

# miR-145-5p inhibits the proliferation and migration of bladder cancer cells by targeting TAGLN2

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**Abstract.** MicroRNA-145-5p (miR-145-5p) is found to be involved in tumor development and progression. However, there are few studies on the effects of miR-145-5p on bladder cancer (BC). The role of miR-145-5p in BC was predicted by analysis of cell proliferation and migration in this study. The miR-145-5p and transgelin-2 (TAGLN2) expressions were evaluated via reverse transcription-quantitative PCR (RT-qPCR) or western blot analysis. The MTT and Transwell assay assessed cell proliferation and migration. TAGLN2 targeted to miR-145-5p was determined using luciferase assays. The results showed that the miR-145-5p downregulation was found in BC. miR-145-5p overexpression inhibited cell proliferation and migration in BC. Moreover, miR-145-5p directly targeted TAGLN2, and TAGLN2 expression was increased in BC. In addition, the high expression of TAGLN2 promoted cell proliferation and migration in BC. miR-145-5p appeared to regulate TAGLN2 in BC, and it also inhibited the cell proliferation and migration. The novel miR-145-5p/TAGLN2 axis may provide new therapeutic implications for BC.

## Introduction

As a typical malignant urogenital tract cancer, bladder cancer (BC) is the sixth most prevalent cancer (1) and the second most common cause of death for cancer in the urinary system for men (2). About 330,000 new BC cases occur around the world resulting in 130,000 deaths annually (3). The main pathogenic factors of bladder are environmental and genetic factors (4). Although surgical operation, radiotherapy and

chemotherapy have made great improvement in treatment, BC is still a common disease with a high mortality rate (5). Moreover, there are many limitations for the established BC biomarkers to diagnose the various cancers (6). Thus efficient gene therapies should be developed to use for early diagnosis of cancer.

MicroRNAs (miRNAs) act as tumor suppressors or oncogenes by regulating gene expression at the post-transcriptional levels to get involved in tumor formation (7). Especially, dysregulation of miRNAs in BC was closely related with bladder tumorigenesis (8). Therefore, miRNAs could be used to diagnose cancer. There are many studies on the role of miRNAs which influenced cell physiological activity in BC, such as cell migration, invasion, proliferation and survival. For example, tumor suppressors including miR-106a, miR-223, and miR-613 accommodated BC by regulating the MAPKs, NCOA1 and SphK1, respectively (9-11). In addition, tumor oncogenes containing miR-130, miR-200c, and miR-556 regulate BC by regulating the PTEN, RECK and DAB2IP, respectively (12-14). However, research on the function of miR-145-5p is still rare in the pathogenesis of BC.

Transgelin-2 (TAGLN2) belonging to the ABP family was firstly discovered in 1998 (15). Recently, the dysregulated expression of TAGLN2 was identified in various cancers. TAGLN2 function as an oncogene to promote cancer cell proliferation, invasion and migration (16-18). In addition, Yoshino *et al* indicated that TAGLN2 along with miR-1/133a affected cell proliferation, apoptosis, invasion and migration in BC (19). Nonetheless, there are no reports on TAGLN2/miR-145-5p in BC.

This study proposed the hypothesis that miR-145-5p suppressed tumor formation by regulating TAGLN2. In order to verify the hypothesis, miR-145-5p and TAGLN2 expressions were detected in BC. Besides, we also examined cell proliferation and migration to further expound the roles of miR-145-5p and TAGLN2 in BC.

