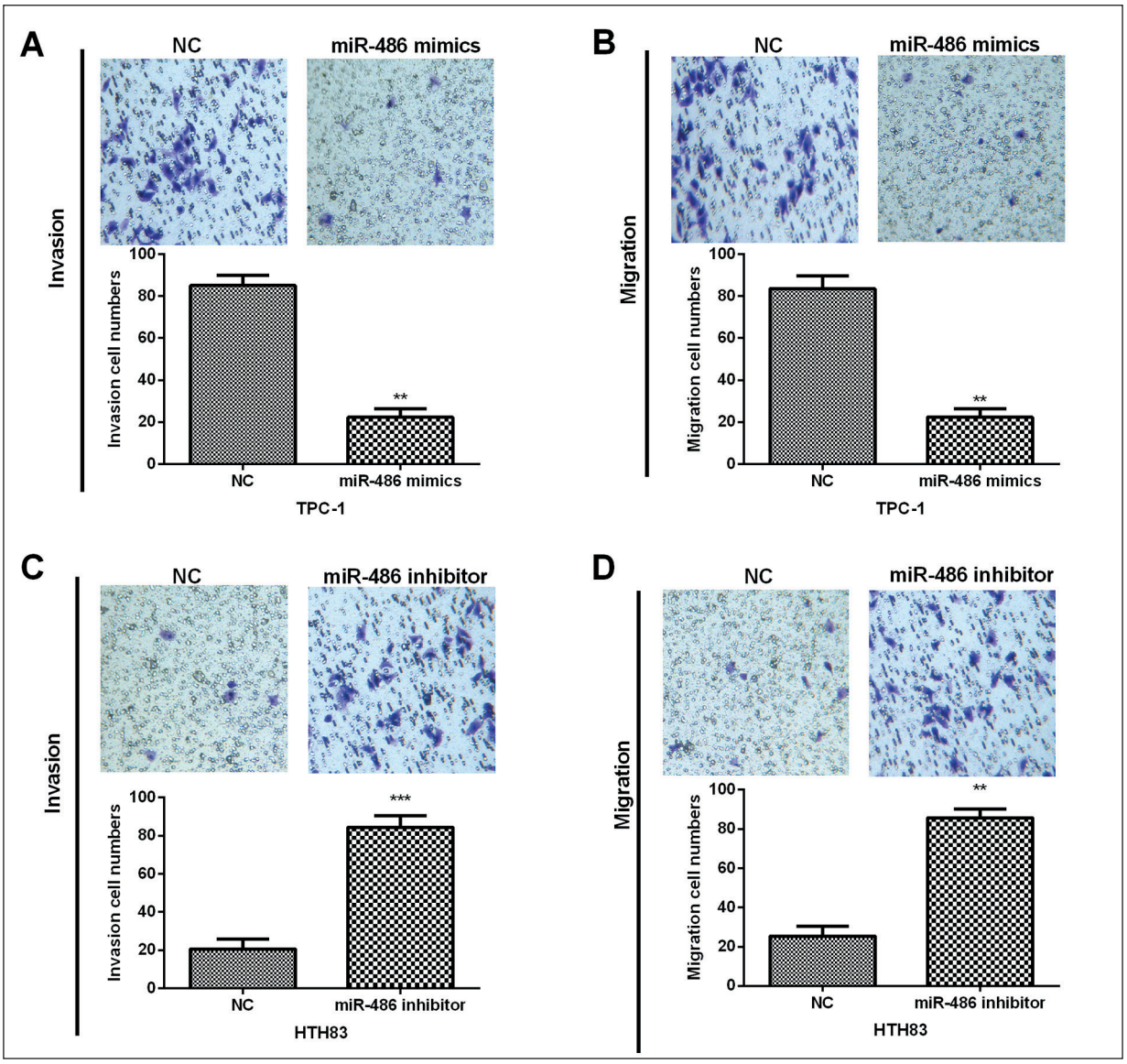

Figure 3. MiR-486 restoration dramatically suppressed the PTC cell invasion and migration capacities (magnification ´ 40). **A, B,** Invasion and migration abilities of TPC-1 treated with miR-486 mimics were measured by transwell assays. **C, D,** Transwell assays were carried out to assess the invasion and migration of HTH83 cells treated with miR-486 inhibitor. $^{**}p<0.01$, $^{***}p<0.001$.

