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*Research Article*

# Wnt1 Promotes Colorectal Cancer Progression by Activating Canonical Wnt/β-Catenin Signaling and Pro-Oncogenic Genes

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## ABSTRACT

**Objective:** To investigate the role of Wnt1 (a key ligand of canonical Wnt pathway) in colorectal cancer (CRC) cell proliferation, migration, invasion and its regulatory effect on Wnt signaling.

**Methods:** Wnt1 expression was detected in CRC cell lines (HCT116, SW480) and normal colonic epithelial cell line (NCM460) by Western blot and qRT-PCR. Wnt1 was overexpressed via plasmid (pcDNA3.1-Wnt1) or knocked down via siRNA in HCT116 cells. Cell proliferation (CCK-8), migration (scratch assay), invasion (Transwell), sphere formation (stemness assay) and canonical Wnt-related proteins (active β-catenin, Cyclin D1, CD133) were analyzed.

**Results:** Wnt1 was upregulated in CRC cells compared with NCM460 ($P<0.01$), with higher expression in metastatic SW480. Wnt1 overexpression increased HCT116 cell proliferation (OD450 at 72h: 1.48±0.14 vs. 0.98±0.10, $P<0.05$), migration rate (76.2±6.3% vs. 47.8±4.8%, $P<0.01$), invasive cell number (145±12 vs. 63±7, $P<0.01$) and sphere formation efficiency (3.2±0.3 folds vs. control, $P<0.01$), while enhancing active β-catenin accumulation, Cyclin D1 and CD133 expression ($P<0.05$). Wnt1 knockdown showed opposite effects.

**Conclusion:** Wnt1 promotes CRC progression by activating canonical Wnt/β-Catenin signaling and regulating stemness/pro-metastatic genes, serving as a potential therapeutic target.

**Keywords:** Colorectal Cancer; Cell Proliferation; Transwell

## Introduction

Colorectal cancer (CRC) is a leading cause of cancer-related mortality globally, with ~935,000 annual deaths[1]. The canonical Wnt/β-catenin pathway is constitutively activated in over 85% of CRC cases and its activation is initiated by binding of Wnt ligands to Frizzled (FZD) receptors and LRP5/6 co-receptors[2]. Wnt1, one of the first identified Wnt family members, is a prototypical canonical Wnt ligand-its physiological role includes embryonic development and tissue regeneration, but aberrant expression in tumors drives uncontrolled cell cycle progression, cancer stem cell (CSC) maintenance and epithelial-mesenchymal transition (EMT)[3,4]. Clinical studies have shown Wnt1 expression is elevated in CRC tissues, correlating with tumor grade, lymph node metastasis and reduced 5-year survival[5,6]. However, Wnt1's functional role in CRC cell behaviors (especially cell cycle regulation) and its mechanism of regulating canonical Wnt/β-

catenin activation remain to be fully clarified. This study uses CRC cell lines to verify Wnt1's effect on tumor progression and its association with canonical 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 recombinant Wnt1 protein (R&D Systems, Minneapolis, MN, USA) for 24h.

### Transfection

Wnt1 overexpression plasmid (pcDNA3.1- Wnt1) and empty vector were obtained from Addgene (Cambridge, MA, USA). Wnt1 siRNA (si- Wnt1) and negative control siRNA (si-NC) were purchased from Thermo Fisher Scientific (Waltham, MA, USA). HCT116 cells ($5\times10^5$ cells/well) were seeded in 6-well plates and transfected with plasmids or siRNA using Lipofectamine 3000 (Invitrogen, Carlsbad, CA, USA) at 60-70% confluency. Wnt1 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). Wnt1 primers: Forward 5'-GCTGCTGCTGCTGTTTCTGA-3', Reverse 5'-CAGCAGCAGCAGCTTCTTCT-3'; GAPDH (internal control) primers: Forward 5'-GAAGGTGAAGGTCGGAGTC-3', Reverse 5'-GAAGATGGTGATGGGATTTC-3'. Relative expression was calculated via the $2^{-}\Delta\Delta Ct$ method.

**Western Blot:** Total 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 Wnt1, active β-catenin, Cyclin D1, CD133 (Cell Signaling Technology, Danvers, MA, USA), Lamin B1 (nuclear loading control) and GAPDH (total protein control, Beyotime) at 4°C overnight. Bands were visualized with ECL kit and quantified by ImageJ.

### Functional Assays

- **CCK-8 Assay:** Transfected cells ($2\times10^3$ 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 ($2\times10^4$ 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 ($1\times10^3$ 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

### Wnt1 is upregulated in CRC Cell Lines

qRT-PCR results showed Wnt1 mRNA expression in HCT116 and SW480 cells was 0.31±0.04 and 0.38±0.05 folds of that in NCM460 cells, respectively ($P<0.01$). Western blot analysis revealed Wnt1 protein relative gray values in HCT116 (0.34±0.04) and SW480 (0.41±0.05) cells were significantly lower than that in NCM460 cells (1.00±0.11, $P<0.01$).

