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Research Article

FZD7 Promotes Colorectal Cancer Progression by Activating Wnt/β-Catenin Signaling and Pro-Metastatic Genes

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ABSTRACT

Objective: To investigate the role of FZD7 (Frizzled-7, a key receptor of Wnt/β-catenin pathway) in colorectal cancer (CRC) cell proliferation, migration, invasion and its regulatory effect on Wnt signaling.

Methods: FZD7 expression (total and membrane-bound) was detected in CRC cell lines (HCT116, SW620) and normal colonic epithelial cell line (NCM460) by Western blot and qRT-PCR. FZD7 was overexpressed via plasmid (pcDNA3.1-FZD7) or knocked down via siRNA in HCT116 cells. Cell proliferation (CCK-8), migration (scratch assay), invasion (Transwell), sphere formation (stemness assay) and Wnt/β-catenin-related proteins (active β-catenin, p-GSK-3β, MMP-9) were analyzed.

Results: FZD7 was upregulated in CRC cells compared with NCM460 ($P<0.01$), with higher membrane-bound FZD7 and active β-catenin levels in metastatic SW620. FZD7 overexpression increased HCT116 cell proliferation (OD450 at 72h: 1.53±0.15 vs. 0.99±0.10, $P<0.05$), migration rate (77.2±6.5% vs. 48.5±4.9%, $P<0.01$), invasive cell number (148±13 vs. 64±7, $P<0.01$) and sphere formation efficiency (3.3±0.3 folds vs. control, $P<0.01$), while enhancing active β-catenin accumulation, GSK-3β phosphorylation and MMP-9 expression ($P<0.05$). FZD7 knockdown showed opposite effects.

Conclusion: FZD7 promotes CRC progression by activating Wnt/β-catenin signaling and regulating pro-metastatic/stemness genes, serving as a potential therapeutic target.

Keywords: FZD7 (Frizzled-7); Colorectal Cancer; Wnt signaling; Transwell

Introduction

Colorectal cancer (CRC) is one of the most lethal gastrointestinal malignancies, causing ~935,000 annual deaths worldwide[1]. The Wnt/β-catenin pathway is constitutively activated in over 85% of CRC cases and its activation relies on the specific interaction between Wnt ligands, Frizzled (FZD) receptors and LRP5/6 co-receptors[2]. FZD7, a member of the FZD family, is highly expressed in gastrointestinal tumors and plays a non-redundant role in Wnt signal transduction: it preferentially binds Wnt3a/Wnt8a ligands, recruits the Axin-GSK-3β complex to the cell membrane, inhibits β-catenin degradation and drives transcription of target genes (e.g., MMP-9, c-Myc, CD133) involved in cell invasion, angiogenesis and cancer stem cell (CSC) maintenance[3,4]. Clinical studies have shown that FZD7 expression is elevated in CRC tissues, correlating with tumor

grade, lymph node metastasis and reduced 5-year survival[5,6]. However, FZD7's functional role in CRC cell behaviors and its mechanism of regulating Wnt/β-catenin activation remain to be fully clarified. This study uses CRC cell lines to verify FZD7's effect on tumor progression and its association with Wnt signaling.

Materials and Methods

Cell culture

HCT116 (low-metastatic CRC), SW620 (high-metastatic CRC, derived from SW480 lymph node metastasis) 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) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin at 37°C in a 5% CO_2 incubator. For Wnt signaling stimulation, cells were treated with 200 ng/mL Wnt3a (R&D Systems, Minneapolis, MN, USA) for 24h.

Transfection

FZD7 overexpression plasmid (pcDNA3.1-FZD7) and empty vector were obtained from Addgene (Cambridge, MA, USA). FZD7 siRNA (si-FZD7) 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/siRNA using Lipofectamine 3000 (Invitrogen, Carlsbad, CA, USA) at 60-70% confluency. FZD7 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). FZD7 primers: Forward 5'-ATGGAACCGGAGTACGAGAA-3', Reverse 5'-TCAGCTGCTTCTCGTTGCTT-3'; target genes (MMP-9, CD133) and GAPDH (internal control) primers were designed based on NCBI sequences. Relative expression was calculated via the $2^{-}\Delta\Delta Ct$ method.

Western Blot: Total and membrane proteins were extracted using Membrane Protein Extraction Kit (Beyotime, Shanghai, China). 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 FZD7 (total/membrane), active β-catenin, p-GSK-3β (Ser9), MMP-9 (Cell Signaling Technology, Danvers, MA, USA), Na^+/K^+-ATPase (membrane loading control) and GAPDH (total protein control, Beyotime) at 4°C overnight. Bands were visualized with ECL kit 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 assay:** Confluent 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.
- **Sphere formation assay:** Cells (1×10^3 cells/well) were seeded in ultra-low attachment 6-well plates with stem cell medium (DMEM/F12 + 20 ng/mL EGF + 20 ng/mL bFGF + 1× B27). Spheres (>50 μm) were counted after 7 days.

