European Review for Medical and Pharmacological Sciences 2020; 24: 4361-4367

LncRNA FALEC promotes proliferation, migration, and invasion of PTC cells through regulating Wnt/β-catenin signaling pathway

X. XIAO[1], L. LI[1], J.-C. CUI[1], Y. WANG[2]

[1]Department of Thyroid and Breast Surgery, The People's Hospital of Liaoning Province, Shenyang, China
[2]Department of Anesthesiology, Liaoning Cancer Hospital & Institute, Shenyang, China

Abstract. – OBJECTIVE: The aim of this study was to explore the expression of long non-coding ribonucleic acid (lncRNA) FALEC (hereinafter referred to as FALEC) in papillary thyroid carcinoma (PTC) and its effects on the proliferation, invasion, and metastasis of PTC cells.

PATIENTS AND METHODS: Quantitative Reverse Transcription-Polymerase Chain Reaction (qRT-PCR) was performed to measure the expression level of FALEC in 48 cases of PTC tissues and cells. The small interfering (si)-FALEC was synthesized and transfected into PTC cells. Interference efficiency was confirmed *via* qRT-PCR assay. Subsequently, the effect of FALEC on the proliferation of PTC cells was determined by cell counting kit-8 (CCK-8) assay. Wound healing and transwell assays were conducted to detect the effects of FALEC on the invasion, migration, and metastasis of PTC cells. Additionally, changes in the protein expression of Wnt/β-catenin signaling pathway molecular markers was detected via Western blotting.

RESULTS: The expression level of FALEC was significantly higher in PTC tissues than that of adjacent normal tissues. FALEC expression was significantly up-regulated in PTC cell lines, as well. CCK-8 assay revealed that the proliferation ability of PTC cells was remarkably weakened after down-regulation of FALEC *in vitro*. Wound healing and transwell assays demonstrated that, compared with si-normal control (NC) group, the migration and invasion capabilities declined significantly in si-FALEC group. Furthermore, the Western blotting analysis indicated that the expression of Wnt/β-catenin signaling pathway molecular markers was changed after the interference in FALEC expression.

CONCLUSIONS: FALEC expression was up-regulated in PTC tissues and cell lines. Highly expressed FALEC facilitated the proliferation, migration, and invasion of PTC by regulating the Wnt/β-catenin signaling pathway.

Key Words:
Papillary thyroid carcinoma (PTC), LncRNA FALEC, Biological function, Wnt/β-catenin pathway

Introduction

Thyroid cancer is a common endocrine gland malignancy, of which thyroid papillary carcinoma (PTC) accounts for about 80%[1,2]. Over the past few years, the incidence rate of PTC has increased dramatically[3]. The prognosis of PTC patients treated with c onventional therapeutic regimen (surgery + I131 + thyroxine) is relatively good. However, some patients still have poor therapeutic effects4. Currently, exploration of the biological properties and new therapeutic approaches of PTC has been concerned by clinicians.

Long non-coding ribonucleic acids (lncRNAs) are a class of RNA molecules with a transcript length of over 200 nucleotides. LncRNAs have been found to play vital roles in regulating the life activities of organisms. Meanwhile, they are closely correlated with the development of many diseases, including malignant tumors[5,6]. LncRNA PICART1 promotes the proliferation and metastasis of non-small cell lung cancer cells through targeted regulation on AKT1 signaling pathway[7]. Moreover, highly expressed LOC554202 facilitates the proliferation and invasion of gastric cancer cells by modulating the expressions of P21 and E-cadherin[8].

Many lncRNAs are differentially expressed in PTC, which can also affect the development and progression of PTC. Li et al[9] have discovered that lncRNA n340790 has a highly differential expression in thyroid cancer. It promotes the proliferation

Corresponding Author: Xuan Xiao, BM; e-mail: brex@sina.com

of thyroid cancer cells by directly adsorbing miR-1254. Li et al[10] have found that lncRNA PANDAR is highly expressed in 64 cases of PTC tissues. Cytology experiments *in vitro* have also indicated that lncRNA PANDAR is able to promote the proliferation and invasion, and inhibit the apoptosis of PTC cells. LncRNAs play a crucial regulatory role in thyroid cancer; however, no report has focused on the expression of FALEC in PTC tissues and cells. In the present study, we discovered for the first time that FALEC expression was significantly up-regulated in PTC tissues and cells. Furthermore, *in vitro* experiments confirmed that FALEC facilitated the proliferation, migration, and invasion of PTC cells.

