ISSN: 2584-0355
DOI: doi.org/10.51219/MCCRJ/Houhong-Wang/351

URF PUBLISHERS
connect with research world

Medical & Clinical Case Reports Journal

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

Vol: 3 & Iss: 3

Research Article

ACVR1B Regulates Colorectal Cancer Progression via Mediating the TGF-β/Activin Signaling Pathway

Houhong Wang*

Department of General Surgery, The Affiliated Bozhou Hospital of Anhui Medical University, China

Citation: Wang H. ACVR1B Regulates Colorectal Cancer Progression via Mediating the TGF-β/Activin Signaling Pathway. *Medi Clin Case Rep J* 2025;3(3):1274-1276. DOI: doi.org/10.51219/MCCRJ/Houhong-Wang/351

Received: 02 October, 2024; **Accepted:** 04 November, 2024; **Published:** 05 December, 2025

***Corresponding author:** Houhong Wang. Department of General Surgery, The Affiliated Bozhou Hospital of Anhui Medical University, China

ABSTRACT

Objective: To investigate the role of ACVR1B (activin A receptor type 1B) in colorectal cancer (CRC) cell proliferation, migration, invasion, and its regulation of the TGF-β/activin signaling pathway.

Methods: ACVR1B expression in CRC cell lines (HCT116, SW480) and normal colonic epithelial cell line (NCM460) was detected by Western blot and qRT-PCR. ACVR1B was overexpressed via plasmid or knocked down via siRNA in HCT116 cells. Cell proliferation (CCK-8), migration (scratch assay), invasion (Transwell), and TGF-β/activin-related proteins (p-Smad2, p-Smad3, Smad4, Activin A) were analyzed.

Results: ACVR1B was upregulated in CRC cells ($P<0.01$). ACVR1B overexpression increased proliferation (OD450 at 72h: 1.38±0.13 vs. 0.91±0.09, $P<0.05$), migration (24h rate: 71.5±5.9% vs. 42.8±4.3%, $P<0.01$), invasion (cell number: 128±10 vs. 55±6, $P<0.01$), and upregulated p-Smad2, p-Smad3, Activin A ($P<0.05$). ACVR1B knockdown showed opposite effects.

Conclusion: ACVR1B promotes CRC progression via activating TGF-β/activin signaling, serving as a potential therapeutic target.

Keywords: ACVR1B (activin A receptor type 1B); Colorectal Cancer; Cell Proliferation; TGF-β/activin

Introduction

Colorectal cancer (CRC) causes ~935,000 annual deaths globally, with dysregulated signaling pathways driving its malignant progression[1]. The TGF-β/activin signaling pathway plays context-dependent roles in CRC: while TGF-β often suppresses early tumors, activin-A-mediated signaling (via ACVR1B) can promote advanced CRC progression[2,3]. ACVR1B, a type I receptor of the TGF-β superfamily, binds activin A and forms a complex with type II receptors, triggering Smad2/Smad3 phosphorylation and downstream oncogenic signaling[4]. ACVR1B is upregulated in gastric, pancreatic, and CRC, correlating with poor patient prognosis[5-7]. However, ACVR1B's functional role in regulating CRC cell behaviors and its impact on TGF-β/activin pathway activation remain incompletely clarified. This study explores ACVR1B's effect on CRC cells and its association with the TGF-β/activin 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 activin A stimulation, cells were treated with 50 ng/mL recombinant human activin A (R&D Systems, Minneapolis, MN, USA) for 24h.

Transfection

ACVR1B overexpression plasmid (pcDNA3.1-ACVR1B) and empty vector were obtained from Addgene (Cambridge, MA, USA). ACVR1B siRNA (si-ACVR1B) and negative control siRNA (si-NC) were purchased from Thermo Fisher Scientific (Waltham, MA, USA). HCT116 cells (5×10^2 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. ACVR1B 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). ACVR1B 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 ACVR1B, p-Smad2 (Ser465/467), p-Smad3 (Ser423/425), Smad4, Activin A (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

ACVR1B is upregulated in CRC cell lines

qRT-PCR results showed ACVR1B mRNA expression in HCT116 and SW480 cells was 3.92±0.36 and 3.35±0.31 folds of that in NCM460 cells, respectively ($P<0.01$). Western blot analysis revealed ACVR1B protein relative gray values in HCT116 (2.98±0.27) and SW480 (2.52±0.23) cells were significantly higher than that in NCM460 cells (1.00±0.10, $P<0.01$).

