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Research Article

Smad2 Mediates Colorectal Cancer Progression via Regulating the TGF-β/Smad Signaling Pathway

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ABSTRACT

Objective: To investigate the role of Smad2 in colorectal cancer (CRC) cell proliferation, migration, invasion and its regulation of the TGF-β/Smad signaling pathway.

Methods: Smad2 expression in CRC cell lines (HCT116, SW480) and normal colonic epithelial cell line (NCM460) was detected by Western blot and qRT-PCR. Smad2 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-Smad2, Smad4, PAI-1) were analyzed.

Results: Smad2 was downregulated in CRC cells ($P<0.01$). Smad2 overexpression reduced proliferation (OD450 at 72h: 0.65±0.06 vs. 1.30±0.11, $P<0.05$), migration (24h rate: 29.8±3.7% vs. 67.5±5.5%, $P<0.01$), invasion (cell number: 40±5 vs. 121±9, $P<0.01$) and upregulated p-Smad2, Smad4, PAI-1 ($P<0.05$). Smad2 knockdown showed opposite effects.

Conclusion: Smad2 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) accounts for ~935,000 annual deaths globally, with dysregulated signaling pathways driving its malignant progression[1]. The TGF-β/Smad pathway plays context-dependent roles in CRC: suppressing early tumorigenesis while promoting metastasis in advanced stages[2,3]. Smad2, a core mediator of this pathway, is phosphorylated by activated TGF-β receptors, then forms heteromeric complexes with Smad4 to translocate to the nucleus and activate tumor-suppressive target genes (e.g., PAI-1)[4]. Smad2 is frequently downregulated in gastric, pancreatic and CRC, correlating with poor patient prognosis[5-7]. However, Smad2's functional role in regulating CRC cell behaviors and its impact on TGF-β/Smad pathway activation remain incompletely clarified. This study explores Smad2's effect on CRC cells and its association with the TGF-β/Smad signaling axis.

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

Smad2 overexpression plasmid (pcDNA3.1-Smad2) and empty vector were obtained from Addgene (Cambridge, MA, USA). Smad2 siRNA (si-Smad2) 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 or siRNA using Lipofectamine 3000 (Invitrogen, Carlsbad, CA, USA) at 60-70% confluency. Smad2 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). Smad2 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 Smad2, p-Smad2 (Ser465/467), 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 24h, 48h and 72h after adding 10μL CCK-8 solution (Dojindo, Kumamoto, Japan).
- **Scratch Wound Healing Assay:** Confluent transfected 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.

Statistical analysis

Data were presented as mean ± standard deviation (SD, triplicate experiments). 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

Smad2 is Downregulated in CRC cell lines

qRT-PCR results showed Smad2 mRNA expression in HCT116 and SW480 cells was 0.28±0.03 and 0.35±0.04 folds of that in NCM460 cells, respectively ($P<0.01$). Western blot analysis revealed Smad2 protein relative gray values in HCT116 (0.31±0.04) and SW480 (0.38±0.05) cells were significantly lower than that in NCM460 cells (1.00±0.10, $P<0.01$).

Smad2 inhibits CRC cell proliferation

Smad2 overexpression reduced HCT116 cell OD450 at 48h (0.57±0.07 vs. 0.92±0.08, $P<0.05$) and 72h (0.65±0.06 vs. 1.30±0.11, $P<0.05$). Smad2 knockdown increased OD450 at 48h (1.10±0.09 vs. 0.89±0.07, $P<0.05$) and 72h (1.41±0.12 vs. 1.27±0.10, $P<0.05$).

Smad2 Inhibits CRC cell invasion

Transwell assay revealed Smad2 overexpression reduced invasive cell number to 40±5, significantly less than the control group (121±9, $P<0.01$). Smad2 knockdown increased invasive cells to 137±11, more than the si-NC group (117±8, $P<0.01$).

Smad2 activates the TGF-β/Smad signaling pathway

Smad2 overexpression upregulated p-Smad2 (1.98±0.18 vs. 1.00±0.09, $P<0.05$), Smad4 (1.85±0.17 vs. 1.00±0.08, $P<0.05$) and PAI-1 (1.80±0.16 vs. 1.00±0.07, $P<0.05$). Smad2 knockdown showed opposite effects. TGF-β1 stimulation further enhanced these changes, confirming Smad2's role in pathway activation.

Discussion

Smad2 is downregulated in CRC cells and its overexpression inhibits CRC cell proliferation, migration and invasion by activating the TGF-β/Smad pathway-consistent with its tumor-suppressive role in other gastrointestinal cancers[5-7]. Mechanistically, Smad2 phosphorylation and complex formation with Smad4 drive activation of tumor-suppressive 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 Smad2's crosstalk with pathways like Wnt/β-catenin[8]. Restoring Smad2 expression to reactivate TGF-β/Smad signaling may be a promising CRC therapeutic strategy[9,10].

Conclusion

Smad2 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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