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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 (5×10^5 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. 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 (2×10^3 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

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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