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Research Article

Wnt3a Promotes Colorectal Cancer Progression by Activating Canonical Wnt/β-Catenin Signaling and Stemness-Associated Genes

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ABSTRACT

Objective: To investigate the role of Wnt3a (a key ligand of canonical Wnt pathway) in colorectal cancer (CRC) cell proliferation, migration, invasion, and its regulatory effect on Wnt signaling.

Methods: Wnt3a expression was detected in CRC cell lines (HCT116, SW480) and normal colonic epithelial cell line (NCM460) by Western blot and qRT-PCR. Wnt3a was overexpressed via plasmid (pcDNA3.1-Wnt3a) 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: Wnt3a was upregulated in CRC cells compared with NCM460 ($P<0.01$), with higher expression in metastatic SW480. Wnt3a 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 ($P<0.05$). Wnt3a knockdown showed opposite effects.

Conclusion: Wnt3a 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]. Wnt3a, 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 Wnt3a expression is elevated in CRC tissues, correlating with tumor grade, lymph node metastasis and reduced 5-year survival[5,6]. However, Wnt3a'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 Wnt3a'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 Wnt3a protein (R&D Systems, Minneapolis, MN, USA) for 24h.

Transfection

Wnt3a overexpression plasmid (pcDNA3.1- Wnt3a) and empty vector were obtained from Addgene (Cambridge, MA, USA). Wnt3a siRNA (si- Wnt3a) 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 or siRNA using Lipofectamine 3000 (Invitrogen, Carlsbad, CA, USA) at 60-70% confluency. Wnt3a 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). Wnt3a 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 Wnt3a, 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 (2×10^3 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

Wnt3a is upregulated in CRC Cell Lines

qRT-PCR results showed Wnt3a 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 Wnt3a 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, $P<0.01$).

Wnt3a Inhibits CRC Cell Proliferation

Wnt3a 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, $P<0.05$). Wnt3a 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, $P<0.05$).

Wnt3a Suppresses CRC Cell Migration and Invasion

Wnt3a 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, $P<0.01$). Wnt3a 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, $P<0.01$).

Wnt3a Maintains CRC Cell Stemness

Wnt3a 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, $P<0.05$). Wnt3a 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, $P<0.05$).

Wnt3a Activates Canonical Wnt/β-Catenin Signaling

Wnt3a 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, $P<0.05$). Wnt3a 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 Wnt3a-induced proliferation and stemness ($P<0.05$).

Discussion

This study confirms Wnt3a 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, Wnt3a 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 Wnt3a's crosstalk with the PI3K-AKT pathway in CRC[9], as both pathways frequently co-activate to promote tumor progression. Targeting Wnt3a (e.g., via neutralizing antibodies or small-molecule inhibitors of Wnt3a-FZD interaction) may be a promising strategy for CRC treatment[10].

Conclusion

Wnt3a 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.

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