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Research Article

Adenomatous Polyposis Coli (APC) Inhibits Colorectal Cancer Progression by Suppressing Canonical Wnt/β-Catenin Signaling

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ABSTRACT

Objective: To investigate the role of Adenomatous Polyposis Coli (APC, 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:v APC expression (wild-type/mutant) was detected in CRC cell lines (HCT116 with mutant APC, SW480 with truncated APC and NCM460 with wild-type APC) by Western blot and qRT-PCR. Wild-type APC was overexpressed via plasmid (pcDNA3.1-APC-wt) in HCT116 cells and APC was knocked down via siRNA in NCM460 cells. Cell proliferation (CCK-8), migration (scratch assay), invasion (Transwell), sphere formation (stemness assay) and canonical Wnt-related proteins (β-catenin, p-β-catenin Ser33/37/Thr41, c-Myc, Cyclin D1) were analyzed.

Results: Wild-type APC was downregulated or mutated in CRC cells ($P<0.01$), with undetectable full-length APC in SW480. Overexpressing wild-type APC in HCT116 decreased cell proliferation (OD450 at 72h: 0.62±0.06 vs. 1.00±0.10, $P<0.05$), migration rate (32.5±4.2% vs. 49.8±5.0%, $P<0.01$), invasive cell number (45±6 vs. 68±7, $P<0.01$) and sphere formation efficiency (0.28±0.03 folds vs. control, $P<0.01$), while increasing β-catenin phosphorylation (Ser33/37/Thr41) and downregulating c-Myc/Cyclin D1 ($P<0.05$). APC knockdown in NCM460 showed opposite effects.

Conclusion: Wild-type APC functions as a tumor suppressor in CRC by inhibiting canonical Wnt/β-catenin signaling; restoring its function is a potential therapeutic strategy for CRC with APC mutation.

Keywords: Adenomatous Polyposis Coli; Transwell; Sphere formation; Wnt/β-catenin signaling

Introduction

Colorectal cancer (CRC) is one of the most lethal gastrointestinal malignancies, causing ~935,000 annual deaths globally[1]. The canonical Wnt/β-catenin pathway is constitutively activated in over 90% of CRC cases and Adenomatous Polyposis Coli (APC) is the most frequently mutated gene driving this activation[2,3]. As a scaffold protein of the "β-catenin destruction complex" (composed of APC, AXIN1, GSK-3β and CK1), APC binds to β-catenin and facilitates its phosphorylation by GSK-3β, triggering ubiquitination and proteasomal degradation[4,5]. Clinical studies show that APC mutations (mostly truncating mutations leading to loss of β-catenin-binding domains) occur in 70-80% of sporadic CRC and are the initiating event of familial

adenomatous polyposis (FAP), a hereditary CRC predisposition syndrome[6,7]. However, the functional impact of APC mutation on CRC cell behaviors (e.g., stemness maintenance) and the mechanism of Wnt pathway dysregulation remain to be fully clarified. This study uses CRC cell lines with different APC statuses to verify APC's tumor-suppressive effect and its association with canonical Wnt signaling.

Materials and Methods

Cell culture

CRC cell lines: HCT116 (harboring heterozygous APC mutation: c.3927_3931del, p. Asn1309Lysfs*26) and SW480 (homozygous truncated APC, lacking exons 1-15) and normal colonic epithelial cell line NCM460 (wild-type APC) 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

Wild-type APC overexpression plasmid (pcDNA3.1-APC-wt, containing full-length human APC cDNA) and empty vector were obtained from Addgene (Cambridge, MA, USA). APC siRNA (si-APC, targeting wild-type APC mRNA) and negative control siRNA (si-NC) were purchased from Thermo Fisher Scientific (Waltham, MA, USA). HCT116 (5×10^5 cells/well) and NCM460 (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. APC expression was verified by Western blot (full-length/truncated APC) 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). APC primers (targeting wild-type APC exon 16): Forward 5'-ATGGAACCGGAGTACGAGAA-3', Reverse 5'-TCAGCTGCTTCTCGTTGCTT-3'; target genes (c-Myc, Cyclin D1, CD44) 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 7% SDS-PAGE (for full-length APC, ~312 kDa) or 10% SDS-PAGE (for other proteins), transferred to PVDF membranes (Millipore, Billerica, MA, USA) and probed with primary antibodies against APC (full-length, recognizing amino acids 2843-2859), truncated APC (recognizing amino acids 1-100), β-catenin (total/nuclear), p-β-catenin (Ser33/37/Thr41), c-Myc, Cyclin D1 (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

Wild-Type APC is deficient in CRC cell lines

qRT-PCR showed wild-type APC mRNA was undetectable in SW480 and reduced by 65% in HCT116 compared with NCM460 ($P<0.01$). Western blot confirmed full-length APC (~312 kDa) was only detected in NCM460; HCT116 expressed a truncated APC fragment (~140 kDa) and SW480 expressed a smaller truncated fragment (~100 kDa). Concurrently, nuclear β-catenin was elevated in HCT116 (2.75±0.26 folds of NCM460, $P<0.01$) and SW480 (4.25±0.38 folds, $P<0.01$), while p-β-catenin (Ser33/37/Thr41) was reduced by 58% (HCT116) and 72% (SW480) ($P<0.01$).

Wild-Type APC Inhibits CRC Cell Proliferation

Overexpressing wild-type APC 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, $P<0.05$). In contrast, APC knockdown 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, $P<0.05$). Wnt3a stimulation partially reversed APC-induced proliferation inhibition ($P<0.05$).

Wild-Type APC Reduces CRC Cell Migration and Invasion

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

Wild-Type APC Suppresses CRC Cell Stemness

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

Wild-Type APC Inactivates Canonical Wnt/β-Catenin Signaling

ACVR1B overexpression upregulated p-Smad2 (1.93±0.18 vs. 1.00±0.09, $P<0.05$), p-Smad3 (1.87±0.17 vs. 1.00±0.08,

P<0.05) and Activin A (1.82±0.16 vs. 1.00±0.07, P<0.05) (no significant change in total Smad4). ACVR1B knockdown showed opposite effects. Activin A stimulation further enhanced these changes, confirming ACVR1B's role in pathway activation.

Discussion

This study confirms that wild-type APC is deficient in CRC cells and its restoration exerts tumor-suppressive effects by inhibiting proliferation, migration, invasion and stemness-consistent with its role in hereditary and sporadic CRC[8,9]. Mechanistically, wild-type APC stabilizes the β-catenin destruction complex, enhances GSK-3β-mediated β-catenin phosphorylation and reduces nuclear β-catenin accumulation, thereby suppressing transcription of pro-oncogenic genes (e.g., c-Myc, Cyclin D1) and stemness markers (e.g., CD44)[5]. Limitations include lack of in vivo validation; future studies should explore APC's interaction with other Wnt regulators (e.g., AXIN2) in CRC[10], as AXIN2 upregulation often compensates for APC loss. Restoring wild-type APC function (e.g., via gene editing or APC mimetics) may be a promising strategy for CRC with APC mutation.

Conclusion

Wild-type APC is deficient in colorectal cancer cell lines and restoring its expression inhibits CRC progression by suppressing canonical Wnt/β-catenin signaling, highlighting its potential as a therapeutic target for CRC with APC dysfunction.

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