gression were further investigated. First, we performed the IHC assays to determine the expressions of TENM1 in PTC tissues, and results indicated that TENM1 was significantly upregulated and mainly localized at the nucleus and cytoplasm (Figures 5A and 5B). Furthermore, we analyzed the overall survival rate of PTC patients with different TENM1 expressions by the Kaplan-Meier analysis. Kaplan-Meier analysis data showed that the overall survival rate of TENM1 highly expressing PTC patients was poorer than those who had low TENM1 expressions (Figure 5C). Then, the ERK and AKT signaling pathways and EMT were analyzed by performing Western blot. Findings of Western blots demonstrated a significant decrease in the expressions of p-ERK and p-AKT in TPC-1 cells which were treated with miR-486 mimics. On the contrary, the miR-486 inhibition notably upregulated the p-ERK and p-AKT expressions in HTH83 cells (Figure 5D). Moreover, EMT has been considered to be an important regulator of tumor initiation and metastasis. We next measured the expressions of EMT

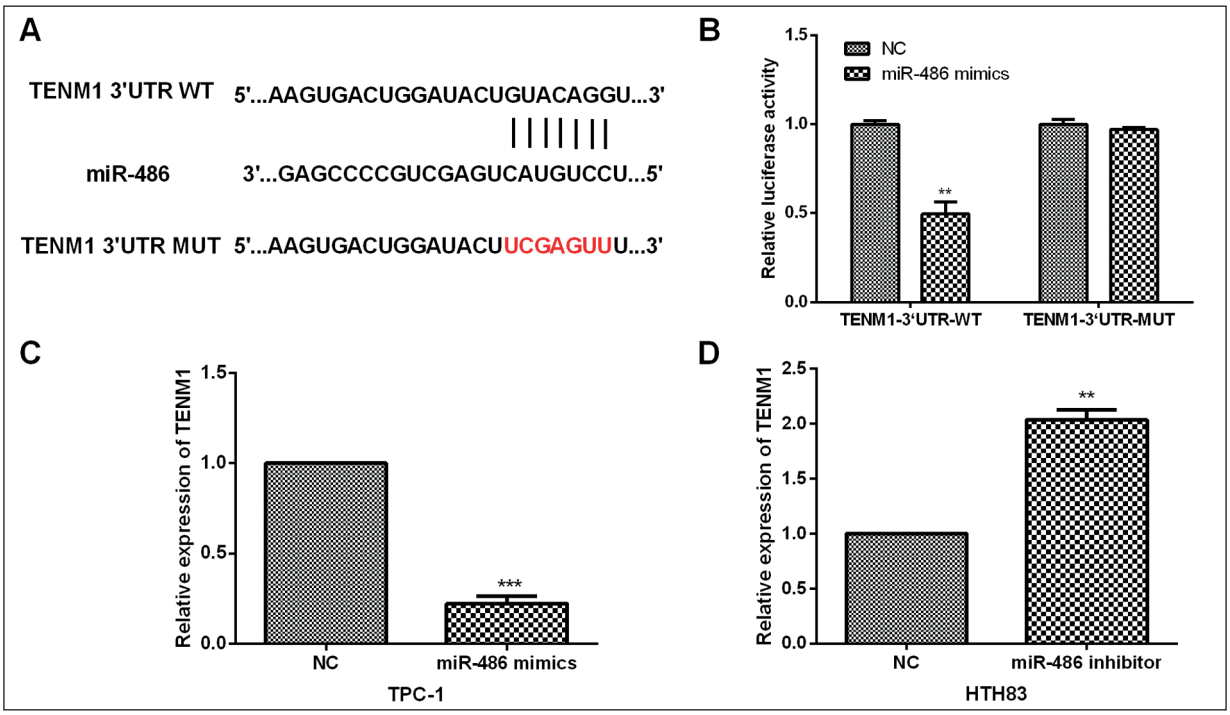

Figure 4. TENM1 was confirmed as a functional target of miR-486. **A,** Putative binding sequences of miR-486 in TENM1 3'UTR. **B,** Luciferase activities of PTC cells co-transfected with the TENM1 3'UTR-WT or MUT and miR-486 mimics were analyzed. **C, D,** TENM1 expressions were measured in TPC-1 or HTH83 treated with miR-486 mimics or inhibitor. $^{**}p<0.01$, $^{***}p<0.001$.

markers and found that miR-486 overexpression significantly promoted the E-cadherin expressions in TPC-1 cells, while notably inhibited the N-cadherin and vimentin expressions. In the meantime, the results also demonstrated that miR-486 inhibition in HTH83 cells remarkably suppressed the E-cadherin expressions and enhanced the vimentin and N-cadherin expression (Figure 5D). These results suggest that miR-486 inhibits the activation of the ERK/AKT signaling pathways pathway in PTC, which may partially explain the mechanism underlying the functions of miR-486.

MiR-486 Inhibited PTC Tumorigenicity In Vivo

To further assess the underlying effects of miR-486 on tumor growth *in vivo*, TPC-1 cells stably expressing miR-486 were subcutaneously injected into nude mice. Results revealed that the tumor growth was prominently inhibited and tumor volume was significantly decreased by miR-486 overexpression (Figures 6A and 6B). These data indicated that miR-486 suppressed tumor growth *in vivo*.

