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Research Article

LEF1 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 LEF1 (lymphoid enhancer-binding factor 1, a key transcription factor of Wnt/β-catenin pathway) in colorectal cancer (CRC) cell proliferation, migration, invasion and its regulatory effect on Wnt signaling.

Methods: LEF1 expression (total and nuclear) was detected in CRC cell lines (HCT116, SW480) and normal colonic epithelial cell line (NCM460) by Western blot and qRT-PCR. LEF1 was overexpressed via plasmid (pcDNA3.1-LEF1) 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 (nuclear β-catenin, c-Myc, CD44) were analyzed.

Results: LEF1 was upregulated in CRC cells compared with NCM460 ($P<0.01$), with higher nuclear LEF1 and β-catenin levels in metastatic SW480. LEF1 overexpression increased HCT116 cell proliferation (OD450 at 72h: 1.45±0.14 vs. 0.96±0.10, $P<0.05$), migration rate (74.5±6.2% vs. 46.8±4.7%, $P<0.01$), invasive cell number (138±12 vs. 61±7, $P<0.01$) and sphere formation efficiency (2.8±0.3 folds vs. control, $P<0.01$), while enhancing nuclear LEF1-β-catenin complex formation and c-Myc/CD44 expression ($P<0.05$). LEF1 knockdown showed opposite effects.

Conclusion: LEF1 promotes CRC progression by activating Wnt/β-catenin signaling and regulating stemness-associated genes, serving as a potential therapeutic target.

Keywords: Colorectal Cancer; Cell Proliferation; Transwell; Lymphoid Enhancer-Binding Factor 1

Introduction

Colorectal cancer (CRC) is a leading cause of cancer-related deaths globally, with ~935,000 annual fatalities[1]. The Wnt/β-catenin pathway is constitutively activated in over 80% of CRC cases, driving tumor initiation, progression and metastasis[2]. LEF1, a member of the TCF/LEF transcription factor family, is the key effector of Wnt signaling: in the presence of Wnt ligands, LEF1 dissociates from inhibitory complexes, translocates to the nucleus and forms a complex with β-catenin to transcribe downstream target genes (e.g., c-Myc, Cyclin D1, CD44) involved in cell cycle progression, stem cell maintenance and invasion[3,4]. Clinical studies have shown elevated LEF1 expression in CRC tissues, correlating with tumor stage, lymph node metastasis and poor 5-year survival[5,6]. However, LEF1'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 LEF1'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) with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin at 37°C, 5% CO_2. For Wnt signaling stimulation, cells were treated with 200 ng/mL Wnt3a (R&D Systems, Minneapolis, MN, USA) for 24h.

Transfection

LEF1 overexpression plasmid (pcDNA3.1-LEF1) and empty vector were from Addgene (Cambridge, MA, USA). LEF1 siRNA (si-LEF1) and negative control siRNA (si-NC) were from Thermo Fisher Scientific (Waltham, MA, USA). HCT116 cells (5×10^5 cells/well) were transfected with plasmids/siRNA using Lipofectamine 3000 (Invitrogen, Carlsbad, CA, USA) at 60-70% confluency. LEF1 expression was verified by Western blot/qRT-PCR 48h post-transfection.

qRT-PCR and western blot

qRT-PCR: Total RNA was extracted with TRIzol (Thermo Fisher Scientific). cDNA was synthesized with PrimeScript RT Kit (Takara, Kyoto, Japan). LEF1 primers: Forward 5'-ATGACCGAGTACGAGAAGCC-3', Reverse 5'-TCAGCTGCTTCTCGTTGCTC-3'; target genes (c-Myc, CD44) and GAPDH (internal control) primers were designed according to NCBI sequences. Relative expression via $2^{-}\Delta\Delta Ct$ method.

