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Research Article

Smad7 Promotes Colorectal Cancer Progression via Inhibiting the TGF-β/Smad Signaling Pathway

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ABSTRACT

Objective: To investigate the role of Smad7 in colorectal cancer (CRC) cell proliferation, migration, invasion, and its regulation of the TGF-β/Smad signaling pathway.

Methods: Smad7 expression in CRC cell lines (HCT116, SW480) and normal colonic epithelial cell line (NCM460) was detected by Western blot and qRT-PCR. Smad7 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 (Smad2, p-Smad2, Smad3, p-Smad3) were analyzed.

Results: Smad7 was upregulated in CRC cells ($P<0.01$). Smad7 overexpression increased proliferation (OD450 at 72h: 1.35±0.11 vs. 0.89±0.08, $P<0.05$), migration (24h rate: 70.2±5.8% vs. 41.5±4.2%, $P<0.01$), invasion (cell number: 120±9 vs. 52±6, $P<0.01$), and downregulated p-Smad2/p-Smad3 ($P<0.05$). Smad7 knockdown showed opposite effects.

Conclusion: Smad7 promotes CRC progression via inhibiting 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, ranking second in cancer-related mortality[1]. The TGF-β/Smad signaling pathway plays dual roles in CRC: suppressing early tumorigenesis but promoting progression in advanced stages[2,3]. Smad7, an inhibitory Smad, negatively regulates TGF-β/Smad signaling by blocking Smad2/Smad3 phosphorylation and nuclear translocation[4]. Smad7 is upregulated in liver, gastric, and pancreatic cancers, correlating with poor prognosis[5-7]. However, Smad7's functional role in CRC and its impact on TGF-β/Smad signaling remain understudied. This study explores Smad7'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) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin at 37°C in a 5% CO_2 humidified incubator. For TGF-β stimulation,

cells were treated with 10 ng/mL recombinant human TGF-β1 (R&D Systems, Minneapolis, MN, USA) for 24h.

Transfection

Smad7 overexpression plasmid (pcDNA3.1-Smad7) and negative control plasmid (pcDNA3.1) were obtained from Addgene (Cambridge, MA, USA). Smad7 siRNA (si-Smad7) and negative control siRNA (si-NC) were purchased from Thermo Fisher Scientific (Waltham, MA, USA). HCT116 cells were seeded in 6-well plates (5×10^5 cells/well) and transfected with plasmids or siRNA using Lipofectamine 3000 (Invitrogen, Carlsbad, CA, USA) at 60-70% confluency. Smad7 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). Smad7 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: Cells were lysed with RIPA buffer (Beyotime, Shanghai, China) containing protease inhibitors. Protein concentration was measured by BCA assay (Beyotime). 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 Smad7, Smad2, p-Smad2 (Ser465/467), Smad3, p-Smad3 (Ser423/425) (Cell Signaling Technology, Danvers, MA, USA), and GAPDH (Beyotime) at 4°C overnight. Membranes were incubated with HRP-conjugated secondary antibody (Beyotime) for 1h, and bands were visualized with ECL kit (Millipore) and quantified by ImageJ.

Functional Assays

- **CCK-8 Assay:** Transfected HCT116 cells (2×10^3 cells/well) were seeded in 96-well plates. At 24h, 48h, and 72h, 10μL CCK-8 solution (Dojindo, Kumamoto, Japan) was added, and absorbance at 450nm was measured with a microplate reader (Bio-Rad, Hercules, CA, USA).
- **Scratch Wound Healing Assay:** Confluent transfected cells were scratched with a 200μL pipette tip. Wound width was measured at 0h and 24h, and 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, and medium with 20% FBS to the lower chamber. After 24h, invasive cells on the lower membrane were fixed, stained with 0.1% crystal violet, and counted under a microscope (five random fields).

Statistical analysis

All experiments were performed in triplicate. Data were presented as mean ± standard deviation (SD). Statistical analysis was conducted using SPSS 26.0 software (IBM, Armonk, NY, USA) with independent samples t-test. $P<0.05$ was considered statistically significant.

Results

Smad7 is Upregulated in CRC cell lines

qRT-PCR results showed that Smad7 mRNA expression in HCT116 and SW480 cells was 3.85±0.35 and 3.22±0.29 folds of that in NCM460 cells, respectively ($P<0.01$). Western blot analysis revealed that Smad7 protein relative gray values in HCT116 (2.92±0.26) and SW480 (2.45±0.22) cells were significantly higher than that in NCM460 cells (1.00±0.10, $P<0.01$).

Smad7 Promotes CRC Cell Proliferation

Smad7 overexpression increased the OD450 value of HCT116 cells at 48h (1.08±0.09 vs. 0.72±0.06, $P<0.05$) and 72h (1.35±0.11 vs. 0.89±0.08, $P<0.05$). In contrast, Smad7 knockdown reduced the OD450 value at 48h (0.55±0.07 vs. 0.88±0.07, $P<0.05$) and 72h (0.68±0.07 vs. 1.32±0.10, $P<0.05$).

Smad7 Enhances CRC Cell Migration

Scratch wound healing assay showed that the migration rate of HCT116 cells in the Smad7 overexpression group was 70.2±5.8% at 24h, significantly higher than that in the control group (41.5±4.2%, $P<0.01$). Smad7 knockdown reduced the migration rate to 32.6±4.0%, which was lower than that in the si-NC group (68.8±5.5%, $P<0.01$).

Smad7 Promotes CRC Cell Invasion

Transwell invasion assay revealed that the number of invasive HCT116 cells in the Smad7 overexpression group was 120±9, significantly more than that in the control group (52±6, $P<0.01$). Smad7 knockdown reduced the number of invasive cells to 45±5, which was less than that in the si-NC group (115±8, $P<0.01$).

Smad7 Inhibits the TGF-β/Smad Signaling Pathway

Western blot analysis showed that Smad7 overexpression downregulated the relative gray values of p-Smad2 (0.42±0.05 vs. 1.00±0.08, $P<0.05$) and p-Smad3 (0.39±0.04 vs. 1.00±0.07, $P<0.05$) (with no significant change in total Smad2/Smad3). Smad7 knockdown showed opposite effects: p-Smad2 (1.85±0.16 vs. 1.00±0.08, $P<0.05$) and p-Smad3 (1.78±0.15 vs. 1.00±0.07, $P<0.05$) were upregulated. TGF-β1 stimulation further enhanced Smad2/Smad3 phosphorylation in Smad7-knockdown cells, confirming Smad7's inhibitory role in TGF-β/Smad signaling.

Discussion

Smad7 is upregulated in CRC cells, and its overexpression promotes CRC cell proliferation, migration, and invasion by inhibiting the TGF-β/Smad pathway-consistent with its oncogenic role in other gastrointestinal cancers[5-7]. Mechanistically, Smad7 binds to activated TGF-β receptors to block Smad2/Smad3 phosphorylation[4], aligning with our data showing downregulated p-Smad2/p-Smad3 in Smad7-overexpressing cells. Limitations include lack of in vivo validation and clinical sample analysis; future studies should explore Smad7's crosstalk with other pathways (e.g., Wnt/β-catenin[8]). Targeting Smad7 may be a promising CRC therapeutic strategy[9,10].

Conclusion

Smad7 is upregulated in colorectal cancer cell lines. It promotes CRC cell proliferation, migration, and invasion by

inhibiting the TGF-β/Smad signaling pathway, indicating its potential as a therapeutic target for CRC.

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