## Materials and methods

**Clinical tissues.** Clinical tissues were collected from 22 patients with BC who were undergoing transurethral resection or cystectomy at Beijing Ditan Hospital, Capital Medical University (Beijing, China). The study was approved by the

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**Key words:** miR-145-5p, transgelin-2, proliferation, migration, bladder cancer

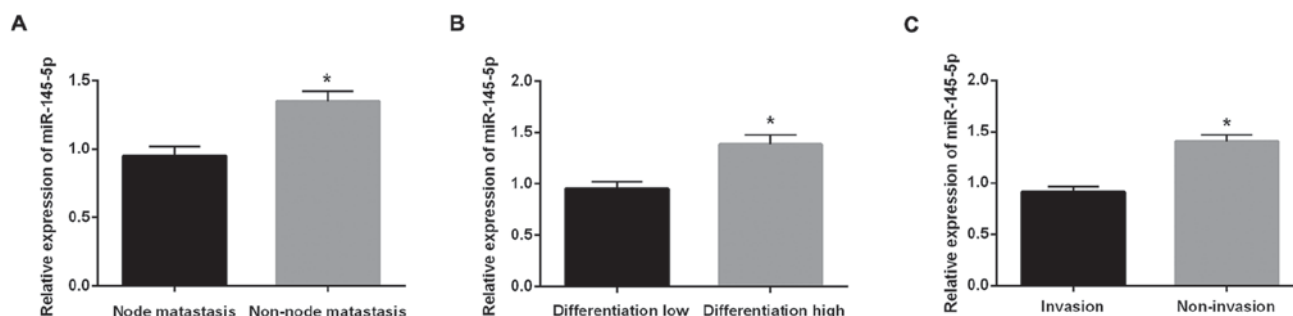


Figure 1. The relationship between the miR-145-5p expression and the clinicopathological features was analyzed in BC. (A) The correlation between miR-145-5p and lymph node metastasis. (B) The association between miR-145-5p and vessel invasion. (C) The association between miR-145-5p and differentiation. \* $P < 0.05$ ; BC, bladder cancer.

Ethics Committee of Beijing Ditan Hospital, Capital Medical University. Signed written informed consents were obtained from the patients or guardians. BC adjacent tissues were obtained from areas about 2 cm away from tumor lesions.

**Cell culture and transfection.** The human BC cell lines T24 and 5637, and the immortalized urothelial cell line SV-HUC-1 were applied in this study. All the cell lines were obtained from the Shanghai Institute of Cell Biology, Chinese Academy of Sciences (Shanghai, China). The cells were grown in RPMI-1640 medium supplemented by 10% fetal bovine serum (FBS). The cells were incubated at 37°C, with 5% CO<sub>2</sub> atmosphere.

The miR-145-5p mimic and inhibitor, TAGLN2 siRNA were purchased from RiBoBio Co., Ltd. (Guangzhou, China) and then they were transferred into T24 or 5637 cells with Lipofectamine® 2000 (Invitrogen; Thermo Fisher Scientific, Inc., Carlsbad, CA, USA) according to manufacturer's instructions.

**RNA extraction and reverse transcription-quantitative PCR (RT-qPCR).** TRIzol reagent (Invitrogen; Thermo Fisher Scientific, Inc.) was applied for extracting total RNA containing miRNA to quantitate the miR-145-5p expression in BC tissues and cell lines. RNA was reverse transcribed using the RevertAid First Strand cDNA kit (Thermo Fisher Scientific, Inc.) according to the manufacturer's introduction. The reactions were incubated at 25°C for 5 min, 42°C for 60 min and 70°C for 5 min. The miR-145-5p reverse transcription primer is 5'-AGTCCAGTTTTCCCAGGAATCCCT-3'. RT-qPCR was performed through the SYBR Premix Ex Taq (Takara Bio, Inc., Otsu, Japan) on an ABI PRISM 7500 Real-time PCR system (Applied Biosystems, Thermo Fisher Scientific, Inc.). The reactions were incubated at 94°C for 10 min, followed by 45 cycles at 95°C for 15 sec and 60°C for 30 sec. The primers were designed as follows: miR-145-F 5'-CCTTGTCCTCACGGTCCAGT-3', and R 5'-AACCATGACCTCAAGAACAGTATT-3'; TAGLN2-F 5'-CTACCTGAAGCCGGTG TCC-3', and R 5'-ATCCCCAGAGAAGAGCCCAT-3'; U6-F 5'-GCTTCGCGCAGCACATATACTAAAAT -3', and R 5'-CGCTTACGAATTTGCGTGTTCAT -3'; GAPDH-F, 5'-GAGTCAACGGATTTGGTCGT-3' and R, 5'-TTGATTTTGGAGGGATCTCG-3'. U6 and GAPDH were used as control of miR-145 and TAGLN2. The miR-145-5p and TAGLN2 levels were analyzed using the 2<sup>-ΔΔC<sub>q</sub></sup> method (20).

