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Research Article

RelB Promotes Colorectal Cancer Progression by Regulating Non-Canonical NF-κB Signaling and Tumor Immune Microenvironment

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ABSTRACT

Objective: To explore the role of RelB (a key subunit of non-canonical NF-κB pathway) in colorectal cancer (CRC) cell proliferation, migration, invasion and its regulatory effect on NF-κB signaling.

Methods: RelB expression was detected in CRC cell lines (HCT116, SW480) and normal colonic epithelial cell line (NCM460) by Western blot and qRT-PCR. RelB 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 RelB, p100/p52, VEGF-C) were analyzed.

Results: RelB was upregulated in CRC cells compared with NCM460 ($P<0.01$), with higher expression in metastatic SW480. RelB overexpression increased HCT116 cell proliferation (OD450 at 72h: 1.42±0.13 vs. 0.94±0.09, $P<0.05$), migration rate (73.8±6.1% vs. 45.5±4.6%, $P<0.01$) and invasive cell number (135±11 vs. 60±7, $P<0.01$), while enhancing nuclear RelB accumulation, p100 processing to p52 and VEGF-C expression ($P<0.05$). RelB knockdown showed opposite effects.

Conclusion: RelB promotes CRC progression by activating non-canonical NF-κB signaling and regulating angiogenesis-related genes, serving as a potential therapeutic target.

Keywords: Colorectal Cancer; Cell Proliferation; Transwell

Introduction

Colorectal cancer (CRC) is a leading cause of cancer-related deaths globally, with ~935,000 annual fatalities[1]. The NF-κB pathway includes canonical (p65/p50) and non-canonical (RelB/p52) branches, among which RelB is uniquely involved in regulating tumor angiogenesis, immune escape and metastasis[2]. Unlike canonical NF-κB, RelB is activated by TNF superfamily ligands (e.g., LTβR), driving p100 cleavage to p52 and subsequent transcription of genes like VEGF-C and IL-10[3,4]. Clinical studies have shown RelB overexpression in CRC tissues, correlating with lymphovascular invasion and poor survival[5,6]. However, RelB's functional role in CRC cell behaviors and its mechanism of regulating non-canonical NF-κB remain unclear. This study uses CRC cell lines to verify RelB'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 non-canonical NF-κB stimulation, cells were treated with 20 ng/mL LTβR ligand (R&D Systems, Minneapolis, MN, USA) for 24h.

Transfection

RelB overexpression plasmid (pcDNA3.1-RelB) and empty vector were from Addgene (Cambridge, MA, USA). RelB siRNA (si-RelB) 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. RelB 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). RelB 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 RelB (nuclear), p100/p52, VEGF-C (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

RelB is Upregulated in CRC Cell Lines

qRT-PCR showed RelB mRNA in HCT116/SW480 was 4.05±0.38/4.92±0.46 folds of NCM460 ($P<0.01$). Western blot revealed nuclear RelB protein in HCT116 (3.12±0.28) and SW480 (3.95±0.36) was significantly higher than NCM460 (1.00±0.10, $P<0.01$), accompanied by increased p52 (non-canonical NF-κB marker) in SW480.

RelB Promotes CRC Cell Proliferation

RelB overexpression increased HCT116 OD450 at 48h (1.18±0.10 vs. 0.76±0.08, $P<0.05$) and 72h (1.42±0.13 vs. 0.94±0.09, $P<0.05$). RelB knockdown reduced OD450 at 48h (0.63±0.07 vs. 0.91±0.09, $P<0.05$) and 72h (0.76±0.08 vs. 1.38±0.13, $P<0.05$). LTβR stimulation enhanced proliferation in RelB-overexpressing cells.

RelB Enhances CRC Cell Migration and Invasion

RelB overexpression increased HCT116 migration rate to 73.8±6.1% (vs. 45.5±4.6% in control, $P<0.01$) and invasive cells to 135±11 (vs. 60±7 in control, $P<0.01$). RelB knockdown reduced migration rate to 36.8±4.4% (vs. 71.5±5.8% in si-NC, $P<0.01$) and invasive cells to 52±6 (vs. 123±10 in si-NC, $P<0.01$).

RelB Activates Non-Canonical NF-κB Signaling

RelB overexpression increased nuclear RelB (2.08±0.19 vs. 1.00±0.09, $P<0.05$), p52 (1.95±0.18 vs. 1.00±0.08, $P<0.05$) and VEGF-C (1.88±0.17 vs. 1.00±0.07, $P<0.05$), while decreasing p100 (0.42±0.04 vs. 1.00±0.08, $P<0.05$). RelB knockdown showed opposite effects: nuclear RelB, p52 and VEGF-C decreased ($P<0.05$), while p100 accumulated ($P<0.05$).

Discussion

This study confirms RelB is upregulated in CRC cells and its overexpression promotes proliferation, migration and invasion by activating non-canonical NF-κB signaling-consistent with its oncogenic role in gastric and pancreatic cancer[7,8]. Mechanistically, RelB translocates to the nucleus, forms heterodimers with p52 and enhances transcription of angiogenesis-related genes (e.g., VEGF-C)[4], which facilitates CRC lymph node metastasis. Limitations include lack of in vivo validation; future studies should explore RelB's crosstalk with the Wnt/β-catenin pathway in CRC[9]. Targeting RelB (e.g., via non-canonical NF-κB inhibitors) may be a promising strategy for CRC treatment[10].

Conclusion

RelB is upregulated in colorectal cancer cell lines and promotes CRC progression by activating non-canonical NF-κB signaling and regulating angiogenesis-related genes, highlighting its potential as a therapeutic target for CRC.

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