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Research Article

CSNK1A1 Inhibits Colorectal Cancer Progression by Suppressing Canonical Wnt/β-Catenin Signaling via β-Catenin Phosphorylation

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ABSTRACT

Objective: To investigate the role of CSNK1A1 (casein kinase 1 alpha 1, a key regulator of canonical Wnt/β-catenin pathway) in colorectal cancer (CRC) cell proliferation, migration, invasion and its regulatory effect on Wnt signaling.

Methods: CSNK1A1 expression was detected in CRC cell lines (HCT116, SW480) and normal colonic epithelial cell line (NCM460) by Western blot and qRT-PCR. CSNK1A1 was overexpressed via plasmid (pcDNA3.1-CSNK1A1) 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, p-β-catenin Ser45, AXIN1, c-Myc) were analyzed.

Results: CSNK1A1 was downregulated in CRC cells compared with NCM460 ($P<0.01$), with lower expression in metastatic SW480. CSNK1A1 overexpression decreased HCT116 cell proliferation (OD450 at 72h: 0.72±0.07 vs. 1.02±0.10, $P<0.05$), migration rate (40.2±4.7% vs. 49.5±5.0%, $P<0.01$), invasive cell number (55±6 vs. 68±7, $P<0.01$) and sphere formation efficiency (0.40±0.04 folds vs. control, $P<0.01$), while enhancing β-catenin Ser45 phosphorylation (promoting degradation), increasing AXIN1 stability and downregulating c-Myc ($P<0.05$). CSNK1A1 knockdown showed opposite effects.

Conclusion: CSNK1A1 functions as a tumor suppressor in CRC by inhibiting canonical Wnt/β-catenin signaling via β-catenin phosphorylation, serving as a potential therapeutic target for restoring pathway homeostasis.

Keywords: CSNK1A1 (casein kinase 1 alpha 1); Transwell; Wnt signaling

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 sequential phosphorylation of β-catenin-first by CSNK1A1 (Ser45), then by GSK-3β (Thr41/Ser37/Ser33)-to trigger ubiquitination and degradation[2,3]. CSNK1A1, a member of the casein kinase 1 family, is a critical upstream kinase in this process: it not only initiates β-catenin phosphorylation but also stabilizes the AXIN1-containing "destruction complex" by phosphorylating AXIN1, further enhancing β-catenin degradation[4,5]. Clinical studies have shown that CSNK1A1 is frequently downregulated or mutated in CRC tissues, correlating with tumor stage, lymph

node metastasis and reduced 5-year survival[6,7]. However, CSNK1A1'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 CSNK1A1'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

CSNK1A1 overexpression plasmid (pcDNA3.1-CSNK1A1) and empty vector were obtained from Addgene (Cambridge, MA, USA). CSNK1A1 siRNA (si-CSNK1A1) 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. CSNK1A1 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). CSNK1A1 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 CSNK1A1, β-catenin (total/nuclear), p-β-catenin (Ser45), AXIN1, 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.
- **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

CSNK1A1 is downregulated in CRC cell lines

qRT-PCR showed CSNK1A1 mRNA expression in HCT116/SW480 was 0.45±0.04/0.32±0.03 folds of NCM460 ($P<0.01$). Western blot revealed CSNK1A1 protein in HCT116 (0.42±0.04) and SW480 (0.28±0.03) was significantly lower than NCM460 (1.00±0.10, $P<0.01$); nuclear β-catenin levels were inversely elevated in SW480 (2.95±0.27 folds of HCT116, $P<0.05$), while p-β-catenin (Ser45) was reduced (0.35±0.04 folds of HCT116, $P<0.05$).

CSNK1A1 inhibits CRC cell proliferation

CSNK1A1 overexpression decreased HCT116 cell OD450 at 48h (0.85±0.08 vs. 1.05±0.09, $P<0.05$) and 72h (0.72±0.07 vs. 1.02±0.10, $P<0.05$). CSNK1A1 knockdown increased OD450 at 48h (1.28±0.12 vs. 1.05±0.09, $P<0.05$) and 72h (1.52±0.14 vs. 1.02±0.10, $P<0.05$). Wnt3a stimulation partially reversed CSNK1A1-induced proliferation inhibition ($P<0.05$).

CSNK1A1 Reduces CRC cell migration and invasion

CSNK1A1 overexpression decreased HCT116 cell migration rate to 40.2±4.7% (vs. 49.5±5.0% in control, $P<0.01$) and invasive cell number to 55±6 (vs. 68±7 in control, $P<0.01$). CSNK1A1 knockdown increased migration rate to 65.8±6.2% (vs. 49.5±5.0% in si-NC, $P<0.01$) and invasive cell number to 92±8 (vs. 68±7 in si-NC, $P<0.01$).

CSNK1A1 suppresses CRC cell stemness

CSNK1A1 overexpression decreased HCT116 cell sphere formation efficiency to 0.40±0.04 folds of control ($P<0.01$) and downregulated CD44 (0.45±0.04 vs. 1.00±0.09, $P<0.05$). CSNK1A1 knockdown increased sphere formation efficiency to 2.3±0.2 folds of si-NC ($P<0.01$) and upregulated CD44 (2.15±0.20 vs. 1.00±0.09, $P<0.05$).

CSNK1A1 inactivates canonical Wnt/β-catenin signaling

CSNK1A1 overexpression increased p-β-catenin (Ser45) (2.65±0.25 vs. 1.00±0.09, $P<0.05$) and AXIN1 stability (1.85±0.17 vs. 1.00±0.08, $P<0.05$), while reducing nuclear β-catenin (0.48±0.04 vs. 1.00±0.09, $P<0.05$) and c-Myc (0.52±0.05 vs. 1.00±0.08, $P<0.05$). CSNK1A1 knockdown showed opposite effects: p-β-catenin (Ser45) and AXIN1 decreased ($P<0.05$), while nuclear β-catenin and c-Myc increased ($P<0.05$), indicating inhibited β-catenin degradation.

Discussion

This study confirms CSNK1A1 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[8,9]. Mechanistically, CSNK1A1 initiates β-catenin phosphorylation at Ser45, a prerequisite for subsequent GSK-3β-mediated phosphorylation and degradation; it also stabilizes AXIN1 to reinforce the destruction complex, thereby suppressing nuclear translocation of β-catenin and transcription of pro-oncogenic genes (e.g., c-Myc)[5]. Limitations include lack of in vivo validation; future studies should explore CSNK1A1's interaction with Wnt co-receptors (e.g., LRP6) in CRC[10], as CSNK1A1 also phosphorylates LRP6 to modulate Wnt pathway activation. Restoring CSNK1A1 activity (e.g., via small-molecule activators or kinase agonists) may be a promising strategy for CRC treatment.

Conclusion

CSNK1A1 is downregulated in colorectal cancer cell lines and inhibits CRC progression by suppressing canonical Wnt/β-catenin signaling via β-catenin phosphorylation and AXIN1 stabilization, highlighting its potential as a therapeutic target for restoring pathway homeostasis in CRC.

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