**Cell proliferation and migration assays.** The cell proliferation and migration were performed using MTT and Transwell assays to investigate the role of the miR-145-5p and TAGLN2 using T24 or 5637 cells. The experimental procedures were performed according to a previous study (18).

**Dual luciferase report assays.** The wt 3'-UTR of TAGLN2 or mut 3'-UTR of TAGLN2 were inserted into the pGL3 promoter vector (GenScript, Nanjing, China) for luciferase reporter experiments. Then, the vector and miR-145-5p mimic were transfected into 5637 cells. Cells were cultured in a 24-well plate. About 48 h after transfection, the dual luciferase reporter assay system (Promega Corporation, Madison, WI, USA) was applied to perform luciferase assays.

**Western blot analysis.** The protein samples were obtained using RIPA buffer. Proteins were separated through SDS-PAGE and then incubated with 5% non-fat milk blocked membranes at room temperature. Next we incubated the membranes overnight at 4°C with rabbit polyclonal TAGLN2 antibody (cat. no. ab233478; 1:1,000), rabbit polyclonal GAPDH antibody (cat. no. ab9485; 1:1,000) and subsequently incubated with matched goat polyclonal secondary antibody to rabbit IgG - H&L (cat. no. ab150077; 1:1,000) all from Abcam (Cambridge, MA, USA). The protein expression levels were measured by a gel imaging system (JS-780; Pei Qing Technology Co., Ltd., Shanghai, China).

**Statistical analysis.** The experimental data are presented as the mean ± SD. Enumeration data were analyzed using Student's t-test and Chi-square test. Statistical analysis was analyzed with GraphPad Prism 6.0 (GraphPad Software, Inc., La Jolla, CA, USA) and SPSS 17.0 (SPSS, Inc., Chicago, IL, USA).  $P < 0.05$  was considered to indicate a statistically significant difference.

## Results

**The relationship between miR-145-5p expression and the clinicopathological features was analyzed in BC.** In Fig. 1, the relationships between clinicopathological characteristics and the expression of miR-145-5p are summarized. The miR-145-5p expression was closely related with lymph node metastasis (Fig. 1A), differentiation (Fig. 1B) and vascular invasion (Fig. 1C;  $P < 0.01$ ).

