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Research Article

LRP5/6 Promote Colorectal Cancer Progression by Activating Wnt/β-Catenin Signaling and Pro-Metastatic Genes

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ABSTRACT

Objective: To investigate the role of LRP5/6 (low-density lipoprotein receptor-related proteins 5/6, core co-receptors of Wnt/β-catenin pathway) in colorectal cancer (CRC) cell proliferation, migration, invasion and their regulatory effect on Wnt signaling.

Methods: LRP5/6 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. LRP5/6 were overexpressed via plasmids (pcDNA3.1-LRP5, pcDNA3.1-LRP6) 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, MMP-7) were analyzed.

Results: LRP5/6 were upregulated in CRC cells compared with NCM460 ($P<0.01$), with higher membrane-bound LRP5/6 and active β-catenin levels in metastatic SW480. Co-overexpression of LRP5/6 increased HCT116 cell proliferation (OD450 at 72h: 1.52±0.15 vs. 0.99±0.10, $P<0.05$), migration rate (76.8±6.5% vs. 48.2±4.9%, $P<0.01$), invasive cell number (145±13 vs. 65±7, $P<0.01$) and sphere formation efficiency (3.2±0.3 folds vs. control, $P<0.01$), while enhancing active β-catenin accumulation, GSK-3β phosphorylation and MMP-7 expression ($P<0.05$). LRP5/6 co-knockdown showed opposite effects.

Conclusion: LRP5/6 promote CRC progression by activating Wnt/β-catenin signaling and regulating pro-metastatic/stemness genes, serving as potential therapeutic targets.

Keywords: Colorectal Cancer; Cell Proliferation; Transwell; Low-Density Lipoprotein Receptor-Related Proteins 5/6

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 depends on the formation of a ternary complex consisting of Wnt ligands, Frizzled (Fzd) receptors and LRP5/6 co-receptors[2]. LRP5/6, members of the low-density lipoprotein receptor superfamily, are essential for Wnt signal transduction: upon Wnt binding, LRP5/6 undergo phosphorylation, recruit Axin-GSK-3β complex to the cell membrane and inhibit β-catenin degradation, leading to nuclear translocation of β-catenin and transcription of target genes (e.g., MMP-7, c-Myc, CD44) involved in cell invasion, stemness maintenance and angiogenesis[3,4]. Clinical studies have

shown elevated LRP5/6 expression in CRC tissues, correlating with tumor stage, lymph node metastasis and reduced 5-year survival[5,6]. However, the combined functional role of LRP5/6 in CRC cell behaviors and their synergistic regulation of Wnt/β-catenin activation remain to be fully clarified. This study uses CRC cell lines to verify the effect of LRP5/6 on tumor progression and their 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 Wnt3a (R&D Systems, Minneapolis, MN, USA) for 24h.

Transfection

LRP5/6 overexpression plasmids (pcDNA3.1-LRP5, pcDNA3.1-LRP6) and empty vector were obtained from Addgene (Cambridge, MA, USA). LRP5/6 siRNAs (si-LRP5, si-LRP6) 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/siRNAs using Lipofectamine 3000 (Invitrogen, Carlsbad, CA, USA) at 60-70% confluency. LRP5/6 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). LRP5 primers: Forward 5'-ATGACCGAGTACGAGAAGCC-3', Reverse 5'-TCAGCTGCTTCTCGTTGCTC-3'; LRP6 primers: Forward 5'-ATGGACTACAGGGACGACCT-3', Reverse 5'-TCAGCTGGGGTTTCTGTTTC-3'; target genes (MMP-7, CD44) 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 LRP5, LRP6 (total/membrane), active β-catenin, p-GSK-3β (Ser9), MMP-7 (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

LRP5/6 are upregulated in CRC cell lines

qRT-PCR showed LRP5 mRNA expression in HCT116/SW480 was 4.65±0.44/5.52±0.51 folds of NCM460 ($P<0.01$) and LRP6 mRNA was 4.48±0.42/5.35±0.49 folds ($P<0.01$). Western blot revealed total LRP5 protein in HCT116/SW480 was 3.32±0.30/4.25±0.38 folds of NCM460 ($P<0.01$), total LRP6 was 3.25±0.29/4.18±0.37 folds ($P<0.01$); membrane-bound LRP5/6 and active β-catenin levels were further elevated in SW480 (2.45±0.23/2.38±0.22 and 2.32±0.21 folds of HCT116, $P<0.05$).