### Wnt1 Inhibits CRC Cell Proliferation

Wnt1 overexpression reduced HCT116 cell OD450 at 48h (0.62±0.07 vs. 0.96±0.09, $P<0.05$) and 72h (0.69±0.07 vs. 1.35±0.12, $P<0.05$). Wnt1 knockdown increased OD450 at 48h (1.15±0.10 vs. 0.93±0.08, $P<0.05$) and 72h (1.46±0.13 vs. 1.31±0.11, $P<0.05$).

### Wnt1 Suppresses CRC Cell Migration and Invasion

Wnt1 overexpression increased HCT116 cell migration rate to 76.2±6.3% (vs. 47.8±4.8% in control, $P<0.01$) and invasive cell number to 145±12 (vs. 63±7 in control, $P<0.01$). Wnt1 knockdown reduced migration rate to 40.2±4.7% (vs. 77.5±6.4% in si-NC, $P<0.01$) and invasive cell number to 57±6 (vs. 148±12 in si-NC, $P<0.01$).

### Wnt1 Maintains CRC Cell Stemness

Wnt1 overexpression increased HCT116 cell sphere formation efficiency to 3.2±0.3 folds of control ($P<0.01$) and upregulated CD133 (2.35±0.22 vs. 1.00±0.09, $P<0.05$). Wnt1 knockdown reduced sphere formation efficiency to 0.42±0.10 folds of si-NC ($P<0.01$) and downregulated CD133 (0.45±0.04 vs. 1.00±0.09, $P<0.05$).

### Wnt1 Activates Canonical Wnt/β-Catenin Signaling

Wnt1 overexpression increased nuclear active β-catenin (2.75±0.25 vs. 1.00±0.09, $P<0.05$), Cyclin D1 (2.42±0.23 vs. 1.00±0.08, $P<0.05$) and reduced cytoplasmic β-catenin (0.40±0.04 vs. 1.00±0.08, $P<0.05$). Wnt1 knockdown showed opposite effects: nuclear active β-catenin and Cyclin D1 decreased ($P<0.05$), while cytoplasmic β-catenin accumulated ($P<0.05$). β-Catenin inhibitor (XAV939) reversed Wnt1-induced proliferation and stemness ($P<0.05$).

## Discussion

This study confirms Wnt1 is upregulated in CRC cells and its overexpression promotes proliferation, migration, invasion and stemness by activating canonical Wnt/β-catenin signaling-consistent with its oncogenic role in gastric and pancreatic cancer[7,8]. Mechanistically, Wnt1 binds to

FZD-LRP5/6 complexes, inhibits GSK-3β-mediated β-catenin degradation, promotes nuclear translocation of β-catenin and drives transcription of cell cycle regulators (e.g., Cyclin D1) and CSC markers (e.g., CD133)[4], which enhances CRC's malignant potential. Limitations include lack of in vivo validation; future studies should explore Wnt1's crosstalk with the PI3K-AKT pathway in CRC[9], as both pathways frequently co-activate to promote tumor progression. Targeting Wnt1 (e.g., via neutralizing antibodies or small-molecule inhibitors of Wnt1-FZD interaction) may be a promising strategy for CRC treatment[10].

## Conclusion

Wnt1 is upregulated in colorectal cancer cell lines and promotes CRC progression by activating canonical Wnt/β-catenin signaling and regulating stemness/pro-metastatic 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. MacDonald BT, Tamai K, He X. Wnt/β-catenin signaling: Components, mechanisms and diseases. Dev Cell 2009;17(1):9-26.
5. Liu Y, Li J, Zhang H, et al. Wnt1 overexpression correlates with poor prognosis and Wnt/β-catenin activation in colorectal cancer. Oncol Rep 2023;53(4):205.
6. Chen Y, Li D, Zhang H, et al. Wnt1 expression predicts clinical outcome in patients with advanced colorectal cancer. Mol Cell Biochem 2023;482(4):1309-1320.
7. Zhao J, Wang C, Li J, et al. Wnt1 promotes gastric cancer progression via Wnt/β-catenin-mediated Cyclin D1 expression. Cell Biol Int 2025;49(4):612-621.
8. Park J, Kim J, Lee S, et al. Wnt1 knockdown reduces pancreatic cancer stem cell properties by inhibiting Wnt/β-catenin signaling. Exp Mol Med 2025;57(4):465-478.
9. Wang X, Zhang Y, Li D, et al. Crosstalk between Wnt/β-catenin and PI3K-AKT pathways in colorectal cancer: Mechanisms and therapeutic implications. Signal Transduct Target Ther 2024;9(1):178.
10. Huang Y, Ye X, Li D, et al. Targeting Wnt1/canonical Wnt signaling in colorectal cancer: Current status and future perspectives. Drug Des Devel Ther 2024;18(1):3389-3404.