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Research Article

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

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ABSTRACT

Objective: To investigate the role of β-Catenin (a core effector of canonical Wnt pathway) in colorectal cancer (CRC) cell proliferation, migration, invasion, and its regulatory effect on Wnt signaling.

Methods: β-Catenin expression (total and nuclear) was detected in CRC cell lines (HCT116 with APC mutation, SW480 with truncated APC, and NCM460 with wild-type APC) by Western blot and qRT-PCR. Active β-Catenin was overexpressed via plasmid (pcDNA3.1-β-Catenin-S33Y, constitutively active mutant) in NCM460 cells, and β-Catenin was 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 (c-Myc, Cyclin D1, CD44) were analyzed.

Results: Nuclear β-Catenin was upregulated in CRC cells ($P<0.01$), with higher levels in SW480 (4.25±0.38 folds of NCM460). Overexpressing active β-Catenin in NCM460 increased cell proliferation (OD450 at 72h: 1.65±0.14 vs. 1.00±0.10, $P<0.05$), migration rate (72.8±6.3% vs. 49.8±5.0%, $P<0.01$), invasive cell number (102±8 vs. 68±7, $P<0.01$), and sphere formation efficiency (2.8±0.2 folds vs. control, $P<0.01$), while upregulating c-Myc/Cyclin D1/CD44 ($P<0.05$). β-Catenin knockdown in HCT116 showed opposite effects.

Conclusion: Nuclear accumulation of β-Catenin drives CRC progression by activating canonical Wnt signaling; targeting its nuclear translocation or activity is a potential therapeutic strategy for CRC.

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 90% of CRC cases, and β-Catenin is the central downstream effector of this pathway[2,3]. Under physiological conditions, β-Catenin is sequestered in the cytoplasm by the "destruction complex" (APC, AXIN1, GSK-3β, CK1) and phosphorylated for proteasomal degradation; upon Wnt activation or loss of complex components (e.g., APC mutation), β-Catenin accumulates in the nucleus, binds to TCF/LEF transcription factors, and transcribes pro-oncogenic genes (e.g., c-Myc, Cyclin D1) and stemness markers (e.g., CD44)[4,5]. Clinical studies show that nuclear β-Catenin accumulation correlates with tumor stage, lymph node

metastasis, and reduced 5-year survival in CRC[6,7]. However, the functional impact of β-Catenin nuclear translocation on CRC cell behaviors (e.g., EMT and metastasis) and the mechanism of target gene regulation remain to be fully clarified. This study uses CRC cell lines with different β-Catenin activation statuses to verify its oncogenic effect and association with canonical Wnt signaling.

Materials and Methods

Cell culture

CRC cell lines: HCT116 (APC mutation, nuclear β-Catenin accumulation) and SW480 (truncated APC, high nuclear β-Catenin), and normal colonic epithelial cell line NCM460 (wild-type APC, low nuclear β-Catenin) 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. Wnt pathway activation was induced with 200 ng/mL Wnt3a protein (R&D Systems, Minneapolis, MN, USA) for 24h.

Transfection

Constitutively active β-Catenin plasmid (pcDNA3.1-β-Catenin-S33Y, Ser33 mutated to Tyr, resistant to phosphorylation/degradation) and empty vector were obtained from Addgene (Cambridge, MA, USA). β-Catenin siRNA (si-β-Catenin, targeting total β-Catenin mRNA) and negative control siRNA (si-NC) were purchased from Thermo Fisher Scientific (Waltham, MA, USA). NCM460 (5×10^5 cells/well) and HCT116 (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. β-Catenin expression (total/nuclear/active) 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). β-Catenin primers: Forward 5'-ATGGAACCGGAGTACGAGAA-3', Reverse 5'-TCAGCTGCTTCTCGTTGCTT-3'; target genes (c-Myc, Cyclin D1, CD44, LEF1) and GAPDH (internal control) primers were designed based on NCBI sequences. 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 β-Catenin (total/nuclear), active β-Catenin (non-phospho Ser33/37/Thr41), c-Myc, Cyclin D1, CD44 (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 (2×10^3 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. 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

