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Role of *TEAD4* in colorectal cancer cell proliferation and analysis of its mechanism

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Abstract

Objective: Colorectal cancer is one of the major causes of tumor-related deaths worldwide. Transcriptional factor *TEAD4* is a key factor in the YAP–TAZ signaling pathway. The aim of the present study was to investigate the effect and mechanism of *TEAD4* knockdown on colorectal cancer cell proliferation.

Methods: Transient transfection and a lentivirus system were used to achieve transient or stable *TEAD4* knockdown in Caco2 and HT-29 cells. Western blotting was used to detect transfection efficiency and related protein expression. The sulforhodamine B method and EdU Imaging Kits were used to determine cell viability and DNA synthesis.

Results: Western blotting showed that knockdown of *TEAD4* could increase the expression of the cell cycle protein *p27* in Caco2 ($F = 45.78$, $P < 0.001$) and HT-29 ($F = 40.71$, $P < 0.001$) cells. Sulforhodamine B assays showed that cell viability with *TEAD4* knockdown was significantly decreased in Caco2 and HT-29 cells. Results of EdU assays showed that DNA synthesis was significantly inhibited in Caco2 ($P_{si\#2} = 0.001$, $P_{si\#3}$ $P < 0.001$) and HT-29 cells ($P_{si\#2} = 0.001$, $P_{si\#3} = 0.011$) with knockdown of *TEAD4*.

Conclusions: The present study showed that *TEAD4* plays an important role in cell proliferation in the Caco2 and HT-29 colorectal cancer cell lines, partially through upregulation of *p27*, suggesting that *TEAD4* is a potential target and biomarker for the development of novel therapeutics for colorectal cancer.

KEYWORDS

cell proliferation, colorectal cancer, *TEAD4*

1 | INTRODUCTION

Colon cancer is one of the most common and fatal malignancies, with the third highest incidence and fourth highest mortality worldwide.[1] Over the past 30 years, especially in big cities in China, the incidence of colon cancer has been increasing year by year, as diets have changed from Chinese to European and American diets. TEA domain family members (TEADs) are important transcription factors in the YAP–TAZ signaling pathway, which can promote cell proliferation, survival, migration, and tumorigenesis.[2,3] Recent studies have shown that TEADs are involved in the development of a variety of tumors and are expressed in all types of tissues.[4] Some researchers have found that the high expression of TEAD1 is associated with a poor clinical prognosis in prostate cancer, and the knockdown TEAD1 in PC3 of human prostate cancer cells can significantly inhibit the formation of glandular vesicles.[5,6] In addition, *TEAD4* can promote the proliferation of triple-negative breast cancer cells.[7] Accordingly, researchers have also found high expression and overexpression of *TEAD4* in tubal cancer and testicular germ cell tumors.[8–10] However, the role of *TEAD4* in promoting colorectal tumorigenesis remains to be explored. In the present study, we knocked down *TEAD4* in the Caco2 and HT29 colorectal cancer cell lines to assess its effects on cell proliferation and related protein expression, providing a new theoretical basis for targeted treatment of colorectal cancer.

2 | METHODS

2.1 | Cell culture and transfection

We purchased the human colon cancer cell lines, Caco2 and HT-29, from the American Type Culture Collection (Manassas, VA, USA). Both cell lines were maintained as monolayer cultures in the conditions recommended by the American Type Culture Collection. Caco2 cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum (HyClone, Logan, Utah, USA). HT-29 cells were cultured in RPMI-1640 supplemented with 10% fetal bovine serum (HyClone). Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) was used for all transfections with siRNA and plasmids according to the manufacturer's instructions.

2.2 | Plasmid construction and stable *TEAD4* overexpression

The human *TEAD4* gene was amplified from MCF10A cDNA with the pfu enzymes by PCR using the primers 5′-GATCGGATCCT TGGAGGGCACGGCCGGCAC-3′ and 5′-GATCGAATTCTCATTCTTT CACCAGCCTGT-3′. The PCR products were digested with Bam HI/Eco RI and subcloned into the pBabe vector and verified by DNA sequencing. To generate the pBabe-*TEAD4* plasmid, we amplified the full-length *TEAD4* gene into the pBabe-puro retroviral vector. The pBabe-*TEAD4* plasmid was transfected into AMP293 packaging cells to produce retroviruses. At 72 h after transfection, retroviruses were used to transduce Caco2 and HT-29 cells. After 48 h, 2 μg/mL puromycin was used for selecting drug-resistant cell populations.