ACVR1B promotes CRC cell proliferation

ACVR1B overexpression increased HCT116 cell OD450 at 48h (1.12±0.10 vs. 0.74±0.07, $P<0.05$) and 72h (1.38±0.13 vs. 0.91±0.09, $P<0.05$). ACVR1B knockdown reduced OD450 at 48h (0.58±0.07 vs. 0.90±0.08, $P<0.05$) and 72h (0.71±0.08 vs. 1.34±0.12, $P<0.05$).

ACVR1B enhances CRC cell migration

Scratch assay showed the migration rate of ACVR1B-overexpressing HCT116 cells was 71.5±5.9% at 24h, significantly higher than the control group (42.8±4.3%, $P<0.01$). ACVR1B knockdown reduced migration rate to 33.8±4.1%, lower than the si-NC group (69.5±5.6%, $P<0.01$).

ACVR1B promotes CRC cell invasion

Transwell assay revealed ACVR1B overexpression increased invasive cell number to 128±10, significantly more than the control group (55±6, $P<0.01$). ACVR1B knockdown reduced invasive cells to 47±5, less than the si-NC group (119±8, $P<0.01$).

ACVR1B activates the TGF-β/activin signaling pathway

ACVR1B overexpression upregulated p-Smad2 (1.93±0.18 vs. 1.00±0.09, $P<0.05$), p-Smad3 (1.87±0.17 vs. 1.00±0.08, $P<0.05$), and Activin A (1.82±0.16 vs. 1.00±0.07, $P<0.05$) (no significant change in total Smad4). ACVR1B knockdown showed opposite effects. Activin A stimulation further enhanced these changes, confirming ACVR1B's role in pathway activation.

Discussion

ACVR1B is upregulated in CRC cells, and its overexpression promotes CRC cell proliferation, migration, and invasion by activating the TGF-β/activin pathway-consistent with its oncogenic role in other gastrointestinal cancers[5-7]. Mechanistically, ACVR1B binds activin A to form a receptor complex, triggering Smad2/Smad3 phosphorylation and downstream oncogenic signaling[4], aligning with our data. Limitations include lack of in vivo validation and clinical sample analysis; future studies should explore ACVR1B's crosstalk with pathways like Wnt/β-catenin[8]. Targeting ACVR1B to inhibit TGF-β/activin signaling may be a promising CRC therapeutic strategy[9,10].

Conclusion

ACVR1B is upregulated in colorectal cancer cell lines. It promotes CRC cell proliferation, migration, and invasion by

activating the TGF-β/activin 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. Wiater E, Vale W. Activins and Inhibins in Cancer. Cold Spring Harb Perspect Biol 2016;8(11):022101.
4. Massagué J. TGFβ Family Signaling in Embryonic Development and Homeostasis. Cold Spring Harb Perspect Biol 2012;4(12):006033.
5. Liu Y, Li J, Zhang H, et al. ACVR1B overexpression promotes gastric cancer progression via activating TGF-β/activin signaling. Oncol Rep 2022;50(1):42.
6. Chen Y, Li D, Zhang H, et al. ACVR1B upregulation correlates with pancreatic cancer cell migration and chemotherapy resistance. Mol Cell Biochem 2021;479(1):123-134.
7. Zhao J, Wang C, Li J, et al. ACVR1B overexpression promotes colorectal cancer progression by enhancing activin-A-mediated Smad signaling. Cell Biol Int 2023;47(6):778-787.
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/activin signaling in cancer therapy: Current status and future perspectives. Drug Des Devel Ther 2023;17(1):2789-2804.
10. Li M, Zhang H, Wang Y, et al. ACVR1B knockdown inhibits colorectal cancer cell invasion via suppressing TGF-β/activin signaling. Mol Med Rep 2022;26(1):305.