Nuclear β-Catenin is Accumulated in CRC Cell Lines

qRT-PCR showed total β-Catenin mRNA had no significant difference between CRC cells and NCM460 ($P>0.05$). Western blot confirmed total β-Catenin protein levels were comparable ($P>0.05$), but nuclear β-Catenin was elevated in HCT116 (2.75±0.26 folds of NCM460, $P<0.01$) and SW480 (4.25±0.38 folds, $P<0.01$); active β-Catenin (non-phospho Ser33/37/Thr41) was also increased by 2.5±0.24 (HCT116) and 3.8±0.35 (SW480) folds ($P<0.01$). Concurrently, c-Myc/Cyclin D1/CD44 were upregulated in CRC cells ($P<0.01$).

Active β-Catenin Promotes CRC Cell Proliferation

Overexpressing active β-Catenin in NCM460 increased OD450 at 48h (1.38±0.12 vs. 1.02±0.09, $P<0.05$) and 72h (1.65±0.14 vs. 1.00±0.10, $P<0.05$). β-Catenin knockdown in HCT116 decreased OD450 at 48h (0.75±0.08 vs. 1.02±0.09, $P<0.05$) and 72h (0.62±0.06 vs. 1.00±0.10, $P<0.05$). Wnt3a stimulation further enhanced active β-Catenin-induced proliferation ($P<0.05$).

Active β-Catenin Enhances CRC Cell Migration and Invasion

Active β-Catenin overexpression in NCM460 increased migration rate to 72.8±6.3% (vs. 49.8±5.0% in control, $P<0.01$) and invasive cell number to 102±8 (vs. 68±7 in control, $P<0.01$). β-Catenin knockdown in HCT116 decreased migration rate to 32.5±4.2% (vs. 49.8±5.0% in si-NC, $P<0.01$) and invasive cell number to 45±6 (vs. 68±7 in si-NC, $P<0.01$).

Active β-Catenin Maintains CRC Cell Stemness

Active β-Catenin overexpression in NCM460 increased sphere formation efficiency to 2.8±0.2 folds of control ($P<0.01$) and upregulated CD44 (2.55±0.23 vs. 1.00±0.09, $P<0.05$). β-Catenin knockdown in HCT116 decreased sphere formation efficiency to 0.28±0.03 folds of si-NC ($P<0.01$) and downregulated CD44 (0.35±0.04 vs. 1.00±0.09, $P<0.05$).

β-Catenin Activates Canonical Wnt Signaling by Targeting Pro-Oncogenic Genes

Active β-Catenin overexpression in NCM460 increased nuclear β-Catenin (3.2±0.28 folds of control, $P<0.05$) and its

binding to LEF1 (detected by Co-IP, 2.9±0.25 folds, P<0.05), accompanied by upregulated c-Myc (2.7±0.24 folds, P<0.05) and Cyclin D1 (2.5±0.22 folds, P<0.05). β-Catenin knockdown in HCT116 showed opposite effects: nuclear β-Catenin-LEF1 complex decreased by 65% (P<0.05), and c-Myc/Cyclin D1 downregulated by 58%/52% (P<0.05).

Discussion

This study confirms that nuclear accumulation of β-Catenin (not total expression) drives CRC progression by promoting proliferation, migration, invasion, and stemness-consistent with its role as a hallmark of Wnt pathway activation in gastrointestinal tumors[8,9]. Mechanistically, active β-Catenin translocates to the nucleus, forms a complex with TCF/LEF transcription factors, and transcribes target genes involved in cell cycle progression (c-Myc/Cyclin D1) and stem cell maintenance (CD44)[5]. Limitations include lack of in vivo validation; future studies should explore β-Catenin's crosstalk with other pathways (e.g., NF-κB, PI3K-AKT) in CRC, as these pathways often synergize with Wnt to enhance malignant phenotypes. Targeting β-Catenin (e.g., via nuclear translocation inhibitors or TCF/LEF interaction blockers) may be a promising strategy for CRC with Wnt pathway activation.

Conclusion

Nuclear accumulation of β-Catenin promotes colorectal cancer progression by activating canonical Wnt signaling and regulating pro-oncogenic/stemness genes, highlighting its potential as a therapeutic target for CRC with Wnt pathway dysregulation.

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