2.3 | RNA interference

The siRNA (shRNA) target sequences used in the present study were as follows: *TEAD4* #2: siRNA/shRNA, 5′-CCCATGATGTGAAGCCTTT-3′; #3: siRNA/shRNA: 5′-AGACAGAGTATGCTCGCTAT-3′; and p27siRNA[11]: 5′-GGAGCAATGCGCAG GAAUATT-3′. All siRNAs were synthesized by Ribobio (Guangzhou, China), and were transfected at a final concentration of 10 nM. The pSIH1-H1-puro lentiviral shRNA vector was used to express all shRNAs in Caco2 and HT-29 cells.

2.4 | Sulforhodamine B colorimetric assays

The sulforhodamine B assay was carried out to measure cell viability in 96-well plates. After treatment, cells were fixed with 10% trichloroacetic acid and stained with sulforhodamine B for 30 min. The excess dye was washed off with 1% acetic acid. The protein-bound dye was dissolved in 10 mM Tris base solution for reading at OD530 using a microplate reader.

2.5 | Cell proliferation assays

Cell proliferation was measured using the EdU Incorporation assay using Click-iT EdU Alexa Fluor 488 Imaging Kits (Invitrogen, Grand Island, NY, USA). Cells were labeled with Edu (10 mM) for 6 h and fixed with 3.7% formaldehyde solution (soluble in phosphate-buffered saline [PBS]) for 15 min at room temperature. Then, 0.5% Triton X-100 in PBS solution was added to the cells followed by incubation for 20 min at room temperature. The cells were then incubated with Click-iT reaction cocktail for 30 min and Hoechst 33342 solution (1:2000 soluble in PBS) for 30 min at room temperature in the dark. Once the cells were ready, images were taken using a fluorescence microscope.

2.6 | Antibodies and western blotting

The anti-*TEAD4* (Cat#sc-101184) antibody was purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). The anti-p27 (Cat# 610241) antibody was purchased from BD Bioscience (San Diego, CA, USA). The anti-β-actin (Cat# A5441) antibody was obtained from Sigma-Aldrich (St. Louis, MO, USA). Cells were washed three times in cold 16 PBS and collected with a lysis buffer (50 mmol/L Tris-Cl pH 7.4, 150 mmol/L NaCl, 1 mmol/L EDTA, 1% Triton X-100). Equal amounts of protein lysate were used for western blot analyses with the indicated antibodies. Horseradish peroxidase-coupled goat anti-mouse and horseradish peroxidase-coupled goat anti-rabbit secondary antibodies (Santa Cruz Biotechnology) were used. Images were collected using an ImageQuant LAS 4000 system (GE HealthCare, Pittsburgh, PA, USA).

2.7 | Statistical analysis

All analyses were carried out using the SPSS 13.0 statistical software package (SPSS Inc., Chicago, IL, USA). Sample size was chosen using SPSS 13.0 statistical software to ensure adequate power to detect a respecified effect size. All experiments were carried out at least three times, and data are reported as the mean $\pm$ SD. The variance was similar between the groups that were statistically compared. Differences between two given groups were analyzed by *t*-tests. *P*-values of 0.05 were considered to be statistically significant.

3 | RESULTS

3.1 | Knocked down *TEAD4* increases the expression of the *p27* protein level in Caco2 and HT-29 cells

As *TEAD4* predominately promotes the growth of breast cancer cells,[7] we hypothesized that *TEAD4* might regulate the *p27*-related signaling pathway, which is known to promote both cell proliferation and the cell cycle. The present results showed that *TEAD4* was knocked down in the Caco2 and HT-29 colon cancer cell lines by four different siRNAs, Caco2/TEADsi#2, Caco2/TEADsi#3, HT-29/TEADsi#2, and HT-29/TEADsi#3, and we observed that silencing *TEAD4* resulted in the upregulation of *p27* protein levels in both cell lines (Figure 1).