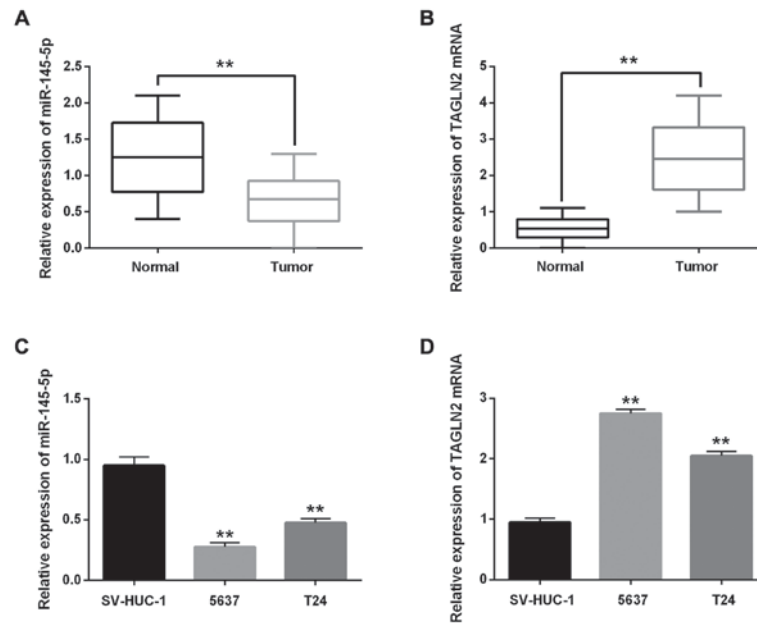


Figure 2. The expression of miR-145-5p and TAGLN2 in BC. (A and B) The miR-145-5p and TAGLN2 expression in tumor tissues and corresponding non-tumor tissues. (C and D) The miR-145-5p and TAGLN2 expressions in T24, 5637 and SV-HUC-1 cells (control). \*\*P<0.01; BC, bladder cancer.

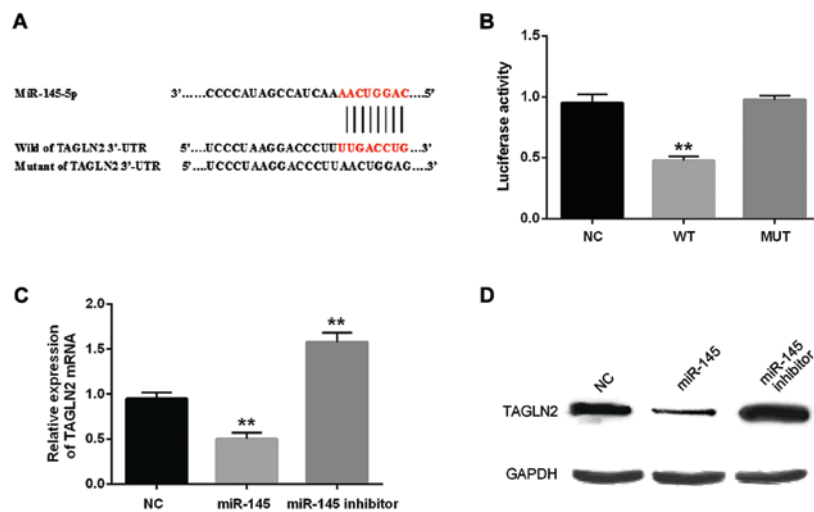


Figure 3. miR-145-5p directly targets TAGLN2 in BC cells. (A) The binding sites of miR-145-5p and TAGLN2. (B) Luciferase reporter assay. (C and D) The mRNA and protein expression of TAGLN2 were analyzed in cells containing miR-145-5p mimic or inhibitor. \*\*P<0.01; BC, bladder cancer.

*miR-145-5p and TAGLN2 expression in BC tissues and cells.* The miR-145-5p and TAGLN2 expression in BC tissues was detected using RT-qPCR. The miR-145-5p expression was lower than that of normal tissues in BC tissues but higher than expression of TAGLN2 (Fig. 2A and B; P<0.01). Furthermore, the miR-145-5p and TAGLN2 expression in BC cell lines was detected simultaneously. The BC cell lines (5637 and T24) had low expression for miR-145-5p but high expression for TAGLN2 mRNA (Fig. 2C and D; P<0.01). Because the expression levels of 5637 were considered to have more difference than T24, 5637 cell line was selected in the following experiments.

*miR-145-5p directly targets TAGLN2 in BC cells.* TargetScan database predicted that TAGLN2 3'UTR has a binding site for

miR-145-5p (Fig. 3A). To verify the above result, we conducted luciferase reporter assay in the 5637 cells. As predicted, luciferase activity in the cells transfected with miR-145-5p mimic and wild-type TAGLN2 was distinctly decreased compared to the control group (P<0.01). Additionally, little change was found in cells containing mutated TAGLN2 and miR-145-5p mimic (Fig. 3B; P>0.05). Furthermore, miR-145-5p overexpression downregulated TAGLN2 mRNA and protein expression significantly (Fig. 3C and D).

*miR-145-5p suppresses the cell proliferation and migration in BC.* The function of miR-145-5p for regulating the cell proliferation and migration in BC were investigated in the present study. The high transfection efficiency was detected in cells containing miR-145-5p mimics or inhibitor (Fig. 4A).

