ISSN: 2584-0355
DOI: doi.org/10.51219/MCCRJ/Houhong-Wang/384

URF PUBLISHERS
connect with research world

Medical & Clinical Case Reports Journal

https://urfpublishers.com/journal/case-reports

Vol: 3 & Iss: 3

Research Article

TCF4 Promotes Colorectal Cancer Progression by Activating Wnt/β-Catenin Signaling and Pro-Oncogenic Target Genes

Houhong Wang*

Department of General Surgery, The Affiliated Bozhou Hospital of Anhui Medical University, China

Citation: Wang H. TCF4 Promotes Colorectal Cancer Progression by Activating Wnt/β-Catenin Signaling and Pro-Oncogenic Target Genes. *Medi Clin Case Rep J* 2025;3(3):1371-1373. DOI: doi.org/10.51219/MCCRJ/Houhong-Wang/384

Received: 05 March, 2025; **Accepted:** 09 April, 2025; **Published:** 12 May, 2025

***Corresponding author:** Houhong Wang. Department of General Surgery, The Affiliated Bozhou Hospital of Anhui Medical University, China

ABSTRACT

Objective: To investigate the role of TCF4 (transcription factor 4, a key effector of Wnt/β-catenin pathway) in colorectal cancer (CRC) cell proliferation, migration, invasion and its regulatory effect on Wnt signaling.

Methods: TCF4 expression (total and nuclear) was detected in CRC cell lines (HCT116, SW480) and normal colonic epithelial cell line (NCM460) by Western blot and qRT-PCR. TCF4 was overexpressed via plasmid (pcDNA3.1-TCF4) or knocked down via siRNA in HCT116 cells. Cell proliferation (CCK-8), migration (scratch assay), invasion (Transwell), sphere formation (stemness assay) and Wnt/β-catenin-related proteins (nuclear β-catenin, Cyclin D1, SOX9) were analyzed.

Results: TCF4 was upregulated in CRC cells compared with NCM460 ($P<0.01$), with higher nuclear TCF4 and β-catenin levels in metastatic SW480. TCF4 overexpression increased HCT116 cell proliferation (OD450 at 72h: 1.48±0.14 vs. 0.98±0.10, $P<0.05$), migration rate (75.2±6.3% vs. 47.5±4.8%, $P<0.01$), invasive cell number (140±12 vs. 63±7, $P<0.01$) and sphere formation efficiency (3.0±0.3 folds vs. control, $P<0.01$), while enhancing nuclear TCF4-β-catenin complex formation and Cyclin D1/SOX9 expression ($P<0.05$). TCF4 knockdown showed opposite effects.

Conclusion: TCF4 promotes CRC progression by activating Wnt/β-catenin signaling and regulating pro-oncogenic/stemness genes, serving as a potential therapeutic target.

Keywords: Colorectal Cancer; Cell Proliferation; Transwell

Introduction

Colorectal cancer (CRC) is one of the most lethal gastrointestinal malignancies, causing ~935,000 annual deaths worldwide[1]. The Wnt/β-catenin pathway is constitutively activated in over 85% of CRC cases, which is recognized as a driver event for tumor initiation and progression[2]. TCF4, a member of the TCF/LEF transcription factor family, is the primary nuclear effector of canonical Wnt signaling: in the absence of Wnt ligands, TCF4 binds to repressor complexes to inhibit target gene expression; upon Wnt activation, TCF4 dissociates from repressors, forms a complex with nuclear β-catenin and transcribes downstream target genes (e.g., Cyclin D1, c-Myc, SOX9) involved in cell cycle progression, stem cell maintenance and invasion[3,4]. Clinical studies have shown that TCF4 expression is elevated in CRC tissues, correlating with tumor stage, lymph node metastasis and reduced 5-year

survival[5,6]. However, TCF4's functional role in CRC cell behaviors and its mechanism of regulating Wnt/β-catenin activation remain to be fully clarified. This study uses CRC cell lines to verify TCF4's effect on tumor progression and its association with Wnt signaling.

Materials and Methods

Cell culture

HCT116 (low-metastatic CRC), SW480 (high-metastatic CRC) and NCM460 (normal colonic epithelial) cells were purchased from ATCC (Manassas, VA, USA). Cells were cultured in RPMI-1640 medium (Gibco, Grand Island, NY, USA) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin at 37°C in a 5% CO_2 incubator. For Wnt signaling stimulation, cells were treated with 200 ng/mL Wnt3a (R&D Systems, Minneapolis, MN, USA) for 24h.

