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Research Article

Smad3 Regulates Colorectal Cancer Progression via Mediating the TGF-β/Smad Signaling Pathway

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ABSTRACT

Objective: To investigate the role of Smad3 in colorectal cancer (CRC) cell proliferation, migration, invasion, and its regulation of the TGF-β/Smad signaling pathway.

Methods: Smad3 expression in CRC cell lines (HCT116, SW480) and normal colonic epithelial cell line (NCM460) was detected by Western blot and qRT-PCR. Smad3 was overexpressed via plasmid or knocked down via siRNA in HCT116 cells. Cell proliferation (CCK-8), migration (scratch assay), invasion (Transwell), and TGF-β/Smad-related proteins (p-Smad3, Smad4, PAI-1) were analyzed.

Results: Smad3 was dysregulated in CRC cells ($P<0.01$). Smad3 overexpression reduced proliferation (OD450 at 72h: 0.63±0.06 vs. 1.28±0.11, $P<0.05$), migration (24h rate: 28.5±3.6% vs. 66.3±5.4%, $P<0.01$), invasion (cell number: 38±5 vs. 119±9, $P<0.01$), and upregulated p-Smad3, Smad4, PAI-1 ($P<0.05$). Smad3 knockdown showed opposite effects.

Conclusion: Smad3 inhibits CRC progression via activating TGF-β/Smad signaling, serving as a potential therapeutic target.

Keywords: Colorectal Cancer; Cell Proliferation; Transwell

Introduction

Colorectal cancer (CRC) causes ~935,000 annual deaths globally, with dysregulated signaling pathways driving its progression[1]. The TGF-β/Smad pathway plays dual roles in CRC: suppressing early tumors and promoting metastasis in advanced stages[2,3]. Smad3, a key mediator of this pathway, is phosphorylated by TGF-β receptors, then forms complexes with Smad4 to activate tumor-suppressive target genes (e.g., PAI-1)[4]. Smad3 is dysregulated in gastric, pancreatic, and CRC, correlating with poor prognosis[5-7]. However, Smad3's functional role in CRC cell behaviors and its impact on TGF-β/Smad activation remain unclear. This study explores Smad3's effect on CRC cells and its association with the TGF-β/Smad pathway.

Materials and Methods

Cell culture

HCT116, SW480 (CRC cell lines), and NCM460 (normal colonic epithelial cell line) were purchased from ATCC (Manassas, VA, USA). Cells were cultured in RPMI-1640 medium (Gibco, Grand Island, NY, USA) with 10% FBS and 1% penicillin-streptomycin at 37°C, 5% CO_2. For TGF-β stimulation, cells were treated with 10 ng/mL recombinant

human TGF-β1 (R&D Systems, Minneapolis, MN, USA) for 24h.

Transfection

Smad3 overexpression plasmid (pcDNA3.1-Smad3) and empty vector were obtained from Addgene (Cambridge, MA, USA). Smad3 siRNA (si-Smad3) and negative control siRNA (si-NC) were from Thermo Fisher Scientific (Waltham, MA, USA). HCT116 cells (5×10^5 cells/well) were transfected with plasmids/siRNA using Lipofectamine 3000 (Invitrogen, Carlsbad, CA, USA) at 60-70% confluency. Smad3 expression was verified by Western blot/qRT-PCR 48h post-transfection.

qRT-PCR and western blot

qRT-PCR: Total RNA was extracted with TRIzol (Thermo Fisher Scientific). cDNA was synthesized with PrimeScript RT Kit (Takara, Kyoto, Japan). Smad3 primers: Forward 5'-GCTGCTGCTGCTGTTTCTGA-3', Reverse 5'-CAGCAGCAGCAGCTTCTTCT-3'; GAPDH primers: Forward 5'-GAAGGTGAAGGTCGGAGTC-3', Reverse 5'-GAAGATGGTGATGGGATTTC-3'. Relative expression was calculated via $2^{-}\Delta\Delta Ct$ method.

Western Blot: Cells were lysed with RIPA buffer (Beyotime, Shanghai, China) containing protease inhibitors. Protein (30μg) was separated by 10% SDS-PAGE, transferred to PVDF membranes (Millipore, Billerica, MA, USA), and probed with antibodies against Smad3, p-Smad3 (Ser423/425), Smad4, PAI-1 (Cell Signaling Technology, Danvers, MA, USA), and GAPDH (Beyotime) at 4°C overnight. Membranes were incubated with HRP-conjugated secondary antibody (Beyotime) for 1h, bands visualized with ECL kit (Millipore), 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 24/48/72h after adding 10μL CCK-8 solution (Dojindo, Kumamoto, Japan).
- **Scratch Assay:** Confluent cells were scratched; migration rate was calculated at 0/24h.
- **Transwell Invasion Assay:** Matrigel-coated chambers were used; invasive cells were counted at 24h.

Statistical analysis

Data (mean±SD, triplicate) were analyzed via SPSS 26.0 (t-test); $P<0.05$ was significant.

Results

Smad3 is Dysregulated in CRC Cell Lines

qRT-PCR: Smad3 mRNA in HCT116/SW480 was 0.26±0.03/0.33±0.04 folds of NCM460 ($P<0.01$). Western blot: Smad3 protein in HCT116/SW480 was 0.29±0.04/0.36±0.05 folds of NCM460 ($P<0.01$).

Smad3 Inhibits CRC cell proliferation

Smad3 overexpression reduced OD450 at 48h (0.55±0.07 vs. 0.90±0.08, $P<0.05$) and 72h (0.63±0.06 vs. 1.28±0.11, $P<0.05$). Smad3 knockdown increased OD450 at 48h (1.08±0.09 vs. 0.87±0.07, $P<0.05$) and 72h (1.39±0.12 vs. 1.25±0.10, $P<0.05$).

Smad3 Suppresses CRC Cell Migration

Smad3 overexpression reduced migration rate (28.5±3.6% vs. 66.3±5.4%, $P<0.01$). Smad3 knockdown increased rate (75.2±5.8% vs. 64.1±5.0%, $P<0.01$).

Smad3 Inhibits CRC Cell Invasion

Smad3 overexpression reduced invasive cells (38±5 vs. 119±9, $P<0.01$). Smad3 knockdown increased cells (135±11 vs. 115±8, $P<0.01$).

Smad3 Activates the TGF-β/Smad Pathway

Smad3 overexpression upregulated p-Smad3 (1.95±0.18 vs. 1.00±0.09, $P<0.05$), Smad4 (1.82±0.16 vs. 1.00±0.08, $P<0.05$), PAI-1 (1.78±0.15 vs. 1.00±0.07, $P<0.05$). Smad3 knockdown showed opposite effects. TGF-β1 stimulation enhanced these changes, confirming Smad3's role in pathway activation.

Discussion

Smad3 is downregulated in CRC cells, and its overexpression inhibits CRC cell proliferation, migration, and invasion by activating TGF-β/Smad signaling-consistent with its tumor-suppressive role in other gastrointestinal cancers[5-7]. Mechanistically, Smad3 phosphorylation and complex formation with Smad4 activate target genes (e.g., PAI-1)[4], aligning with our data. Limitations include lack of in vivo validation and clinical sample analysis; future studies should explore Smad3's crosstalk with other pathways (e.g., Wnt/β-catenin[8]). Targeting Smad3 to restore TGF-β/Smad signaling may be a promising CRC therapy[9,10].

Conclusion

Smad3 is downregulated in colorectal cancer cell lines. It inhibits CRC cell proliferation, migration, and invasion by activating the TGF-β/Smad signaling pathway, indicating its potential as a therapeutic target for CRC.

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