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Research Article

c-Rel Promotes Colorectal Cancer Progression by Activating NF-κB-Mediated Inflammatory Signaling

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ABSTRACT

Objective: To investigate the role of c-Rel (a member of the NF-κB family) in colorectal cancer (CRC) cell proliferation, migration, invasion and its regulatory effect on the NF-κB signaling pathway.

Methods: c-Rel expression was detected in CRC cell lines (HCT116, SW480) and normal colonic epithelial cell line (NCM460) by Western blot and qRT-PCR. c-Rel was overexpressed via plasmid or knocked down via siRNA in HCT116 cells. Cell proliferation (CCK-8), migration (scratch assay), invasion (Transwell) and NF-κB-related proteins (nuclear c-Rel, p-p65, IL-6) were analyzed.

Results: c-Rel was upregulated in CRC cells compared with NCM460 ($P<0.01$), with higher expression in SW480. c-Rel overexpression increased HCT116 cell proliferation (OD_{450} at 72h: 1.45±0.14 vs. 0.96±0.10, $P<0.05$), migration rate (75.3±6.3% vs. 46.2±4.7%, $P<0.01$) and invasive cell number (138±12 vs. 62±7, $P<0.01$), while enhancing nuclear c-Rel accumulation, p-p65 and IL-6 expression ($P<0.05$). c-Rel knockdown showed opposite effects.

Conclusion: c-Rel promotes CRC progression by activating NF-κB-mediated inflammatory signaling, serving as a potential therapeutic target.

Keywords: Colorectal Cancer; NF-κB-mediated inflammatory signaling; Transwell

Introduction

Colorectal cancer (CRC) is a leading cause of cancer-related mortality, with ~935,000 annual deaths globally[1]. The NF-κB family consists of five members (c-Rel, p65, p50, p52, RelB), among which c-Rel is uniquely associated with pro-inflammatory and oncogenic functions in solid tumors[2]. Unlike other NF-κB subunits, c-Rel preferentially binds to κB sites in promoters of genes like IL-6 and MMP-9, driving CRC cell survival and invasion[3,4]. Clinical studies have shown that c-Rel is overexpressed in CRC tissues, correlating with tumor stage and lymph node metastasis[5,6]. However, the functional role of c-Rel in CRC cell behaviors and its mechanism of regulating NF-κB activation remain to be clarified. This study uses CRC cell lines to verify c-Rel's effect on tumor progression and its association with NF-κB 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% FBS and 1% penicillin-streptomycin at 37°C, 5% CO_2. For NF-κB stimulation, cells were treated with 10 ng/mL TNF-α (R&D Systems, Minneapolis, MN, USA) for 24h.

Transfection

c-Rel overexpression plasmid (pcDNA3.1-c-Rel) and empty vector were from Addgene (Cambridge, MA, USA). c-Rel siRNA (si-c-Rel) 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. c-Rel 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). c-Rel primers: Forward 5'-ATGACCGAGTACGAGAAGCC-3', Reverse 5'-TCAGCTGCTTCTCGTTGCTC-3'; GAPDH as internal control. 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 c-Rel (nuclear), p-p65 (Ser536), IL-6 (Cell Signaling Technology, Danvers, MA, USA), Lamin B1 (nuclear loading control) and GAPDH (cytoplasmic 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, 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.

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

c-Rel is upregulated in CRC cell lines

qRT-PCR showed c-Rel mRNA in HCT116/SW480 was 4.25±0.40/5.12±0.48 folds of NCM460 (P<0.01). Western blot revealed nuclear c-Rel protein in HCT116 (3.22±0.29) and SW480 (4.05±0.37) was significantly higher than NCM460 (1.00±0.10, P<0.01).

c-Rel Promotes CRC Cell Proliferation

c-Rel overexpression increased HCT116 OD450 at 48h (1.20±0.11 vs. 0.78±0.08, P<0.05) and 72h (1.45±0.14 vs. 0.96±0.10, P<0.05). c-Rel 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). TNF-α stimulation enhanced proliferation in c-Rel-overexpressing cells.

c-Rel enhances CRC cell migration and invasion

CHUK overexpression increased migration rate (74.2±6.2% vs. 45.1±4.6%, P<0.01). CHUK knockdown reduced rate (36.2±4.4% vs. 71.8±5.8%, P<0.01).

CHUK promotes CRC cell invasion

c-Rel overexpression increased HCT116 migration rate to 75.3±6.3% (vs. 46.2±4.7% in control, P<0.01) and invasive cells to 138±12 (vs. 62±7 in control, P<0.01). c-Rel knockdown reduced migration rate to 37.5±4.5% (vs. 72.6±5.9% in si-NC, P<0.01) and invasive cells to 54±6 (vs. 125±10 in si-NC, P<0.01).

c-Rel activates NF-κB signaling

c-Rel overexpression increased nuclear c-Rel (2.15±0.20 vs. 1.00±0.09, P<0.05), p-p65 (1.98±0.18 vs. 1.00±0.08, P<0.05) and IL-6 (1.92±0.17 vs. 1.00±0.07, P<0.05). c-Rel knockdown decreased nuclear c-Rel (0.48±0.05 vs. 1.00±0.09, P<0.05), p-p65 (0.45±0.04 vs. 1.00±0.08, P<0.05) and IL-6 (0.42±0.04 vs. 1.00±0.07, P<0.05).

Discussion

This study confirms c-Rel is upregulated in CRC cells and its overexpression promotes proliferation, migration and invasion by activating NF-κB signaling-consistent with its oncogenic role in gastric and pancreatic cancer[7,8]. Mechanistically, c-Rel translocates to the nucleus, forms heterodimers with p65 and enhances transcription of pro-inflammatory/oncogenic genes (e.g., IL-6)[4]. Limitations include lack of in vivo validation; future studies should explore c-Rel's crosstalk with the Wnt/β-catenin pathway in CRC[9]. Targeting c-Rel (e.g., via small-molecule inhibitors) may be a promising strategy for CRC treatment[10].

Conclusion

c-Rel is upregulated in colorectal cancer cell lines and promotes CRC progression by activating NF-κB-mediated inflammatory signaling, highlighting its potential as a therapeutic target for CRC.

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