Patients and Methods

Tissue Specimens

A total of 48 pairs of PTC tissue specimens and corresponding adjacent normal tissues were surgically resected in The People's Hospital of Liaoning Province from January 2014 to December 2017. All tissue samples were put in liquid nitrogen immediately after surgical resection and stored in a refrigerator at –80°C for use. The diagnosis was made based on pathological section detections. No patients received treatments such as I131 therapy before surgery. This investigation was approved by the Medical Ethics Committee of the hospital. Informed consent was obtained from patients and their families before the study.

Cell Culture and Transfection

PTC cell lines were purchased from Cell Bank of Chinese Academy of Sciences (Shanghai, China). All cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) or Roswell Park Memorial Institute-1640 (RPMI-1640) medium (Gibco, Rockville, MD, USA) containing 10% fetal bovine serum (FBS; Gibco, Rockville, MD, USA) in a 5% CO_2 incubator at 37°C and saturated humidity. Thereafter, PTC cells in the logarithmic growth phase were taken, digested, and seeded into 6-well plates at a density of 5×104 cells/well. Then, the cells were cultured routinely. When the fusion reached 60-70%, cell transfection was performed in PTC cells according to the instructions of Lipofectamine 3000 (Invitrogen, Carlsbad, CA, USA). The following three siRNA sequences were designed and synthesized by RiboBio (Guangzhou, China): siRNA-1 F 5′-UCCAAAUGUAUUAU GGAAGCA-3′, R 5′-CUUCCAUAAUACAUUUGGAUG-3′. si-RNA-2 F 5'-AAU UUCAGCCAACAUUUG-GAG-3', R 5'-CCAAAUGUUGGCUGA-3'. siRNA-3 F 5'-UAUGAAUUGCAGACUUUGCAG-3', R 5'-GCAAAGUCUGCAAUUCAUAA U-3'.

Quantitative Reverse Transcription-Polymerase Chain Reaction (qRT-PCR)

All PTC tissues and adjacent normal tissues were first cut into pieces. Total RNA in tissues was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). Subsequently, extracted RNA was reversely transcribed into cDNA based on the instructions of the kit (TaKaRa, Otsu, Shiga, Japan). A real-time fluorescent qPCR system was prepared in accordance with SYBR Green (TaKaRa, Otsu, Shiga, Japan) for PCR. Specific procedure was as follows: 95°C for 3 min: denaturation at 95°C for 5 s and annealing at 60°C for 30 s, for a total of 40 cycles. Primers used in this study were: LncRNA FALEC: F 5'-GCCAAGCTCTUGAAAGGCC-3', R 5'-TUCCACGGAGTAGAGCGAGTC-3'; glyceraldehyde 3-phosphate dehydrogenase (GAPDH): R 5'-CATGGAATCCTGTGGCATCC-37; F: 5'-TGATCTFC ATGGTGCTGGGA-3'.

Cell Proliferation Assay

Cell Counting Kit-8 (CCK-8) assay (Dojindo Laboratories, Kumamoto, Japan) was carried out to determine cell proliferation. Transfection sequences small interfering (si)-FALEC and si-normal control (NC) were added to a 96-well plate. Cell proliferation was detected at 0, 24, 48, and 72 h, respectively. Briefly, 10 μL of CCK-8 solution was added to each well, followed by culture at 37°C for 2 h in the dark. Optical density (OD) value at the wavelength of 450 nm was measured by a micro-plate reader. Finally, cell growth curve was plotted.

