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Research Article

AXIN1 Inhibits Colorectal Cancer Progression by Suppressing Canonical Wnt/β-Catenin Signaling and Pro-Oncogenic Genes

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ABSTRACT

Objective: To investigate the role of AXIN1 (a key negative regulator of canonical Wnt/β-catenin pathway) in colorectal cancer (CRC) cell proliferation, migration, invasion and its regulatory effect on Wnt signaling.

Methods: AXIN1 expression was detected in CRC cell lines (HCT116, SW480) and normal colonic epithelial cell line (NCM460) by Western blot and qRT-PCR. AXIN1 was overexpressed via plasmid (pcDNA3.1-AXIN1) 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 (β-catenin, GSK-3β, c-Myc) were analyzed.

Results: AXIN1 was downregulated in CRC cells compared with NCM460 ($P<0.01$), with lower expression in metastatic SW480. AXIN1 overexpression decreased HCT116 cell proliferation (OD450 at 72h: 0.68±0.06 vs. 0.99±0.10, $P<0.05$), migration rate (38.5±4.6% vs. 48.2±4.9%, $P<0.01$), invasive cell number (52±6 vs. 65±7, $P<0.01$) and sphere formation efficiency (0.35±0.04 folds vs. control, $P<0.01$), while reducing β-catenin accumulation, enhancing GSK-3β activity and downregulating c-Myc ($P<0.05$). AXIN1 knockdown showed opposite effects.

Conclusion: AXIN1 functions as a tumor suppressor in CRC by inhibiting canonical Wnt/β-catenin signaling, serving as a potential therapeutic target for restoring pathway homeostasis.

Keywords: Colorectal Cancer; Western Blot; Cell Proliferation; Canonical Wnt-related Proteins

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 activity is tightly regulated by the "destruction complex" composed of AXIN1, APC, GSK-3β and CK1[2]. AXIN1 (Axin-1) acts as a scaffold protein in this complex, facilitating GSK-3β-mediated phosphorylation and degradation of β-catenin-thus preventing nuclear translocation of β-catenin and transcription of pro-oncogenic target genes (e.g., c-Myc, Cyclin D1)[3,4]. Clinical studies have shown that AXIN1 is frequently downregulated or mutated in CRC tissues, correlating with tumor stage, lymph node metastasis and reduced 5-year survival[5,6]. However, AXIN1's functional role

in CRC cell behaviors (especially pathway suppression) and its mechanism of regulating Wnt/β-catenin homeostasis remain to be fully clarified. This study uses CRC cell lines to verify AXIN1's tumor-suppressive effect 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 pathway activation, cells were treated with 200 ng/mL Wnt3a protein (R&D Systems, Minneapolis, MN, USA) for 24h.

Transfection

AXIN1 overexpression plasmid (pcDNA3.1-AXIN1) and empty vector were obtained from Addgene (Cambridge, MA, USA). AXIN1 siRNA (si-AXIN1) 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. AXIN1 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). AXIN1 primers: Forward 5'-ATGGAACCGGAGTACGAGAA-3', Reverse 5'-TCAGCTGCTTCTCGTTGCTT-3'; target genes (c-Myc, Cyclin D1) 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 AXIN1, β-catenin (total/nuclear), GSK-3β (total/p-Ser9), c-Myc (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.
- **Spere 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

AXIN1 is downregulated in CRC cell lines

qRT-PCR showed AXIN1 mRNA expression in HCT116/SW480 was 0.42±0.04/0.28±0.03 folds of NCM460 ($P<0.01$). Western blot revealed AXIN1 protein in HCT116 (0.38±0.04) and SW480 (0.25±0.03) was significantly lower than NCM460 (1.00±0.10, $P<0.01$); nuclear β-catenin levels were inversely elevated in SW480 (2.85±0.26 folds of HCT116, $P<0.05$).

AXIN1 inhibits CRC cell proliferation

AXIN1 overexpression decreased HCT116 cell OD450 at 48h (0.82±0.08 vs. 1.02±0.09, $P<0.05$) and 72h (0.68±0.06 vs. 0.99±0.10, $P<0.05$). AXIN1 knockdown increased OD450 at 48h (1.25±0.12 vs. 1.02±0.09, $P<0.05$) and 72h (1.48±0.14 vs. 0.99±0.10, $P<0.05$). Wnt3a stimulation partially reversed AXIN1-induced proliferation inhibition ($P<0.05$).

AXIN1 reduces CRC cell migration and invasion

AXIN1 overexpression decreased HCT116 cell migration rate to 38.5±4.6% (vs. 48.2±4.9% in control, $P<0.01$) and invasive cell number to 52±6 (vs. 65±7 in control, $P<0.01$). AXIN1 knockdown increased migration rate to 62.8±6.0% (vs. 48.2±4.9% in si-NC, $P<0.01$) and invasive cell number to 88±8 (vs. 65±7 in si-NC, $P<0.01$).

AXIN1 suppresses CRC cell stemness

AXIN1 overexpression decreased HCT116 cell sphere formation efficiency to 0.35±0.04 folds of control ($P<0.01$) and downregulated CD44 (0.42±0.04 vs. 1.00±0.09, $P<0.05$). AXIN1 knockdown increased sphere formation efficiency to 2.1±0.2 folds of si-NC ($P<0.01$) and upregulated CD44 (2.05±0.19 vs. 1.00±0.09, $P<0.05$).

AXIN1 inactivates canonical Wnt/β-catenin signaling

AXIN1 overexpression reduced nuclear β-catenin (0.45±0.04 vs. 1.00±0.09, $P<0.05$), c-Myc (0.48±0.04 vs. 1.00±0.08, $P<0.05$) and p-GSK-3β (Ser9) (0.52±0.05 vs. 1.00±0.08, $P<0.05$) (indicating enhanced GSK-3β activity). AXIN1 knockdown showed opposite effects: nuclear β-catenin, c-Myc and p-GSK-3β increased ($P<0.05$), while β-catenin degradation was inhibited.

Discussion

This study confirms AXIN1 is downregulated in CRC cells and its overexpression exerts tumor-suppressive effects by inhibiting proliferation, migration, invasion and stemness-consistent with its role in gastric and pancreatic cancer[7,8].

Mechanistically, AXIN1 stabilizes the Wnt destruction complex, enhances GSK-3β-mediated β-catenin phosphorylation and degradation and reduces nuclear translocation of β-catenin, thereby suppressing transcription of pro-oncogenic genes (e.g., c-Myc)[4]. Limitations include lack of in vivo validation; future studies should explore AXIN1's interaction with APC (another destruction complex component) in CRC[9], as concurrent loss of AXIN1 and APC often exacerbates Wnt pathway activation. Restoring AXIN1 expression (e.g., via gene delivery or small-molecule stabilizers) may be a promising strategy for CRC treatment[10].

Conclusion

AXIN1 is downregulated in colorectal cancer cell lines and inhibits CRC progression by suppressing canonical Wnt/β-catenin signaling, highlighting its potential as a therapeutic target for restoring pathway homeostasis in CRC.

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