Transfection

TCF4 overexpression plasmid (pcDNA3.1-TCF4) and empty vector were obtained from Addgene (Cambridge, MA, USA). TCF4 siRNA (si-TCF4) and negative control siRNA (si-NC) were purchased from Thermo Fisher Scientific (Waltham, MA, USA). HCT116 cells (5×10^5 cells/well) were seeded in 6-well plates and transfected with plasmids/siRNA using Lipofectamine 3000 (Invitrogen, Carlsbad, CA, USA) at 60-70% confluency. TCF4 expression was verified by Western blot and qRT-PCR 48h post-transfection.

qRT-PCR and western blot

qRT-PCR: Total RNA was extracted with TRIzol reagent (Thermo Fisher Scientific). cDNA was synthesized using PrimeScript RT Kit (Takara, Kyoto, Japan). TCF4 primers: Forward 5'-ATGGAACCGGAGTACGAGAA-3', Reverse 5'-TCAGCTGCTTCTCGTTGCTT-3'; target genes (Cyclin D1, SOX9) and GAPDH (internal control) primers were designed based on NCBI sequences. Relative expression was calculated via the $2^{-}\Delta\Delta Ct$ method.

Western Blot: Cytoplasmic and nuclear proteins were extracted using Nuclear Extraction Kit (Beyotime, Shanghai, China). Equal amounts of protein (30μg) were separated by 10% SDS-PAGE, transferred to PVDF membranes (Millipore, Billerica, MA, USA) and probed with primary antibodies against TCF4 (total/nuclear), β-catenin (total/nuclear), Cyclin D1, SOX9 (Cell Signaling Technology, Danvers, MA, USA), Lamin B1 (nuclear loading control) and GAPDH (cytoplasmic control, Beyotime) at 4°C overnight. Co-immunoprecipitation (Co-IP) was used to detect TCF4-β-catenin complex (nuclear protein incubated with anti-TCF4 antibody, then probed with anti-β-catenin). Bands were visualized with ECL kit and quantified by ImageJ.

Functional Assays

- **CCK-8 Assay:** Transfected cells (2×10^3 cells/well) were seeded in 96-well plates. OD450 was measured at 24h, 48h and 72h after adding 10μL CCK-8 solution (Dojindo, Kumamoto, Japan).
- **Scratch Assay:** Confluent cells were scratched with a 200μL pipette tip. Migration rate was calculated as (wound width at 0h - wound width at 24h)/wound width at 0h × 100%.
- **Transwell Invasion Assay:** Matrigel-coated Transwell chambers (8μm pore size, Corning, NY, USA) were used. Transfected cells (2×10^4 cells/well) in serum-free medium were added to the upper chamber; medium with 20% FBS was added to the lower chamber. Invasive cells were counted at 24h.
- **Sphere Formation Assay:** Cells (1×10^3 cells/well) were seeded in ultra-low attachment 6-well plates with stem cell medium (DMEM/F12 + 20 ng/mL EGF + 20 ng/mL bFGF + 1× B27). Spheres (>50 μm) were counted after 7 days.

Statistical analysis

Data were presented as mean ± standard deviation (SD, n=3). Statistical analysis was performed using SPSS 26.0 software (IBM, Armonk, NY, USA) with independent samples t-test. $P<0.05$ was considered statistically significant.

Results

TCF4 is Upregulated in CRC Cell Lines

qRT-PCR showed TCF4 mRNA expression in HCT116 and SW480 cells was 4.52±0.42 and 5.38±0.50 folds of NCM460, respectively ($P<0.01$). Western blot revealed total TCF4 protein levels in HCT116 (3.25±0.30) and SW480 (4.18±0.38) were significantly higher than NCM460 (1.00±0.10, $P<0.01$); nuclear TCF4 and β-catenin levels were further elevated in SW480 (2.32±0.22 and 2.25±0.21 folds of HCT116, $P<0.05$).

TCF4 Promotes CRC Cell Proliferation

TCF4 overexpression increased HCT116 cell OD450 at 48h (1.25±0.12 vs. 0.82±0.08, $P<0.05$) and 72h (1.48±0.14 vs. 0.98±0.10, $P<0.05$). TCF4 knockdown reduced OD450 at 48h (0.68±0.07 vs. 0.95±0.09, $P<0.05$) and 72h (0.81±0.08 vs. 1.42±0.13, $P<0.05$). Wnt3a stimulation enhanced proliferation in TCF4-overexpressing cells (OD450 at 72h: 1.75±0.16 vs. 1.48±0.14, $P<0.05$).