Clone Formation Assay

Cells in experimental group and control group were inoculated in 6-well plates at a density of 1×103 cells/well, followed by culture for 2 weeks. During the culture, the medium was replaced by fresh one every 5 d. After that, formed colonies were fixed and stained with crystal violet. Finally, formed colonies were observed under a microscope, and the number of colonies was counted.

Would Healing Assay

A 10 μL pipette tip was utilized to create scratch wounds in each cell culture well (as 0 h). Then, a marker pen was used to mark diverse ob-

servation points on the bottom of the cell culture plate. Thereafter, the cells were carefully rinsed with phosphate-buffered saline (PBS) for 3 times to remove the falling cells. Next, the cells were cultured in serum-free Roswell Park Memorial Institute-l640 (RPMI-l640) medium (HyClone, South Logan, UT, USA) or DMEM for 48 h. Wound healing ability of cells was finally observed using a light microscope.

Invasion and Migration Assay

Transfection sequences si-FALEC and si-NC were transiently transfected into PTC cells. Cell concentration was then adjusted to 5×105 cells/mL. Subsequently, cell suspension was added to the upper layer of a transwell chamber (24-well, 8.0 μL). For invasion assay, Matrigel was placed at the bottom of the upper chamber that was added with 100 mL of FBS-free RPMl-l640 medium. Besides, 500 μL of RPMI-l640 medium or complete DMEM containing 10% FBS was added to the lower chamber. 3 duplicate wells were set for each group. Next, transwell chambers were incubated in a 5% CO2 incubator at 37% for 48 h. Then, the cells were fixed with absolute alcohol for 15 min and stained with crystal violet for 20 min. Cells passing through the semipermeable membrane and attaching to the lower layer of the chamber were finally observed under a microscope.

Western Blotting Analysis

Cells transfected with Si-FALEC and si-NC were first lysed using radio-immunoprecipitation assay (RIPA) lysis buffer (Beyotime, Shanghai, China). Protein samples were separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and electro-transferred onto polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA, USA). After blocking with blocking solution for 2 h or at 4°C overnight, the membranes were incubated with primary antibodies at 4°C overnight. On the next day, the membranes were washed with Tris Buffered Saline and Tween-20 (TBST) for 3 times (10 min/time) and incubated with the corresponding secondary antibody. Finally, the membranes were added with enhanced chemiluminescence (ECL) reagent (Pierce, Rockford, IL, USA) A (1 mL) and B (1 mL), mixed and soaked, followed by development in the dark.

Statistical Analysis

Statistical Product and Service Solutions (SPSS) 16.0 software (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Measurement data were expressed as mean ± standard deviation ($\bar{\chi}\pm s$). The *t*-test was employed to compare the difference between the two groups. Enumeration data were expressed as ratio (%). $\chi 2$-test was used for comparison among groups. $p<0.05$ was considered statistically significant.

Results

FALEC Was Highly Expressed in PTC

48 patients definitely diagnosed with PTC through pathology were enrolled in this study. QRT-PCR assay was carried out to detect the expression level of FALEC in paired PTC tissues and adjacent tissues. The results found that the expression of FALEC was significantly up-regulated in 40 out of 48 cases of PTC tissues (Figure 1A). FALEC expression in PTC cells was measured through qRT-PCR assay, as well. The results showed that the relative expression level of FALEC was markedly elevated in PTC cells (Figure 1B). To study the biological role of FALEC in the development and progression of PTC, si-FALEC was designed, synthesized, and transfected into PTC cells. Interference efficiency was verified by qRT-PCR assay (Figures 1C and 1D).

Si-FALEC Inhibited Proliferation of PTC Cells

CCK-8 assay revealed that compared with si-NC group, the proliferation capability of cells in si-FALEC group was significantly repressed after 48 hours (Figures 2A and 2B). Next, the effect of si-FALEC on the proliferation of PTC cells was investigated *via* colony formation assay. The results indicated that interference in FALEC expression significantly suppressed the proliferation of PTC cells (Figures 2C and 2D).