Discussion

Recently, previous studies[19] demonstrated that some patients with PTC frequently suffered from distant metastases after surgeries. Therefore, we strongly need to investigate the mechanisms about PTC and explore novel therapeutic targets for improvement of PTC treatments. Dysregulations of miRNAs usually occur in various types of malignancies, contributing to initiation and development by affecting the expressions of multiple targets[20,21]. Thus, a comprehensive understanding of the relation between specific miRNA and PTC progression is critical to identify the novel the diagnostic and therapeutic approaches. A number of miRNAs were confirmed to be implicated in cell proliferation, migration, and invasion of PTC. For example, Dong et al[22] found that miR-137 served as a tumor suppressor in PTC *via* regulating CXCL12. Xue et al[23] reported that miR-577 inhibited PTC cell proliferation, invasion, and migration *via* regulating SphK2. Liu et al[24] found that miR-144-3p facilitated PTC metastasis and growth by targeting paired box gene 8.

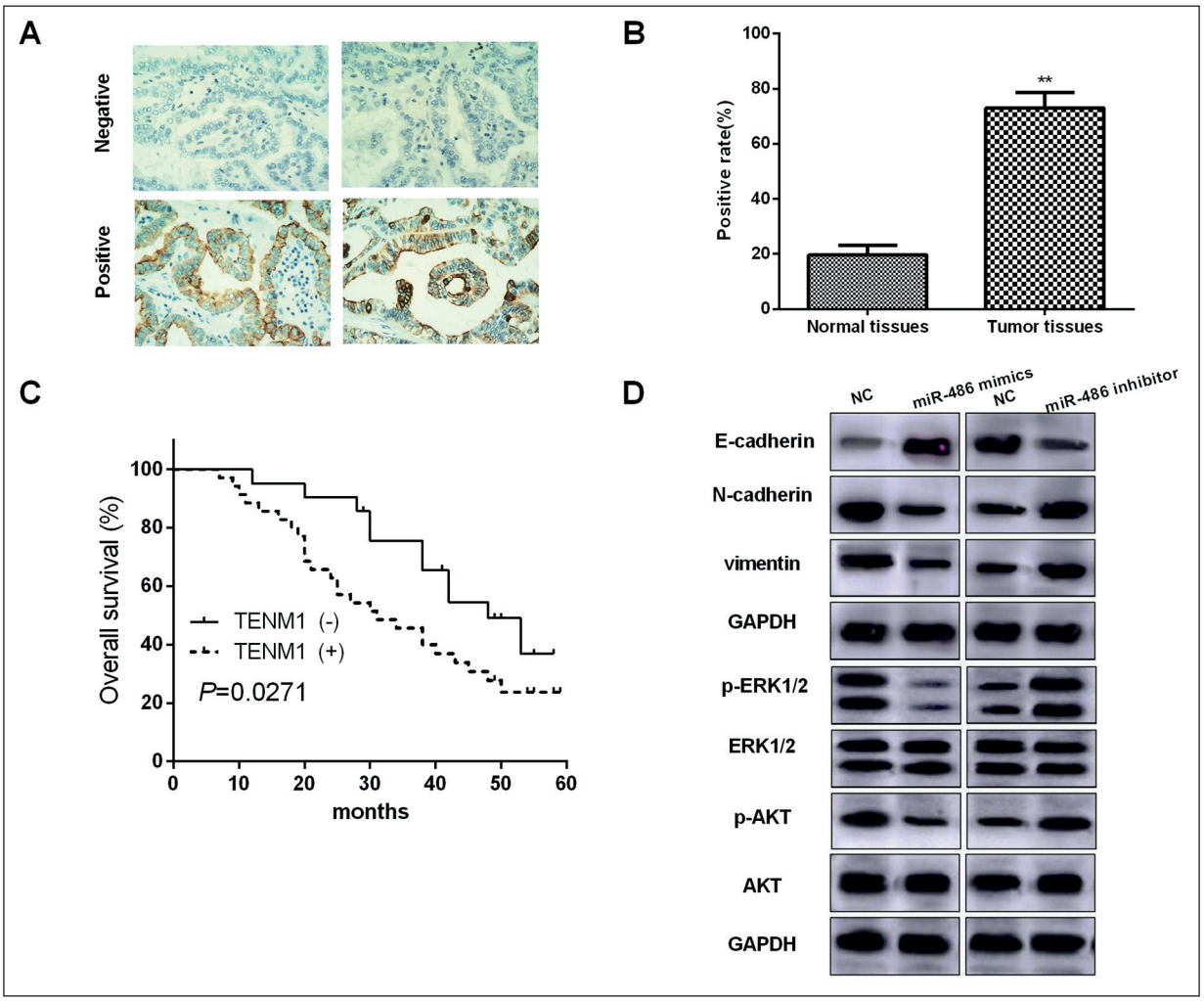

Figure 5. TENM1 was upregulated in PTC tissue samples and miR-486 regulated the AKT and ERK pathways and EMT. **A, B,** TENM1 expressions in PTC tissues were measured by IHC assays (magnification ×40). **C,** Kaplan-Meier analysis of PTC patients with different TENM1 expressions. **D,** Effects of miR-486 on AKT/ERK pathways and EMT in PTC cells. **$p<0.01$.