3.2 | *TEAD4* knockdown inhibits colon cancer cell proliferation in Caco2 and HT-29 cells

To test whether endogenous *TEAD4* also promotes colon cancer cell proliferation, we stably knocked down *TEAD4* in Caco2 and HT-29

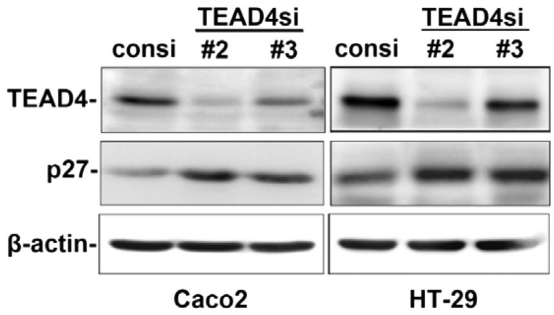

FIGURE 1 Knockdown of *TEAD4* suppresses the protein levels of *p27* in both Caco2 and HT-29 cells. *TEAD4* was silenced by two different siRNAs in both cell lines. The *p27* and *β*-actin protein levels were quantified by IMAGE J software. The normalized *p27* protein levels are shown below the *p27* panel

cells, and as expected, the stable knockdown of *TEAD4* inhibited colon cancer cell proliferation in both Caco2 and HT-29 cells *in vitro* (Figure 2). Furthermore, *TEAD4* knockdown significantly decreased the ratio of EdU-positive cells in both Caco2 and HT-29 cells (Figure 3).

3.3 | *TEAD4* promotes cell proliferation partially through suppressing the *p27* gene in colon cancer cell lines

To determine whether *TEAD4* promotes cell proliferation through *p27*, we carried out a rescue experiment in the Caco2 and HT-29 colon cancer cell lines. We found that knockdown *TEAD4* increased the *p27* protein levels and suppressed cell growth (Figures 1 and 2). After the elevated *p27* protein levels were silenced, *TEAD4* depletion-induced cell growth arrest was significantly rescued (cell viability: Caco2/*TEAD4*si = 0.576 ± 0.039; Caco2/*p27*si = 1.137 ± 0.084; Caco2/*TEAD4*si + *p27*si = 0.912 ± 0.015, no significant differences between group *p27*si [$P = 0.097$] and group *TEAD4*si + *p27*si [$P = 0.076$]; cell viability, HT-29/*TEAD4*si = 0.413 ± 0.013; HT-29/*p27*si = 0.988 ± 0.064; HT-29/*TEAD4*si + *p27*si = 0.927 ± 0.048, no significant differences between group *p27*si [$P = 0.638$] and group *TEAD4*si + *p27*si [$P = 0.567$]). These results suggest that *TEAD4* promotes cell proliferation in part by inhibiting the expression of the *p27* gene.

Furthermore, to test whether *TEAD4* plays a special role in promoting cell proliferation in the Caco2 and HT-29 cell lines, we found that overexpression of *TEAD4* together with the knockdown of *TEAD4* showed no significant difference in promoting cell proliferation compared with knockdown of *TEAD4* only (cell viability: Caco2/TEADsi = 0.400 ± 0.182; Caco2/TEADsi + *TEAD4* = 0.898 ± 0.089; HT-29/TEADsi = 0.453 ± 0.130; HT-29/TEADsi + *TEAD4* = 0.992 ± 0.043). These results suggest that *TEAD4* is unique in promoting cell proliferation in Caco2 and HT-29 cells, excluding interference from other genes in the *TEAD4* family.

4 | DISCUSSION

Colorectal cancer, a common malignant tumor of the digestive system, has the third highest incidence worldwide.[12] In China, the incidence of colorectal cancer has showed an increasing trend year by year,

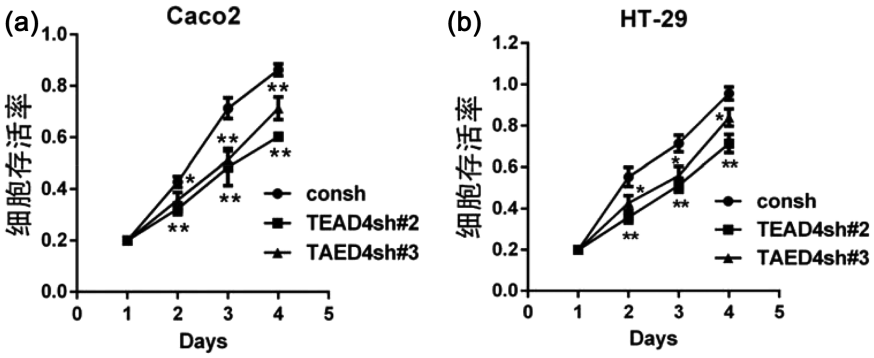

FIGURE 2 Stable knockdown of *TEAD4* significantly suppresses Caco2 and HT-29 cell growth, as determined by the sulforhodamine B assay. $^{**}P < 0.01$, *t*-test