FALEC Regulated Migration and Invasion of PTC Cells Through Wnt/β-Catenin Pathway

To explore the effects of FALEC on the migration and invasion of PTC cells, wound healing assay was performed in this study. The results manifested that after down-regulation of FALEC expression, the migration ability of PTC cells was remarkably suppressed (Figures 3A and 3B). Next, transwell assay demonstrated that cells in si-FALEC group exhibited significantly inhibited migration and invasion abilities when compared with cells in si-NC group (Figures 3C and 3D). Thereafter, the possible mechanism of FALEC in

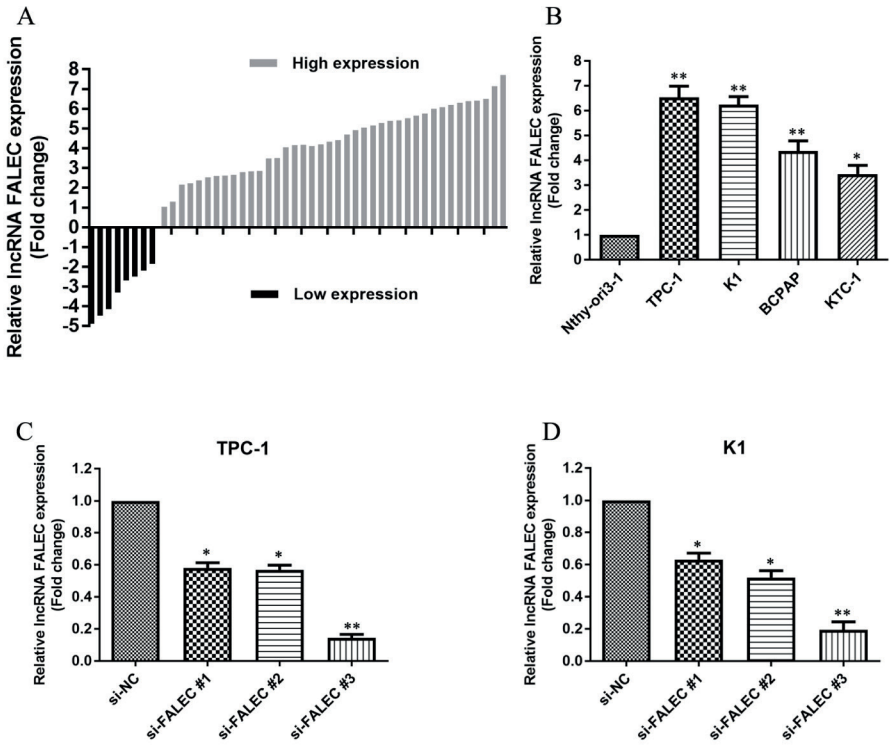

Figure 1. FALEC expression is up-regulated in PTC. **A,** FALEC expression in 48 pairs of PTC tissues and adjacent tissues detected by qRT-PCR assay. The expression level of FALEC is significantly elevated in 40 cases of PTC tissues compared with adjacent tissues. **B,** Relative expression level of FALEC in PTC cells measured through qRT-PCR assay. **C,** and **D,** Interference efficiency of si-FALEC on PTC cells determined *via* qRT-PCR assay.

affecting the migration and invasion of PTC cells was investigated *via* Western blotting analysis. The results discovered that the protein expression of molecular markers of the Wnt/β-catenin pathway was altered after interfering in FALEC expression (Figures 3E and 3F).

The above results implied that FALEC expression was significantly up-regulated in PTC tissues and cells. High expression of FALEC facilitated the proliferation, migration, and invasion of PTC cells by modulating the Wnt/β-catenin pathway.

Discussion

PTC accounts for about 80% of thyroid cancer, which is a common endocrine gland malignancy[1,2]. The development and progression of PTC involve multi-factor and multi-stage changes. Therefore, searching for new therapeutic targets and prognosis and efficacy evaluation markers is expected to provide new directions for the treatment of PTC[11,12].

