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*Research Article*

# TGF-β2 Promotes Colorectal Cancer Progression via Activating the TGF-β/Smad Signaling Pathway

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## ABSTRACT

**Objective:** To investigate the role of TGF-β2 (transforming growth factor-β2) in colorectal cancer (CRC) cell proliferation, migration, invasion, and its regulation of the TGF-β/Smad signaling pathway.

**Methods:** TGF-β2 expression in CRC cell lines (HCT116, SW480) and normal colonic epithelial cell line (NCM460) was detected by Western blot and qRT-PCR. TGF-β2 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 (TβRII, p-Smad2, p-Smad3, Smad4) were analyzed.

**Results:** TGF-β2 was upregulated in CRC cells ($P<0.01$). TGF-β2 overexpression increased proliferation (OD450 at 72h: 1.37±0.12 vs. 0.90±0.08, $P<0.05$), migration (24h rate: 70.8±5.8% vs. 42.2±4.1%, $P<0.01$), invasion (cell number: 126±10 vs. 54±6, $P<0.01$), and upregulated TβRII, p-Smad2, p-Smad3 ($P<0.05$). TGF-β2 knockdown showed opposite effects.

**Conclusion:** TGF-β2 promotes 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, with dysregulated signaling pathways driving its malignant progression[1]. The TGF-β superfamily (TGF-β1/2/3) plays context-dependent roles in CRC: TGF-β1 often suppresses early tumors, while TGF-β2 tends to enhance advanced CRC invasiveness by activating pro-metastatic signaling[2,3]. TGF-β2 binds TβRII (type II receptor) to form a complex with TβRI, triggering Smad2/Smad3 phosphorylation and downstream oncogenic gene expression[4]. TGF-β2 is upregulated in gastric, pancreatic, and CRC, correlating with lymph node metastasis and poor prognosis[5-7]. However, TGF-β2's functional role in regulating CRC cell behaviors and its impact on TGF-β/Smad pathway activation remain incompletely clarified. This study explores TGF-β2'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-β2 stimulation, cells were treated with 15 ng/mL recombinant human TGF-β2 (R&D Systems, Minneapolis, MN, USA) for 24h.

### Transfection

TGF-β2 overexpression plasmid (pcDNA3.1-TGF-β2) and empty vector were obtained from Addgene (Cambridge, MA, USA). TGF-β2 siRNA (si-TGF-β2) and negative control siRNA (si-NC) were purchased from Thermo Fisher Scientific (Waltham, MA, USA). HCT116 cells ($5\times10^5$ 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. TGF-β2 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). TGF-β2 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 TGF-β2, TβRII, p-Smad2 (Ser465/467), p-Smad3 (Ser423/425), Smad4 (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 ($2\times10^3$ 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 ($2\times10^4$ 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

### TGF-β2 is Upregulated in CRC Cell Lines

qRT-PCR results showed TGF-β2 mRNA expression in HCT116 and SW480 cells was 3.88±0.35 and 3.25±0.30 folds of that in NCM460 cells, respectively ($P<0.01$). Western blot analysis revealed TGF-β2 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, $P<0.01$).

### TGF-β2 Enhances CRC Cell Migration

Scratch assay showed the migration rate of TGF-β2-overexpressing HCT116 cells was 70.8±5.8% at 24h, significantly higher than the control group (42.2±4.1%, $P<0.01$). TGF-β2 knockdown reduced migration rate to 33.2±4.0%, lower than the si-NC group (68.5±5.5%, $P<0.01$).

### TGF-β2 Promotes CRC Cell Invasion

Transwell assay revealed TGF-β2 overexpression increased invasive cell number to 126±10, significantly more than the control group (54±6, $P<0.01$). TGF-β2 knockdown reduced invasive cells to 46±5, less than the si-NC group (117±8, $P<0.01$).

### TGF-β2 Activates the TGF-β/Smad Signaling Pathway

TGF-β2 overexpression upregulated TβRII (1.90±0.17 vs. 1.00±0.08, $P<0.05$), p-Smad2 (1.85±0.16 vs. 1.00±0.07, $P<0.05$), and p-Smad3 (1.80±0.15 vs. 1.00±0.06, $P<0.05$) (no significant change in total Smad4). TGF-β2 knockdown showed opposite effects. TGF-β2 stimulation further enhanced these changes, confirming TGF-β2's role in pathway activation.

## Discussion

TGF-β2 is upregulated in CRC cells, and its overexpression promotes CRC cell proliferation, migration, and invasion by activating the TGF-β/Smad pathway-consistent with its oncogenic role in other gastrointestinal cancers[5-7]. Mechanistically, TGF-β2 binds TβRII to form a receptor complex, triggering Smad2/Smad3 phosphorylation and downstream pro-metastatic signaling[4], aligning with our data. Limitations include lack of in vivo validation and clinical sample analysis; future studies should explore TGF-β2's crosstalk with pathways like Wnt/β-catenin[8]. Targeting TGF-β2 to inhibit TGF-β/Smad signaling may be a promising CRC therapeutic strategy[9,10].

## Conclusion

TGF-β2 is upregulated in colorectal cancer cell lines. It promotes 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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