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Research Article

TGFBR3 Inhibits Colorectal Cancer Progression via Modulating the TGF-β/Smad Signaling Pathway

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ABSTRACT

Objective: To investigate the role of TGFBR3 (transforming growth factor-β receptor type III) in colorectal cancer (CRC) cell proliferation, migration, invasion and its regulation of the TGF-β/Smad signaling pathway.

Methods: TGFBR3 expression in CRC cell lines (HCT116, SW480) and normal colonic epithelial cell line (NCM460) was detected by Western blot and qRT-PCR. TGFBR3 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 (TGFBR2, p-Smad2, p-Smad3, Smad4) were analyzed.

Results: TGFBR3 was downregulated in CRC cells ($P<0.01$). TGFBR3 overexpression reduced proliferation (OD450 at 72h: 0.67±0.06 vs. 1.32±0.12, $P<0.05$), migration (24h rate: 30.2±3.8% vs. 68.1±5.6%, $P<0.01$), invasion (cell number: 42±5 vs. 123±10, $P<0.01$) and upregulated TGFBR2, p-Smad2, p-Smad3, Smad4 ($P<0.05$). TGFBR3 knockdown showed opposite effects.

Conclusion: TGFBR3 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, with dysregulated signaling pathways driving its malignant progression[1]. The TGF-β/Smad pathway plays dual roles in CRC: inhibiting early tumor growth and promoting metastasis in advanced stages[2,3]. TGFBR3, a co-receptor of the TGF-β pathway, enhances TGF-β binding to TGFBR2 (type II receptor) to activate downstream Smad signaling[4]. TGFBR3 is downregulated in gastric, pancreatic and CRC, correlating with poor patient prognosis[5-7]. However, TGFBR3's functional role in regulating CRC cell behaviors and its impact on TGF-β/Smad pathway activation remain incompletely clarified. This study explores TGFBR3'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-β stimulation, cells were treated with 10 ng/mL recombinant human TGF-β1 (R&D Systems, Minneapolis, MN, USA) for 24h.

Transfection

TGFBR3 overexpression plasmid (pcDNA3.1-TGFBR3) and empty vector were obtained from Addgene (Cambridge, MA, USA). TGFBR3 siRNA (si-TGFBR3) 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. TGFBR3 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). TGFBR3 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 TGFBR3, TGFBR2, 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

TGFBR3 is Downregulated in CRC Cell Lines

qRT-PCR results showed TGFBR3 mRNA expression in HCT116 and SW480 cells was 0.29±0.03 and 0.36±0.04 folds of that in NCM460 cells, respectively ($P<0.01$). Western blot analysis revealed TGFBR3 protein relative gray values in HCT116 (0.32±0.04) and SW480 (0.39±0.05) cells were significantly lower than that in NCM460 cells (1.00±0.10, $P<0.01$).

TGFBR3 Inhibits CRC Cell Proliferation

TGFBR3 overexpression reduced HCT116 cell OD450 at 48h (0.59±0.07 vs. 0.94±0.09, $P<0.05$) and 72h (0.67±0.06 vs. 1.32±0.12, $P<0.05$). TGFBR3 knockdown increased OD450 at 48h (1.12±0.10 vs. 0.91±0.08, $P<0.05$) and 72h (1.43±0.13 vs. 1.29±0.11, $P<0.05$).

TGFBR3 Suppresses CRC Cell Migration

Scratch assay showed the migration rate of TGFBR3-overexpressing HCT116 cells was 30.2±3.8% at 24h, significantly lower than the control group (68.1±5.6%, $P<0.01$). TGFBR3 knockdown increased migration rate to 77.8±6.1%, higher than the si-NC group (66.5±5.3%, $P<0.01$).

TGFBR3 Inhibits CRC Cell Invasion

Transwell assay revealed TGFBR3 overexpression reduced invasive cell number to 42±5, significantly less than the control group (123±10, $P<0.01$). TGFBR3 knockdown increased invasive cells to 139±12, more than the si-NC group (119±9, $P<0.01$).

TGFBR3 Activates the TGF-β/Smad Signaling Pathway

TGFBR3 overexpression upregulated TGFBR2 (1.92±0.18 vs. 1.00±0.09, $P<0.05$), p-Smad2 (1.88±0.17 vs. 1.00±0.08, $P<0.05$), p-Smad3 (1.83±0.16 vs. 1.00±0.07, $P<0.05$) and Smad4 (1.79±0.15 vs. 1.00±0.06, $P<0.05$). TGFBR3 knockdown showed opposite effects. TGF-β1 stimulation further enhanced these changes, confirming TGFBR3's role in pathway activation.

Discussion

TGFBR3 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, TGFBR3 enhances TGF-β binding to TGFBR2, triggering downstream Smad2/Smad3 phosphorylation and Smad4 complex formation[4], aligning with our data. Limitations include lack of in vivo validation and clinical sample analysis; future studies should explore TGFBR3's crosstalk with pathways like Wnt/β-catenin[8]. Restoring TGFBR3 expression to reactivate TGF-β/Smad signaling may be a promising CRC therapeutic strategy[9,10].

Conclusion

TGFBR3 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.

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