Over 90% of genes in mammalian genomes have no potential of coding, which have been regarded as non-functional genes in the past. With the completion of the human genetic program, non-coding RNAs considered as "transcriptional noises" have been verified to play vital regulatory roles in cellular life activities[13]. Among them, lncRNAs are a type of RNAs with over than 200 bases in length[14]. The development and progression of malignant tumors are related to many aspects, including rapid cell proliferation, inhibited apoptosis, as well as enhanced invasion, and metastasis. Wang et al[15] have pointed out that abnormal expression of lncRNAs changes the above biological processes, playing a key role in the development and progression of tumors.

FALEC expression has been found significantly elevated in many tumors. Zhao et al[16] have uncovered that in prostate cancer, high expression of FALEC indicates poor prognosis of patients. Meanwhile, it promotes the proliferation, migration and metastasis of prostate cancer. In addition, FALEC expression is up-regulated in melanotic tumor. *In-vitro* and *in-vivo* experiments suggest that FALEC recruits EZH2 to apparently silence P21 expression, thus promoting the proliferation

of melanotic tumor[17]. However, the expression and function of FALEC in PTC have not been fully elucidated. In this study, we discovered that the expression of FALEC was up-regulated in PTC tissues and cells. Subsequent *in vitro* experiments confirmed that FALEC promoted the proliferation, migration, and invasion of PTC cells.

The Wnt/β-catenin signaling pathway is a highly conserved signal transduction pathway. Current studies have found that this pathway has a close correlation with the development of various diseases in humans. Meanwhile, it is also abnormally expressed in many tumors, such as liver cancer and lung cancer. Activating Wnt/β-catenin signaling pathway can facilitate the development and progression of tumors. However, repressing such a pathway reduces the proliferation and migration and promotes the apoptosis of tumor cells[18,19]. LncRNAs, important regulators, participate in regulating the activation/deactivation of Wnt/β-catenin. In cervical cancer, lncRNA CALML3-AS1 promotes the proliferation and metastasis of cells by modulating the Wnt/β-catenin pathway[20]. In this research, we proved that FALEC participated in the regulation on the Wnt/β-catenin signaling pathway in PTC *in-vitro*.

Conclusions

In summary, FALEC was highly expressed in PTC tissues and cell lines. High expression of FALEC facilitated the proliferation, migration, and invasion of PTC by regulating the Wnt/β-catenin signaling pathway.