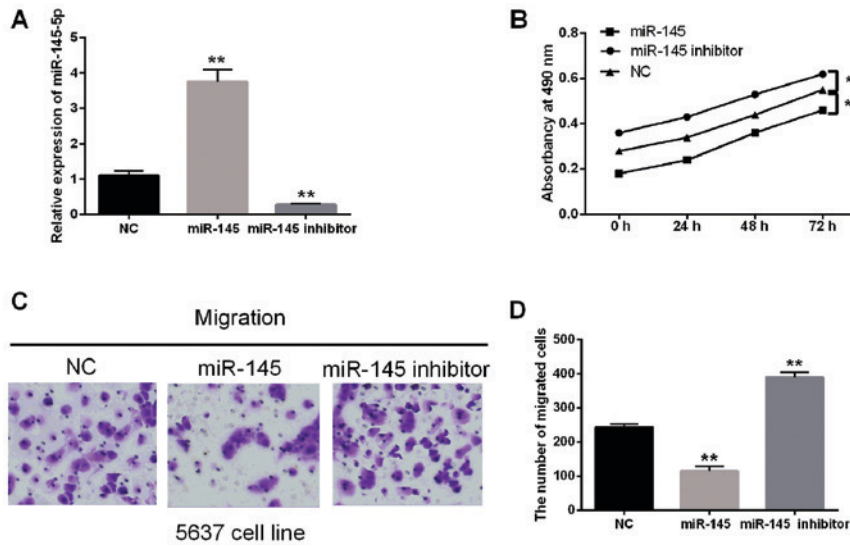


Figure 4. miR-145-5p suppresses BC cell proliferation and migration. (A) miR-145-5p expression was examined in cells contained miR-145-5p mimic or inhibitor via RT-qPCR. (B) The cell proliferation was measured in cells contained miR-145-5p mimic or inhibitor via MTT. (C and D) The cell migration was measured in cells contained miR-145-5p mimic or inhibitor via Transwell analysis. \*P<0.05; \*\*P<0.01; BC, bladder cancer.

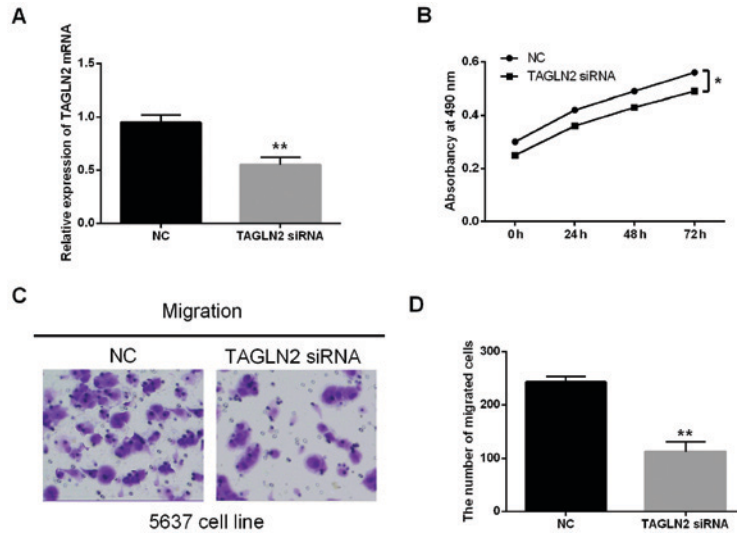


Figure 5. TAGLN2 promotes BC cell proliferation and migration. (A) Expression levels of TAGLN2 were examined in cells contained TAGLN2 siRNA via RT-qPCR. (B) The cell proliferation was measured in cells containing TAGLN2 siRNA via MTT. (C) The cell migration was measured in cells containing TAGLN2 siRNA via Transwell analysis. (D) The number of migrated cells after treatment with TAGLN2 siRNA. \*P<0.05; \*\*P<0.01.