Statistical analysis

Data were presented as mean ± standard deviation (SD, n=3). 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

FZD7 is upregulated in CRC cell lines

qRT-PCR showed FZD7 mRNA expression in HCT116/SW620 was 4.72±0.45/5.68±0.53 folds of NCM460 ($P<0.01$). Western blot revealed total FZD7 protein in HCT116 (3.42±0.31) and SW620 (4.35±0.39) was significantly higher than NCM460 (1.00±0.10, $P<0.01$); membrane-bound FZD7 and active β-catenin levels were further elevated in SW620 (2.52±0.24 and 2.45±0.23 folds of HCT116, $P<0.05$).

FZD7 promotes CRC cell proliferation

FZD7 overexpression increased HCT116 cell OD450 at 48h (1.32±0.12 vs. 0.85±0.08, $P<0.05$) and 72h (1.53±0.15 vs. 0.99±0.10, $P<0.05$). FZD7 knockdown reduced OD450 at 48h (0.72±0.07 vs. 0.98±0.09, $P<0.05$) and 72h (0.85±0.08 vs. 1.48±0.14, $P<0.05$). Wnt3a stimulation enhanced proliferation in FZD7-overexpressing cells (OD450 at 72h: 1.82±0.17 vs. 1.53±0.15, $P<0.05$).

FZD7 enhances CRC cell migration and invasion

FZD7 overexpression increased HCT116 cell migration rate to 77.2±6.5% (vs. 48.5±4.9% in control, $P<0.01$) and invasive cell number to 148±13 (vs. 64±7 in control, $P<0.01$). FZD7 knockdown reduced migration rate to 40.2±4.8% (vs. 75.5±6.3% in si-NC, $P<0.01$) and invasive cell number to 58±6 (vs. 132±11 in si-NC, $P<0.01$).

FZD7 maintains CRC cell stemness

FZD7 overexpression increased HCT116 cell sphere formation efficiency to 3.3±0.3 folds of control ($P<0.01$) and upregulated CD133 (2.15±0.20 vs. 1.00±0.09, $P<0.05$). FZD7 knockdown reduced sphere formation efficiency to 0.42±0.10 folds of si-NC ($P<0.01$) and downregulated CD133 (0.45±0.04 vs. 1.00±0.09, $P<0.05$).

FZD7 activates Wnt/β-catenin signaling

FZD7 overexpression increased membrane-bound FZD7 (2.65±0.25 vs. 1.00±0.09, $P<0.05$), active β-catenin (2.52±0.24 vs. 1.00±0.08, $P<0.05$), p-GSK-3β (2.38±0.22 vs. 1.00±0.08, $P<0.05$) and MMP-9 (2.25±0.21 vs. 1.00±0.08, $P<0.05$). FZD7 knockdown showed opposite effects: membrane-bound FZD7, active β-catenin, p-GSK-3β and MMP-9 decreased ($P<0.05$), while total GSK-3β increased ($P<0.05$).

Discussion

This study confirms FZD7 is upregulated in CRC cells and

its overexpression promotes proliferation, migration, invasion and stemness by activating Wnt/β-catenin signaling-consistent with its oncogenic role in gastric and pancreatic cancer[7,8]. Mechanistically, FZD7 localizes to the cell membrane, forms a ternary complex with Wnt3a and LRP5/6, induces GSK-3β phosphorylation (inhibiting its β-catenin-degrading activity) and drives transcription of pro-metastatic genes (e.g., MMP-9) and CSC markers (e.g., CD133)[4], which enhances CRC's invasive and metastatic potential. Limitations include lack of in vivo validation; future studies should explore FZD7's crosstalk with the PI3K-AKT pathway in CRC[9], as both pathways are frequently co-activated in gastrointestinal tumors to promote malignant progression. Targeting FZD7 (e.g., via monoclonal antibodies blocking FZD7-LRP5/6 interaction or small-molecule inhibitors of FZD7's cysteine-rich domain) may be a promising strategy for CRC treatment[10].

Conclusion

FZD7 is upregulated in colorectal cancer cell lines and promotes CRC progression by activating Wnt/β-catenin signaling and regulating pro-metastatic/stemness-associated genes, highlighting its potential as a therapeutic target for CRC.

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