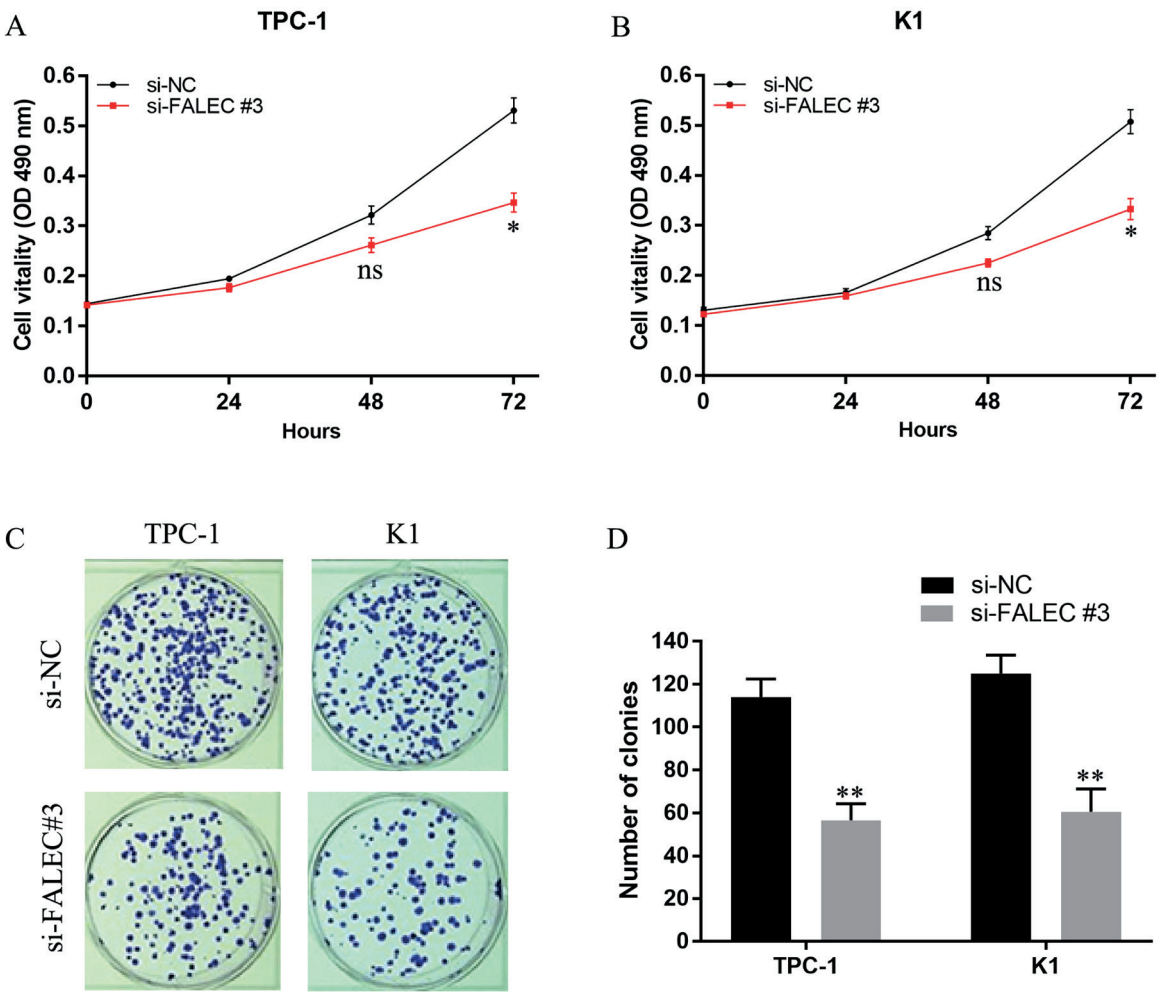

Figure 2. Si-FALEC inhibits PTC cell proliferation. **A,** and **B,** Results of CCK-8 assay. The proliferation ability of cells is weakened after interfering in the expression of FALEC. **C,** and **D,** Results of colony formation assay (magnification × 40). Si-FALEC weakens the proliferation ability of PTC cells.