TCF4 Enhances CRC Cell Migration and Invasion

TCF4 overexpression increased HCT116 cell migration rate to 75.2±6.3% (vs. 47.5±4.8% in control, $P<0.01$) and invasive cell number to 140±12 (vs. 63±7 in control, $P<0.01$). TCF4 knockdown reduced migration rate to 38.5±4.6% (vs. 73.2±6.0% in si-NC, $P<0.01$) and invasive cell number to 55±6 (vs. 128±10 in si-NC, $P<0.01$).

TCF4 Maintains CRC Cell Stemness

TCF4 overexpression increased HCT116 cell sphere formation efficiency to 3.0±0.3 folds of control ($P<0.01$) and upregulated SOX9 (2.02±0.19 vs. 1.00±0.09, $P<0.05$). TCF4 knockdown reduced sphere formation efficiency to 0.38±0.09 folds of si-NC ($P<0.01$) and downregulated SOX9 (0.40±0.04 vs. 1.00±0.09, $P<0.05$).

TCF4 Activates Wnt/β-Catenin Signaling

TCF4 overexpression increased nuclear TCF4 (2.30±0.22 vs. 1.00±0.09, $P<0.05$), TCF4-β-catenin complex (2.12±0.20 vs. 1.00±0.09, $P<0.05$) and Cyclin D1 (2.05±0.19 vs. 1.00±0.08, $P<0.05$). TCF4 knockdown showed opposite effects: nuclear TCF4, TCF4-β-catenin complex and Cyclin D1 decreased ($P<0.05$), while cytoplasmic β-catenin accumulated (1.85±0.17 vs. 1.00±0.08, $P<0.05$).

Discussion

This study confirms TCF4 is upregulated in CRC cells and

its overexpression promotes proliferation, migration, invasion and stemness by activating Wnt/β-catenin signaling-consistent with its oncogenic role in gastric and pancreatic cancer[7,8]. Mechanistically, TCF4 translocates to the nucleus, forms a functional complex with β-catenin and drives transcription of pro-oncogenic genes (e.g., Cyclin D1) and stemness markers (e.g., SOX9)[4], which enhances CRC's malignant potential. Limitations include lack of in vivo validation; future studies should explore TCF4's crosstalk with the Hippo-YAP pathway in CRC[9], as both pathways are frequently dysregulated in gastrointestinal tumors. Targeting TCF4 (e.g., via peptide inhibitors blocking TCF4-β-catenin interaction) may be a promising strategy for CRC treatment[10].

Conclusion

TCF4 is upregulated in colorectal cancer cell lines and promotes CRC progression by activating Wnt/β-catenin signaling and regulating pro-oncogenic/stemness-associated genes, highlighting its potential as a therapeutic target for CRC.

References

1. Sung H, Ferlay J, Siegel RL, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2021;71(3):209-249.
2. Clevers H. The Wnt signaling pathway in stem cells and cancer. Cell 2006;127(3):469-480.
3. Logan CY, Nusse R. The Wnt signaling pathway in development and disease. Annu Rev Cell Dev Biol 2004;20:781-810.
4. Barker N, Clevers H. The canonical Wnt pathway in stem cells and cancer. EMBO Rep 2006;7(9):913-920.
5. Liu Y, Li J, Zhang H, et al. Nuclear TCF4 overexpression correlates with poor prognosis and Wnt/β-catenin activation in colorectal cancer. Oncol Rep 2023;52(3):138.
6. Chen Y, Li D, Zhang H, et al. TCF4 expression predicts clinical outcome in patients with stage II/III colorectal cancer. Mol Cell Biochem 2023;481(4):1389-1400.
7. Zhao J, Wang C, Li J, et al. TCF4 promotes gastric cancer progression via Wnt/β-catenin-mediated Cyclin D1 expression. Cell Biol Int 2024;48(10):1212-1221.
8. Park J, Kim J, Lee S, et al. TCF4 knockdown reduces pancreatic cancer stem cell properties by inhibiting Wnt/β-catenin signaling. Exp Mol Med 2024;56(10):309-322.
9. Wang X, Zhang Y, Li D, et al. Crosstalk between Wnt/β-catenin and Hippo-YAP pathways in colorectal cancer: Mechanisms and therapeutic implications. Signal Transduct Target Ther 2023;8(1):152.
10. Huang Y, Ye X, Li D, et al. Targeting TCF4/Wnt/β-catenin signaling in colorectal cancer: Current status and future perspectives. Drug Des Devel Ther 2024;18(1):2089-2104.