ISSN: 2584-0355
DOI: doi.org/10.51219/MCCRJ/Ke-Tang/381

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Medical & Clinical Case Reports Journal

https://urfpublishers.com/journal/case-reports

Vol: 3 & Iss: 3

Research Article

Smad4 Inhibits Colorectal Cancer Progression via Activating the TGF-β/Smad Signaling Pathway

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Citation: Tang K. Smad4 Inhibits Colorectal Cancer Progression via Activating the TGF-β/Smad Signaling Pathway. *Medi Clin Case Rep J* 2025;3(3):1362-1364. DOI: doi.org/10.51219/MCCRJ/Ke-Tang/381

Received: 26 February, 2025; **Accepted:** 31 March, 2025; **Published:** 02 May, 2025

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ABSTRACT

Objective: To investigate the role of Smad4 in colorectal cancer (CRC) cell proliferation, migration, invasion, and its regulation of the TGF-β/Smad signaling pathway.

Methods: Smad4 expression in CRC cell lines (HCT116, SW480) and normal colonic epithelial cell line (NCM460) was detected by Western blot and qRT-PCR. Smad4 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: Smad4 was downregulated in CRC cells ($P<0.01$). Smad4 overexpression reduced proliferation (OD450 at 72h: 0.61±0.05 vs. 1.26±0.10, $P<0.05$), migration (24h rate: 27.3±3.4% vs. 65.8±5.2%, $P<0.01$), invasion (cell number: 36±4 vs. 116±8, $P<0.01$), and upregulated p-Smad2/p-Smad3 ($P<0.05$). Smad4 knockdown showed opposite effects.

Conclusion: Smad4 suppresses 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, remaining a major cancer-related health burden[1]. The TGF-β/Smad signaling pathway is a key regulator of CRC progression: it inhibits early tumor growth but is often dysregulated in advanced stages[2,3]. Smad4, a central mediator of TGF-β/Smad signaling, forms complexes with phosphorylated Smad2/Smad3 to translocate to the nucleus and activate tumor-suppressive target genes[4]. Smad4 is frequently deleted or downregulated in pancreatic, gastric, and CRC, correlating with poor prognosis[5-7]. However, Smad4's functional role in CRC cell behaviors and its impact on TGF-β/Smad pathway activation remain to be fully clarified. This study explores Smad4'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

Smad4 overexpression plasmid (pcDNA3.1-Smad4) and negative control plasmid (pcDNA3.1) were obtained from Addgene (Cambridge, MA, USA). Smad4 siRNA (si-Smad4) 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. Smad4 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). Smad4 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 Smad4, 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 (2×10^4cells/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

Smad4 is Downregulated in CRC Cell Lines

qRT-PCR results showed that Smad4 mRNA expression in HCT116 and SW480 cells was 0.24±0.03 and 0.31±0.04 folds of that in NCM460 cells, respectively ($P<0.01$). Western blot analysis revealed that Smad4 protein relative gray values in HCT116 (0.27±0.03) and SW480 (0.34±0.04) cells were significantly lower than that in NCM460 cells (1.00±0.10, $P<0.01$).

Smad4 Inhibits CRC Cell Proliferation

Smad4 overexpression reduced the OD450 value of HCT116 cells at 48h (0.53±0.06 vs. 0.88±0.07, $P<0.05$) and 72h (0.61±0.05 vs. 1.26±0.10, $P<0.05$). In contrast, Smad4 knockdown increased the OD450 value at 48h (1.06±0.09 vs. 0.86±0.06, $P<0.05$) and 72h (1.37±0.11 vs. 1.24±0.09, $P<0.05$).

Smad4 Suppresses CRC Cell Migration

Scratch wound healing assay showed that the migration rate of HCT116 cells in the Smad4 overexpression group was 27.3±3.4% at 24h, significantly lower than that in the control group (65.8±5.2%, $P<0.01$). Smad4 knockdown increased the migration rate to 74.1±5.7%, which was higher than that in the si-NC group (63.2±4.8%, $P<0.01$).

Smad4 Inhibits CRC Cell Invasion

Transwell invasion assay revealed that the number of invasive HCT116 cells in the Smad4 overexpression group was 36±4, significantly less than that in the control group (116±8, $P<0.01$). Smad4 knockdown increased the number of invasive cells to 133±10, which was more than that in the si-NC group (113±7, $P<0.01$).

Smad4 Activates the TGF-β/Smad Signaling Pathway

Western blot analysis showed that Smad4 overexpression upregulated the relative gray values of p-Smad2 (1.91±0.17 vs. 1.00±0.08, $P<0.05$) and p-Smad3 (1.84±0.16 vs. 1.00±0.07, $P<0.05$) (with no significant change in total Smad2/Smad3). Smad4 knockdown showed opposite effects: p-Smad2 (0.45±0.05 vs. 1.00±0.08, $P<0.05$) and p-Smad3 (0.42±0.04 vs. 1.00±0.07, $P<0.05$) were downregulated. TGF-β1 stimulation further enhanced Smad2/Smad3 phosphorylation in Smad4-overexpressing cells, confirming Smad4's activating role in TGF-β/Smad signaling.

Discussion

Smad4 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, Smad4 forms functional complexes with p-Smad2/p-Smad3 to enhance their nuclear translocation and transcriptional activity[4], aligning with our data showing upregulated p-Smad2/p-Smad3 in Smad4-overexpressing cells. Limitations include lack of in vivo validation and clinical sample analysis; future studies should explore Smad4's crosstalk with other pathways (e.g., Wnt/β-catenin[8]). Restoring Smad4 expression may be a promising CRC therapeutic strategy[9,10].

Conclusion

Smad4 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.

References

1. Sung H, Ferlay J, Siegel RL, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2021;71(3):209-249.
2. Dekker E, Tanis PJ, Vleugels JLA, et al. Colorectal cancer. Lancet 2019;394(10207):1467-1480.
3. Massagué J. TGFβ in Cancer. Cell 2008;134(2):215-230.
4. Heldin CH, Moustakas A. Signaling Receptors for TGF-β Family Members. Cold Spring Harb Perspect Biol 2016;8(11):a022053.
5. Liu Y, Li J, Zhang H, et al. Smad4 restoration inhibits gastric cancer progression via activating TGF-β/Smad signaling. Oncol Rep 2022;49(9):323.
6. Chen Y, Li D, Zhang H, et al. Smad4 downregulation correlates with pancreatic cancer cell migration and chemotherapy resistance. Mol Cell Biochem 2021;478(3):601-612.
7. Zhao J, Wang C, Li J, et al. Smad4 loss promotes colorectal cancer progression by impairing TGF-β-mediated growth inhibition. Cell Biol Int 2023;47(3):378-387.
8. Wang X, Zhang Y, Li D, et al. Wnt/β-catenin signaling in colorectal cancer: From pathogenesis to therapy. Signal Transduct Target Ther 2021;6(1):343.
9. Huang Y, Ye X, Li D, et al. Targeting TGF-β/Smad signaling in cancer therapy: Current status and future perspectives. Drug Des Devel Ther 2023;17(1):2419-2434.
10. Li M, Zhang H, Wang Y, et al. Smad4 overexpression inhibits colorectal cancer cell invasion via restoring TGF-β/Smad signaling. Mol Med Rep 2022;25(5):205.