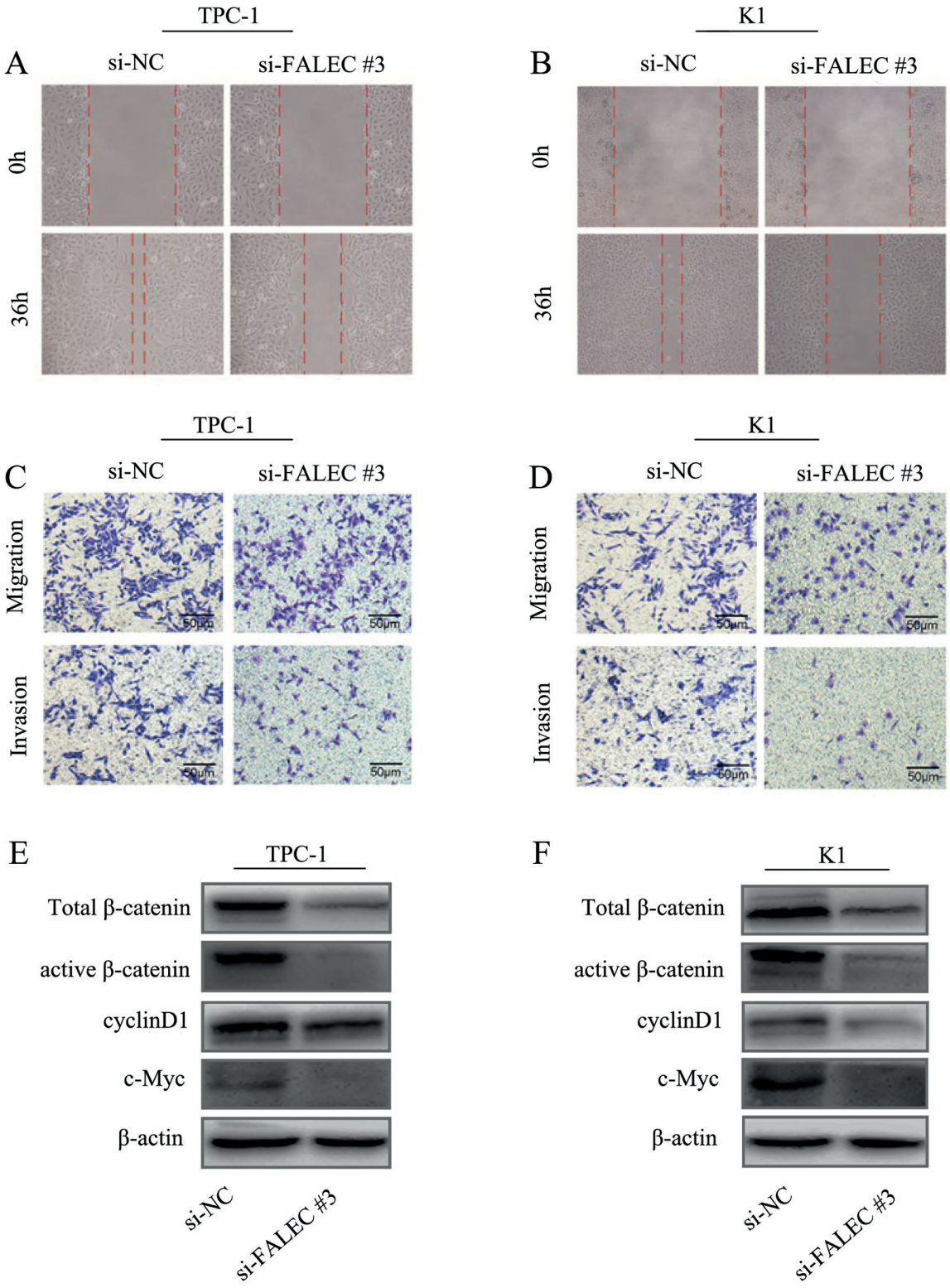

Figure 3. Interfering FALEC expression down-regulates PTC cell migration and invasion through Wnt/β-catenin pathway. **A,** and **B,** Results of wound healing assay. Interfering in FALEC expression weakens the migration ability of PTC cells. **C,** and **D,** Transwell results (magnification × 40). The migration and invasion ability of cells in si-FALEC group declines compared with si-NC group. **E,** and **F,** Results of Western blotting analysis. The expression of WNT/β-catenin signaling pathway molecular markers changes after interfering in FALEC expression in PTC cells.

Conflict of Interests

The Authors declare that they have no conflict of interests.