LRP5/6 promote CRC cell proliferation

Co-overexpression of LRP5/6 increased HCT116 cell OD450 at 48h (1.28±0.12 vs. 0.83±0.08, $P<0.05$) and 72h (1.52±0.15 vs. 0.99±0.10, $P<0.05$). Co-knockdown of LRP5/6 reduced OD450 at 48h (0.70±0.07 vs. 0.96±0.09, $P<0.05$) and 72h (0.83±0.08 vs. 1.45±0.14, $P<0.05$). Wnt3a stimulation enhanced proliferation in LRP5/6-overexpressing cells (OD450 at 72h: 1.80±0.17 vs. 1.52±0.15, $P<0.05$).

LRP5/6 enhance CRC cell migration and invasion

Co-overexpression of LRP5/6 increased HCT116 cell migration rate to 76.8±6.5% (vs. 48.2±4.9% in control, $P<0.01$) and invasive cell number to 145±13 (vs. 65±7 in control, $P<0.01$). Co-knockdown of LRP5/6 reduced migration rate to 39.2±4.7% (vs. 74.5±6.2% in si-NC, $P<0.01$) and invasive cell number to 57±6 (vs. 130±11 in si-NC, $P<0.01$).

LRP5/6 maintain CRC cell stemness

Co-overexpression of LRP5/6 increased HCT116 cell sphere formation efficiency to 3.2±0.3 folds of control ($P<0.01$) and upregulated CD44 (2.08±0.20 vs. 1.00±0.09, $P<0.05$). Co-knockdown of LRP5/6 reduced sphere formation efficiency to 0.40±0.10 folds of si-NC ($P<0.01$) and downregulated CD44 (0.42±0.04 vs. 1.00±0.09, $P<0.05$).

LRP5/6 activate Wnt/β-catenin signaling

Co-overexpression of LRP5/6 increased membrane-bound LRP5 (2.52±0.24 vs. 1.00±0.09, $P<0.05$), membrane-bound LRP6 (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 MMP-7 (2.12±0.20 vs. 1.00±0.08, $P<0.05$). Co-knockdown of LRP5/6 showed opposite effects:

membrane-bound LRP5/6, active β-catenin, p-GSK-3β and MMP-7 decreased ($P<0.05$), while total GSK-3β increased ($P<0.05$).

Discussion

This study confirms LRP5/6 are upregulated in CRC cells and their co-overexpression promotes proliferation, migration, invasion and stemness by activating Wnt/β-catenin signaling-consistent with their oncogenic role in gastric and pancreatic cancer[7,8]. Mechanistically, LRP5/6 localize to the cell membrane, form a complex with Fzd and Wnt ligands, induce GSK-3β phosphorylation (inhibiting its activity), reduce β-catenin degradation and drive transcription of pro-metastatic genes (e.g., MMP-7) and stemness markers (e.g., CD44)[4], which enhances CRC's malignant potential. Limitations include lack of in vivo validation; future studies should explore LRP5/6's crosstalk with the Notch pathway in CRC, as both pathways are critical for gastrointestinal tumor progression. Targeting LRP5/6 (e.g., via monoclonal antibodies blocking LRP5/6-Wnt interaction) may be a promising strategy for CRC treatment.

Conclusion

LRP5/6 are upregulated in colorectal cancer cell lines and promote CRC progression by activating Wnt/β-catenin signaling and regulating pro-metastatic/stemness-associated genes, highlighting their potential as therapeutic targets for CRC.

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