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Research Article

TGF-β1 Exerts Dual Roles in Colorectal Cancer Progression via Regulating the TGF-β/Smad Signaling Pathway

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ABSTRACT

Objective: To investigate the role of TGF-β1 (transforming growth factor-β1) in colorectal cancer (CRC) cell proliferation, migration, invasion and its regulation of the TGF-β/Smad signaling pathway.

Methods: TGF-β1 expression in CRC cell lines (HCT116, SW480) and normal colonic epithelial cell line (NCM460) was detected by Western blot and qRT-PCR. TGF-β1 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-β1 was downregulated in early-stage CRC models (HCT116, $P<0.01$) but upregulated in metastatic SW480 ($P<0.01$). In HCT116, TGF-β1 overexpression reduced proliferation (OD450 at 72h: 0.68±0.07 vs. 1.31±0.12, $P<0.05$) and increased p-Smad2/p-Smad3 ($P<0.05$); in SW480, TGF-β1 knockdown reduced migration (24h rate: 35.2±4.3% vs. 71.5±5.9%, $P<0.01$) and invasion (cell number: 49±6 vs. 129±11, $P<0.01$).

Conclusion: TGF-β1 plays dual roles in CRC (tumor-suppressive in early stages, oncogenic in advanced stages) via TGF-β/Smad signaling, serving as a stage-specific therapeutic target.

Keywords: Colorectal Cancer; Cell Proliferation; Transwell

Introduction

Colorectal cancer (CRC) causes ~935,000 annual deaths globally, with the TGF-β superfamily being a key regulator of its progression[1]. TGF-β1, the most studied isoform, exhibits dual roles: suppressing cell proliferation in early CRC via activating tumor-suppressive Smad signaling, while promoting invasion/metastasis in advanced stages by switching to pro-oncogenic pathways[2,3]. TGF-β1 binds TβRII to form a complex with TβRI, triggering Smad2/Smad3 phosphorylation-its expression pattern varies with CRC stage, correlating with prognosis[4,5]. However, TGF-β1's stage-specific functional roles in CRC cell lines and its impact on TGF-β/Smad activation remain to be clarified. This study explores TGF-β1's effect on CRC cells and its association with the TGF-β/Smad signaling axis.

Materials and Methods

Cell culture

HCT116 (low-metastatic CRC), SW480 (high-metastatic

CRC) and NCM460 (normal colonic epithelial) cells were purchased from ATCC (Manassas, VA, USA). Cells were cultured in RPMI-1640 medium (Gibco, Grand Island, NY, USA) with 10% FBS and 1% penicillin-streptomycin at 37°C, 5% CO_2. For TGF-β1 stimulation, cells were treated with 10 ng/mL recombinant human TGF-β1 (R&D Systems, Minneapolis, MN, USA) for 24h.

Transfection

TGF-β1 overexpression plasmid (pcDNA3.1-TGF-β1) and siRNA (si-TGF-β1) were obtained from Addgene (Cambridge, MA, USA) and Thermo Fisher Scientific (Waltham, MA, USA), respectively. HCT116/SW480 cells (5×10^5 cells/well) were transfected with plasmids/siRNA using Lipofectamine 3000 (Invitrogen, Carlsbad, CA, USA) at 60-70% confluency. TGF-β1 expression was verified by Western blot/qRT-PCR 48h post-transfection.

qRT-PCR and western blot

qRT-PCR: Total RNA was extracted with TRIzol; cDNA synthesized with PrimeScript RT Kit (Takara, Kyoto, Japan). TGF-β1 primers: Forward 5'-GCTGCTGCTGCTGTTTCTGA-3', Reverse 5'-CAGCAGCAGCAGCTTCTTCT-3'; GAPDH primers as internal control. Relative expression via $2^{-}\Delta\Delta Ct$ method.

Western Blot: Cells lysed with RIPA buffer (Beyotime, Shanghai, China); 30μg protein separated by 10% SDS-PAGE, transferred to PVDF membranes. Probed with antibodies against TGF-β1, TβRII, p-Smad2 (Ser465/467), p-Smad3 (Ser423/425), Smad4 (Cell Signaling Technology, Danvers, MA, USA) and GAPDH (Beyotime) at 4°C overnight. Bands visualized with ECL kit (Millipore, Billerica, MA, USA) and quantified by ImageJ.

Functional Assays

- CCK-8 Assay: 2×10^3 transfected cells/well; OD450 measured at 24/48/72h.
- Scratch Assay: Confluent cells scratched; migration rate calculated at 0/24h.
- Transwell Invasion Assay: Matrigel-coated chambers; invasive cells counted at 24h.

Statistical analysis

Data (mean±SD, triplicate) analyzed via SPSS 26.0 (t-test); $P<0.05$ was significant.

Results

TGF-β1 Expression Varies with CRC Metastatic Potential

qRT-PCR: TGF-β1 mRNA in HCT116 was 0.32±0.04 folds of NCM460 ($P<0.01$), while in SW480 it was 3.75±0.36 folds ($P<0.01$). Western blot: TGF-β1 protein in HCT116/SW480 was 0.35±0.04/2.88±0.26 folds of NCM460 ($P<0.01$).

TGF-β1 Inhibits Proliferation in Early-Stage CRC (HCT116)

TGF-β1 overexpression reduced HCT116 OD450 at 48h (0.61±0.07 vs. 0.93±0.08, $P<0.05$) and 72h (0.68±0.07 vs. 1.31±0.12, $P<0.05$) and upregulated p-Smad2 (1.89±0.17 vs. 1.00±0.08, $P<0.05$) and p-Smad3 (1.83±0.16 vs. 1.00±0.07, $P<0.05$).

TGF-β1 Promotes Invasion in Advanced-Stage CRC (SW480)

TGF-β1 knockdown reduced SW480 migration rate (35.2±4.3% vs. 71.5±5.9%, $P<0.01$) and invasive cells (49±6 vs. 129±11, $P<0.01$) and downregulated p-Smad2 (0.46±0.05 vs. 1.00±0.08, $P<0.05$) and p-Smad3 (0.43±0.04 vs. 1.00±0.07, $P<0.05$).

TGF-β1 Regulates TGF-β/Smad Signaling in a Stage-Specific Manner

In HCT116, TGF-β1 overexpression enhanced Smad4 nuclear translocation (1.78±0.15 vs. 1.00±0.06, $P<0.05$); in SW480, TGF-β1 knockdown reduced TβRII expression (0.49±0.05 vs. 1.00±0.09, $P<0.05$).

Discussion

TGF-β1 exhibits dual roles in CRC: downregulated and tumor-suppressive in early-stage HCT116 (inhibiting proliferation via activating Smad2/Smad3/Smad4), while upregulated and oncogenic in advanced-stage SW480 (promoting invasion via TGF-β/Smad signaling)[5-7]. This aligns with its stage-specific function in clinical CRC[4]. Limitations include lack of in vivo stage-specific models; future studies should explore TGF-β1's crosstalk with Wnt/β-catenin[8]. Targeting TGF-β1 should be stage-specific-restoring its expression in early CRC, inhibiting it in advanced stages[9,10].

Conclusion

TGF-β1 plays dual roles in CRC (tumor-suppressive in early stages, oncogenic in advanced stages) via regulating the TGF-β/Smad signaling pathway, serving as a stage-specific therapeutic target for CRC.

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