References

1) Yoon SG, Yi JW, Seong CY, Kim JK, Kim SJ, Chai YJ, Choi JY, Lee KE. Clinical characteristics of papillary thyroid carcinoma arising from the pyramidal lobe. Ann Surg Treat Res 2017; 92: 123-128.
2) Fiore M, Oliveri CG, Caltabiano R, Buffone A, Zuccarello P, Cormaci L, Cannizzaro MA, Ferrante M. Role of emerging environmental risk factors in thyroid cancer: a brief review. Int J Environ Res Public Health 2019; 16: pii: E1185.
3) Tam AA, Ozdemir D, Aydin C, Bestepe N, Ulusoy S, Sungu N, Ersoy R, Cakir B. Association between preoperative thyrotrophin and clinicopathological and aggressive features of papillary thyroid cancer. Endocrine 2018; 59: 565-572.
4) Zambeli-Ljepovic A, Wang F, Dinan MA, Hyslop T, Stang MT, Roman SA, Sosa JA, Scheri RP. Extent of surgery for low-risk thyroid cancer in the elderly: equipoise in survival but not in short-term outcomes. Surgery 2019; 166: 895-900.
5) Do H, Kim W. Roles of oncogenic long non-coding RNAs in cancer development. Genomics Inform 2018; 16: e18.
6) Shin H, Kim Y, Kim M, Lee Y. BC200 RNA: an emerging therapeutic target and diagnostic marker for human cancer. Mol Cells 2018; 41: 993-999.
7) Zhang C, Su C, Song Q, Dong F, Yu S, Huo J. LncRNA PICART1 suppressed non-small cell lung cancer cells proliferation and invasion by targeting AKT1 signaling pathway. Am J Transl Res 2018; 10: 4193-4201.
8) Lin Y, Zhang CS, Li SJ, Li Z, Sun FB. LncRNA LOC554202 promotes proliferation and migration of gastric cancer cells through regulating p21 and E-cadherin. Eur Rev Med Pharmacol Sci 2018; 22: 8690-8697.
9) Li Q, Shen W, Li X, Zhang L, Jin X. The lncRNA n340790 accelerates carcinogenesis of thyroid cancer by regulating miR-1254. Am J Transl Res 2017; 9: 2181-2194.
10) Li Z, Gao B, Hao S, Tian W, Chen Y, Wang L, Zhang X, Luo D. Knockdown of lncRNA-PANDAR suppresses the proliferation, cell cycle and promotes apoptosis in thyroid cancer cells. EXCLI J 2017; 16: 354-362.
11) Qiu J, Zhang W, Xia Q, Liu F, Li L, Zhao S, Gao X, Zang C, Ge R, Sun Y. RNA sequencing identifies crucial genes in papillary thyroid carcinoma (PTC) progression. Exp Mol Pathol 2016; 100: 151-159.
12) Lopez-Campistrous A, Adewuyi EE, Benesch M, Ko YM, Lai R, Thiesen A, Dewald J, Wang P, Chu K, Ghosh S, Williams DC, Vos LJ, Brindley DN, McMullen T. PDGFRα regulates follicular cell differentiation driving treatment resistance and disease recurrence in papillary thyroid cancer. EBioMedicine 2016; 12: 86-97.
13) Kentwell J, Gundara JS, Sidhu SB. Noncoding RNAs in endocrine malignancy. Oncologist 2014; 19: 483-491.
14) Bunch H. Gene regulation of mammalian long non-coding RNA. Mol Genet Genomics 2018; 293: 1-15.
15) Wang W, Zhou R, Wu Y, Liu Y, Su W, Xiong W, Zeng Z. PVT1 promotes cancer progression via microRNAs. Front Oncol 2019; 9: 609.
16) Zhao R, Sun F, Bei X, Wang X, Zhu Y, Jiang C, Zhao F, Han B, Xia S. Upregulation of the long non-coding RNA FALEC promotes proliferation and migration of prostate cancer cell lines and predicts prognosis of PCa patients. Prostate 2017; 77: 1107-1117.
17) Ni N, Song H, Wang X, Xu X, Jiang Y, Sun J. Up-regulation of long noncoding RNA FALEC predicts poor prognosis and promotes melanoma cell proliferation through epigenetically silencing p21. Biomed Pharmacother 2017; 96: 1371-1379.
18) Zhang J, Kang N, Yu X, Ma Y, Pang X. Radial extracorporeal shock wave therapy enhances the proliferation and differentiation of neural stem cells by Notch, PI3K/AKT, and wnt/beta-catenin signaling. Sci Rep 2017; 7: 15321.
19) Chen X, Huan H, Liu C, Luo Y, Shen J, Zhuo Y, Zhang Z, Qian C. Deacetylation of beta-catenin by SIRT1 regulates self-renewal and oncogenesis of liver cancer stem cells. Cancer Lett 2019; 463: 1-10.
20) Liu CN, Zhang HY, Liu CL, Wang CC. Upregulation of lncRNA CALML3-AS1 promotes cell proliferation and metastasis in cervical cancer via activation of the Wnt/beta-catenin pathway. Eur Rev Med Pharmacol Sci 2019; 23: 5611-5620.