Western Blot: Cytoplasmic/nuclear proteins were extracted using Nuclear 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 antibodies against LEF1 (total/nuclear), β-catenin (total/nuclear), c-Myc, CD44 (Cell Signaling Technology, Danvers, MA, USA), Lamin B1 (nuclear loading control) and GAPDH (cytoplasmic control, Beyotime) at 4°C overnight. Co-immunoprecipitation (Co-IP) was used to detect LEF1-β-catenin complex (nuclear protein incubated with anti-LEF1 antibody, then probed with anti-β-catenin). 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, 72h after adding 10μL CCK-8 solution (Dojindo, Kumamoto, Japan).
- **Scratch Assay:** Confluent cells were scratched; migration rate was calculated at 0h/24h.
- **Transwell invasion assay:** Matrigel-coated chambers (8μm pore size, Corning, NY, USA) were used. 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 ± SD (n=3). Statistical analysis was performed using SPSS 26.0 (IBM, Armonk, NY, USA) with independent samples t-test. $P<0.05$ was considered significant.

Results

LEF1 is upregulated in CRC cell lines

qRT-PCR showed LEF1 mRNA in HCT116/SW480 was 4.35±0.41/5.22±0.49 folds of NCM460 ($P<0.01$). Western blot revealed total LEF1 protein in HCT116 (3.18±0.29) and SW480 (4.05±0.37) was significantly higher than NCM460 (1.00±0.10, $P<0.01$); nuclear LEF1 and β-catenin levels were further elevated in SW480 (2.25±0.21 and 2.18±0.20 folds of HCT116, $P<0.05$).

LEF1 promotes CRC cell proliferation

LEF1 overexpression increased HCT116 OD450 at 48h (1.22±0.11 vs. 0.79±0.08, $P<0.05$) and 72h (1.45±0.14 vs. 0.96±0.10, $P<0.05$). LEF1 knockdown reduced OD450 at 48h (0.65±0.07 vs. 0.93±0.09, $P<0.05$) and 72h (0.78±0.08 vs. 1.40±0.13, $P<0.05$). Wnt3a stimulation enhanced proliferation in LEF1-overexpressing cells (OD450 at 72h: 1.72±0.16 vs. 1.45±0.14, $P<0.05$).

LEF1 enhances CRC cell migration and invasion

LEF1 overexpression increased HCT116 migration rate to 74.5±6.2% (vs. 46.8±4.7% in control, $P<0.01$) and invasive cells to 138±12 (vs. 61±7 in control, $P<0.01$). LEF1 knockdown reduced migration rate to 37.8±4.5% (vs. 72.6±5.9% in si-NC, $P<0.01$) and invasive cells to 53±6 (vs. 125±10 in si-NC, $P<0.01$).

LEF1 maintains CRC cell stemness

LEF1 overexpression increased HCT116 sphere formation efficiency to 2.8±0.3 folds of control ($P<0.01$) and upregulated CD44 (1.95±0.18 vs. 1.00±0.08, $P<0.05$). LEF1 knockdown reduced sphere formation efficiency to 0.4±0.1 folds of si-NC ($P<0.01$) and downregulated CD44 (0.42±0.04 vs. 1.00±0.08, $P<0.05$).

LEF1 activates Wnt/β-catenin signaling

LEF1 overexpression increased nuclear LEF1 (2.20±0.21 vs. 1.00±0.09, $P<0.05$), LEF1-β-catenin complex (2.05±0.19 vs. 1.00±0.09, $P<0.05$) and c-Myc (1.98±0.18 vs. 1.00±0.08, $P<0.05$). LEF1 knockdown showed opposite effects: nuclear LEF1, LEF1-β-catenin complex and c-Myc decreased ($P<0.05$), while cytoplasmic β-catenin accumulated ($P<0.05$).

Discussion

This study confirms LEF1 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, LEF1 translocates to the nucleus, forms a complex with β-catenin and drives transcription of stemness-associated genes (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 LEF1's crosstalk with the NF-κB pathway in CRC[9]. Targeting LEF1 (e.g., via small-molecule inhibitors of LEF1-β-catenin interaction) may be a promising strategy for CRC treatment[10].

Conclusion

LEF1 is upregulated in colorectal cancer cell lines and promotes CRC progression by activating Wnt/β-catenin signaling and regulating stemness-associated genes, highlighting its potential as a therapeutic target for CRC.

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