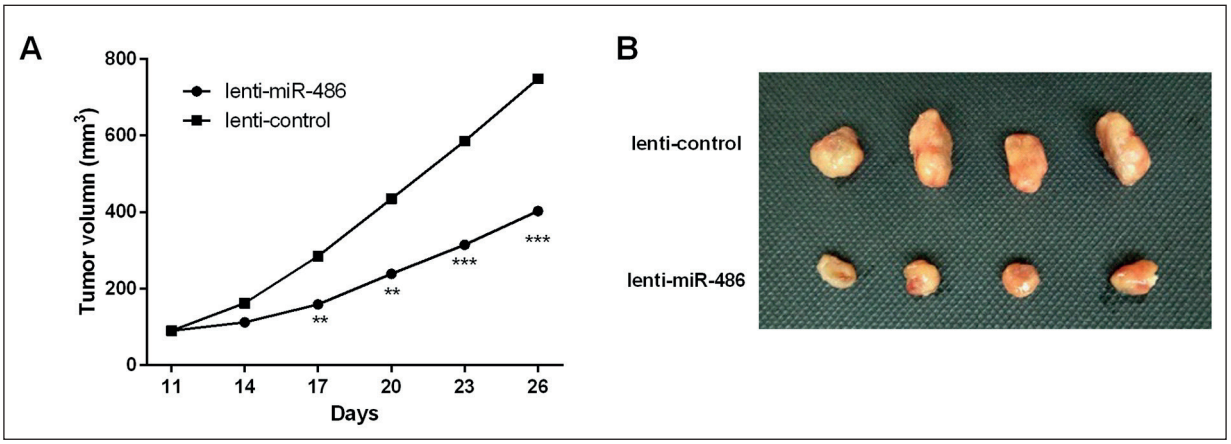

Figure 6. MiR-486 repressed tumor growth *in vivo*. **A,** Growth curve of tumor volumes was drafted. **B,** Representative images of tumors in the different treatment groups. **$p<0.01$, ***$p<0.001$.

Recently, miR-486 has also been studied in multiple human tumors. For instance, miR-486 exerted suppressive functions[25] in esophageal cancer by targeting CDK4/BCAS2. In addition, Sun et al[26] reported that miR-486 regulated hepatocellular carcinoma chemosensitivity and metastasis by targeting CLDN10 and CITRON. He et al[27] found that miR-486 suppressed osteosarcoma development by regulating PKC-delta pathway. In the current research, we focused on the biofunction of miR-486 in PTC progression. Briefly, the results revealed that the expression of miR-486 in PTC tissues was significantly decreased. Moreover, the decreasing miR-486 expressions were observed to be correlated with a worse overall survival rate of PTC patients. Additionally, we found that miR-486 restoration could notably inhibit the proliferation, migration, and invasion of PTC cells through the regulation of the AKT and ERK pathways and EMT. The xenograft assay data also showed that miR-486 overexpression significantly inhibited the PTC growth *in vivo*. All the above data indicated that miR-486 exerted anti-tumor functions in PTC.

The direct functional target of miR-486 was then determined to fully understand the mechanisms of its suppressive influence on PTC progression. TENM1 was identified as a functional target of miR-486 in PTC cells, functioning as an oncogene. Briefly, we found that TENM1 was upregulated in PTC cells and increased TENM1 expression was related to a worse overall survival rate of PTC patients. In addition, Western blot analysis revealed that TENM1 regulated the functions of miR-486 in PTC progression. In general, the above findings were inconsistent with the studies of Cheng et al[28] who found that TENM1 was involved in the PTC development, functioning as a potential biomarker of tumor progression.

Conclusions

MiR-486 was significantly down-regulated in PTC and was related to worse clinicopathologic features and overall survival rate in the current study. On the other hand, we demonstrated that TENM1 was significantly upregulated in PTC cells and tissues. MiR-486 overexpression was found to markedly inhibit the PTC cell proliferation, migration, and invasion by regulating the AKT/ERK pathways and EMT. Besides, we observed that miR-486 overexpression notably suppressed the PTC tumor growth and volume *in vivo*. Further studies to search for their candidate value in other tumors are still needed.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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