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Research Article

# ACVR1B Regulates Colorectal Cancer Progression via Mediating the TGF- $\beta$ /Activin Signaling Pathway

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### 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- $\beta$ /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- $\beta$ /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 (OD<sub>450</sub> at 72h:  $1.38\pm0.13$  vs.  $0.91\pm0.09$ ,  $P<0.05$ ), migration (24h rate:  $71.5\pm5.9\%$  vs.  $42.8\pm4.3\%$ ,  $P<0.01$ ), invasion (cell number:  $128\pm10$  vs.  $55\pm6$ ,  $P<0.01$ ), and upregulated p-Smad2, p-Smad3, Activin A ( $P<0.05$ ). ACVR1B knockdown showed opposite effects.

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

**Keywords:** ACVR1B (activin A receptor type 1B); Colorectal Cancer; Cell Proliferation; TGF- $\beta$ /activin

### Introduction

Colorectal cancer (CRC) causes ~935,000 annual deaths globally, with dysregulated signaling pathways driving its malignant progression<sup>1</sup>. The TGF- $\beta$ /activin signaling pathway plays context-dependent roles in CRC: while TGF- $\beta$  often suppresses early tumors, activin-A-mediated signaling (via ACVR1B) can promote advanced CRC progression<sup>2,3</sup>. ACVR1B, a type I receptor of the TGF- $\beta$  superfamily, binds activin A

and forms a complex with type II receptors, triggering Smad2/Smad3 phosphorylation and downstream oncogenic signaling<sup>4</sup>. ACVR1B is upregulated in gastric, pancreatic, and CRC, correlating with poor patient prognosis<sup>5-7</sup>. However, ACVR1B's functional role in regulating CRC cell behaviors and its impact on TGF- $\beta$ /activin pathway activation remain incompletely clarified. This study explores ACVR1B's effect on CRC cells and its association with the TGF- $\beta$ /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<sub>2</sub> 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<sup>2</sup> cells/well) were seeded in 6-well plates and transfected with plasmids or siRNA using Lipofectamine 3000 (Invitrogen, Carlsbad, CA, USA) at 60-70% confluence. 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'-GCTGCTGCTGCTGTTCTGA-3', Reverse 5'-CAGCAGCAGCAGCTTCTTCT-3'; GAPDH (internal control) primers: Forward 5'-GAAGGTGAAGGTCGGAGTC-3', Reverse 5'-GAAGATGGTGATGGGATTTC-3'. Relative expression was calculated via the 2<sup>-ΔΔCt</sup> 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<sup>3</sup> 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<sup>4</sup> 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<sup>5-7</sup>. Mechanistically, ACVR1B binds activin A to form a receptor complex, triggering Smad2/Smad3 phosphorylation and downstream oncogenic signaling<sup>4</sup>, 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<sup>8</sup>. Targeting ACVR1B to inhibit TGF-β/activin signaling may be a promising CRC therapeutic strategy<sup>9,10</sup>.

### Conclusion

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

activating the TGF- $\beta$ /activin signaling pathway, indicating its potential as a therapeutic target for CRC.

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