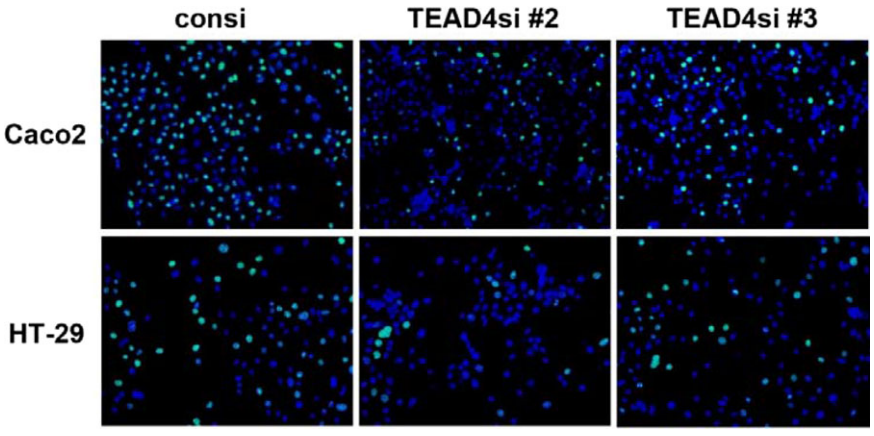

FIGURE 3 *TEAD4* knockdown inhibits DNA synthesis in Caco2 and HT-29 cells, as determined using the Click-iT EdU Alexa Fluor Imaging Kit

and currently, its incidence is fifth among male malignant tumors and fourth among female malignant tumors.[13,14] The human genome encodes four highly homologous TEAD family members (TEAD1-4), which can assemble a transcription complex with YAP–TAZ, thus activating downstream target genes, such as CTGF, Cyr61, Myc, and Gli2,[15] and playing an important role in the process of promoting the occurrence and deterioration of tumors.[16] However, the mechanism of how TEADs promote tumor proliferation needs to be explored.

In the present study, we showed the function and mechanism of *TEAD4* in colon cancer cell proliferation and gene regulation. Our results showed that knockdown of *TEAD4* in Caco2 and HT-29 colon cancer cells increased the protein level of *p27*, which encodes a cell cycle protein that inhibits the cell cycle from G1 to S, suggesting that *TEAD4* might regulate colon cancer cell proliferation through its downstream gene *p27*. Then, we assessed colon cancer cell proliferation by sulforhodamine B and DNA synthesis by EdU, which confirmed that *TEAD4* might regulate cell proliferation and DNA synthesis partially through *p27*, and play an important role in colon tumorigenesis.

Recent studies have shown that TEAD family members could play a crucial role in the progression of multiple malignancies as transcription factors or transcriptional co-activators.[17] Researchers have shown that *TEAD4* and KLF5 are highly expressed in triple-negative breast cancer and can inhibit the development of triple-negative breast cancer by decreasing the expression of *p27* at the transcriptional level.[7,18] In the present study, we found that knockdown of *TEAD4* promoted the expression of the p27 protein in colorectal cancer, thus significantly inhibiting cell proliferation and DNA synthesis, and the mechanism might be associated with the regulation of *TEAD4* in breast cancer.

However, whether *TEAD4* regulates *p27* on its own or by forming transcriptional co-activators with other transcription factors remains unclear. Furthermore, whether *TEAD4* inhibits *p27* expression at the transcriptional level or protein modification level still requires further research.

In the present study, we showed that knockdown of *TEAD4* might inhibit colon cancer cell proliferation and DNA synthesis partially through *p27*. We will further investigate the molecular mechanism of how *TEAD4* affects the proliferation of colorectal cancer cells, and *TEAD4* is expected to become a new target for the clinical diagnosis and treatment of colorectal cancer.

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CONFLICT OF INTEREST

The authors declare that they had read the article and there are no competing interests.

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