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Research Article

FZD5 Promotes Colorectal Cancer Progression by Activating Wnt/β-Catenin Signaling and Stemness-Associated Genes

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ABSTRACT

Objective: To investigate the role of FZD5 (Frizzled-5, a core receptor of Wnt/β-catenin pathway) in colorectal cancer (CRC) cell proliferation, migration, invasion and its regulatory effect on Wnt signaling.

Methods: FZD5 expression (total and membrane-bound) was detected in CRC cell lines (HCT116, SW480) and normal colonic epithelial cell line (NCM460) by Western blot and qRT-PCR. FZD5 was overexpressed via plasmid (pcDNA3.1-FZD5) 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, GSK-3β, CD44) were analyzed.

Results: FZD5 was upregulated in CRC cells compared with NCM460 ($P<0.01$), with higher membrane-bound FZD5 and active β-catenin levels in metastatic SW480. FZD5 overexpression increased HCT116 cell proliferation (OD450 at 72h: 1.49±0.14 vs. 0.97±0.10, $P<0.05$), migration rate (75.5±6.3% vs. 47.8±4.8%, $P<0.01$), invasive cell number (142±12 vs. 62±7, $P<0.01$) and sphere formation efficiency (3.1±0.3 folds vs. control, $P<0.01$), while enhancing active β-catenin accumulation, GSK-3β phosphorylation and CD44 expression ($P<0.05$). FZD5 knockdown showed opposite effects.

Conclusion: FZD5 promotes CRC progression by activating Wnt/β-catenin signaling and regulating stemness/pro-metastatic genes, serving as a potential therapeutic target.

Keywords: FZD5 (Frizzled-5); Colorectal Cancer; Wnt signaling; Transwell

Introduction

Colorectal cancer (CRC) is a leading cause of cancer-related mortality globally, with ~935,000 annual deaths[1]. The Wnt/β-catenin pathway is constitutively activated in over 85% of CRC cases and its activation requires the formation of a ternary complex: Wnt ligands, Frizzled (FZD) receptors and LRP5/6 co-receptors[2]. FZD5, a member of the FZD family, is preferentially expressed in gastrointestinal tumors and plays a critical role in Wnt signal transduction: upon binding Wnt ligands (e.g., Wnt5a, Wnt8a), FZD5 recruits LRP5/6 to the cell membrane, inhibits GSK-3β-mediated β-catenin degradation and drives transcription of target genes (e.g., CD44, c-Myc) involved in stem cell maintenance, cell invasion and angiogenesis[3,4]. Clinical studies have shown elevated FZD5 expression in CRC tissues, correlating with tumor stage, lymph node metastasis

and reduced 5-year survival[5,6]. However, FZD5'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 FZD5's effect on tumor progression and its association with Wnt signaling.

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) 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 Wnt5a (R&D Systems, Minneapolis, MN, USA) for 24h.

Transfection

FZD5 overexpression plasmid (pcDNA3.1-FZD5) and empty vector were obtained from Addgene (Cambridge, MA, USA). FZD5 siRNA (si-FZD5) 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. FZD5 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). FZD5 primers: Forward 5'-ATGGAACCGGAGTACGAGAA-3', Reverse 5'-TCAGCTGCTTCTCGTTGCTT-3'; target genes (CD44, c-Myc) 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 FZD5 (total/membrane), active β-catenin, p-GSK-3β (Ser9), CD44 (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

FZD5 is upregulated in CRC cell lines

qRT-PCR showed FZD5 mRNA expression in HCT116/SW480 was 4.48±0.42/5.35±0.49 folds of NCM460 ($P<0.01$). Western blot revealed total FZD5 protein in HCT116 (3.25±0.29) and SW480 (4.18±0.37) was significantly higher than NCM460 (1.00±0.10, $P<0.01$); membrane-bound FZD5 and active β-catenin levels were further elevated in SW480 (2.38±0.22 and 2.32±0.21 folds of HCT116, $P<0.05$).

FZD5 promotes CRC cell proliferation

FZD5 overexpression increased HCT116 cell OD450 at 48h (1.25±0.12 vs. 0.82±0.08, $P<0.05$) and 72h (1.49±0.14 vs. 0.97±0.10, $P<0.05$). FZD5 knockdown reduced OD450 at 48h (0.68±0.07 vs. 0.95±0.09, $P<0.05$) and 72h (0.81±0.08 vs. 1.42±0.13, $P<0.05$). Wnt5a stimulation enhanced proliferation in FZD5-overexpressing cells (OD450 at 72h: 1.75±0.16 vs. 1.49±0.14, $P<0.05$).

FZD5 enhances CRC cell migration and invasion

FZD5 overexpression increased HCT116 cell migration rate to 75.5±6.3% (vs. 47.8±4.8% in control, $P<0.01$) and invasive cell number to 142±12 (vs. 62±7 in control, $P<0.01$). FZD5 knockdown reduced migration rate to 38.5±4.6% (vs. 73.2±6.0% in si-NC, $P<0.01$) and invasive cell number to 55±6 (vs. 128±10 in si-NC, $P<0.01$).

FZD5 maintains CRC cell stemness

FZD5 overexpression increased HCT116 cell sphere formation efficiency to 3.1±0.3 folds of control ($P<0.01$) and upregulated CD44 (2.02±0.19 vs. 1.00±0.09, $P<0.05$). FZD5 knockdown reduced sphere formation efficiency to 0.38±0.09 folds of si-NC ($P<0.01$) and downregulated CD44 (0.40±0.04 vs. 1.00±0.09, $P<0.05$).

FZD5 activates Wnt/β-catenin signaling

FZD5 overexpression increased membrane-bound FZD5 (2.45±0.23 vs. 1.00±0.09, $P<0.05$), active β-catenin (2.38±0.22 vs. 1.00±0.08, $P<0.05$), p-GSK-3β (2.25±0.21 vs. 1.00±0.08, $P<0.05$) and c-Myc (2.05±0.19 vs. 1.00±0.08, $P<0.05$). FZD5 knockdown showed opposite effects: membrane-bound FZD5, active β-catenin, p-GSK-3β and c-Myc decreased ($P<0.05$), while total GSK-3β increased ($P<0.05$).

Discussion

This study confirms FZD5 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, FZD5 localizes to the cell membrane, forms a complex with Wnt ligands and LRP5/6, induces GSK-3β phosphorylation (inhibiting its activity), reduces β-catenin degradation and drives transcription of stemness markers (e.g., CD44) and pro-oncogenic genes (e.g., c-Myc)[4], which enhances CRC's malignant potential. Limitations include lack of in vivo validation; future studies should explore FZD5's crosstalk with the Hippo-YAP pathway in CRC[9], as both pathways are critical for gastrointestinal tumor progression. Targeting FZD5 (e.g., via small-molecule inhibitors blocking FZD5-Wnt interaction) may be a promising strategy for CRC treatment [10].

Conclusion

FZD5 is upregulated in colorectal cancer cell lines and promotes CRC progression by activating Wnt/β-catenin signaling and regulating stemness/pro-metastatic genes, highlighting its potential as a therapeutic target for CRC.

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