Moreover, the MTT results demonstrated that cell proliferation was suppressed by miR-145-5p overexpression but promoted by the downregulation of miR-145-5p (Fig. 4B). The Transwell analysis suggested that the cell migration was also inhibited by miR-145-5p overexpression while promoted by the miR-145-5p downregulation (Fig. 4C and D; P<0.01).

*The regulated function of TAGLN2 in BC.* To analyze the regulated function of TAGLN2 in BC, the TAGLN2 siRNA was transfected into the 5637 cells (Fig. 5A). Moreover, the MTT and Transwell assay were conducted to identify the proliferation and migration. The results suggested that the knockdown of TAGLN2 significantly inhibited the proliferation and migration of 5637 cells (Fig. 5B-D; P<0.05). It indicated that TAGLN2 might have the carcinogenic effect in BC to some extent.

**Discussion**

Many scholars have proposed that miRNAs act as oncogenes or tumor suppressors through regulating the relative target genes in various cancers (21), and that the change in miRNA expression was related to the pathogenesis, metastasis and progression of human cancer (22). Among them, miR-145 plays a suppressive role in tumor progression and is involved in tumorigenesis. Furthermore, miR-145 with anticancer effect was identified in ovarian carcinoma (23), colorectal carcinoma (24) and non-small cell lung cancer (25). The same conclusion for BC was obtained in this study. Furthermore, it has been reported that miR-145 participated in various physiological and pathological rhythms in BCs, including differentiation (26), apoptosis (27), and invasion (28). Although miR-145 taking part in pathogenesis of BC has been reported



in many studies, its regulated mechanisms on cell proliferation and migration in BC continue to be confused.

This study revealed downregulation of miR-145 in BC tissues. Moreover, the inhibiting effect of miR-145-5p on cell proliferation and migration were found in BC. In brief, all the results indicated that miR-145-5p had inhibitory effects on the pathogenesis of BC. Therefore, it is worth exploring BC relationship between miR-145-5p and its target gene.

In this study, TAGLN2 was found to be a potential target gene of miR-145-5p in BC through bioinformatics analysis. As a member of the calponin family of actin-binding proteins, TAGLN2 is an oncogene. Many investigations have detected that TAGLN2 has modulated cell proliferation, differentiation, migration and apoptosis (29). Additionally, Yoshino *et al* proved that downregulating TAGLN2 inhibited cell proliferation, migration and invasion activity in BC (19). We also affirmed that miR-145-5p directly targeted TAGLN2 in the present study. Moreover, we found that miR-145-5p overexpression brought about downregulation of TAGLN2 in BC. Furthermore, Transwell analysis revealed that TAGLN2 overexpression promoted BC cell proliferation and migration which was in keeping with the previous studies.

Besides, we also identified the relationship between clinicopathological characteristics and miR-145-5p expression in BC. It was found that the expression of miR-145-5p was closely related to lymph node metastasis, differentiation and vessel invasion ( $P < 0.01$ ). Follow-up experiments will be conducted and the prognostic analysis for these patients will continue to be further analyzed.

In conclusion, the present study emphasized that miR-145-5p suppressed TAGLN2 expression and contributed to cell proliferation and migration in BC. This novel miR-145-5p/TAGLN2 axis may provide new therapeutic implications for BC. Future research needs to make full use of the potential impact of miR-145-5p on cancer treatment.

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#### Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

#### Authors' contributions

HZ contributed significantly to data analysis and manuscript preparation. MJ performed the data analyses and wrote the manuscript. QL performed the data analyses. ZH helped perform the analysis with constructive discussions. YZ contributed in the organisation of the experimental data. SJ contributed to the conception of the study. All authors read and approved the final manuscript.

#### Ethics approval and consent to participate

The study was approved by the Ethics Committee of Beijing Ditan Hospital, Capital Medical University (Beijing, China). Signed informed consents were obtained from the patients or guardians.

#### Patient consent for publication

Not applicable.

#### Competing interests

The authors declare that they have no competing interests.

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