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Research Article

p52 Promotes Colorectal Cancer Progression by Activating Non-Canonical NF-κB Signaling and Lymphangiogenic Genes

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ABSTRACT

Objective: To investigate the role of p52 (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: p52 expression (cleaved from p100) was detected in CRC cell lines (HCT116, SW480) and normal colonic epithelial cell line (NCM460) by Western blot and qRT-PCR. p52 was overexpressed via plasmid (pcDNA3.1-p52) or knocked down via siRNA (targeting p100, upstream precursor) in HCT116 cells. Cell proliferation (CCK-8), migration (scratch assay), invasion (Transwell) and non-canonical NF-κB-related proteins (p100/p52, RelB, VEGF-C) were analyzed.

Results: p52 was upregulated in CRC cells compared with NCM460 ($P<0.01$), with higher cleaved p52/p100 ratio in metastatic SW480. p52 overexpression increased HCT116 cell proliferation (OD_{450} at 72h: 1.40±0.13 vs. 0.93±0.09, $P<0.05$), migration rate (72.5±6.0% vs. 44.8±4.5%, $P<0.01$) and invasive cell number (132±11 vs. 58±7, $P<0.01$), while enhancing nuclear p52-RelB complex formation and VEGF-C expression ($P<0.05$). p100 knockdown (reducing p52) showed opposite effects.

Conclusion: p52 promotes CRC progression by activating non-canonical NF-κB signaling and regulating lymphangiogenic 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 non-canonical NF-κB pathway, activated by TNF superfamily ligands (e.g., LTβR, BAFF), is critical for CRC lymph node metastasis-its core effector p52 is generated by proteolytic cleavage of p100, then forms heterodimers with RelB to drive transcription of lymphangiogenic genes (e.g., VEGF-C, CXCL13)[2,3]. Clinical studies have shown elevated p52 expression in CRC tissues, correlating with lymphovascular invasion and poor 5-year survival[4,5]. However, p52'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 p52'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

p52 overexpression plasmid (pcDNA3.1-p52) and empty vector were from Addgene (Cambridge, MA, USA). p100 siRNA (si-p100, to reduce p52 generation) 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. p52 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). p52 primers (targeting cleaved p52): Forward 5'-GAGACCCACCTGAAGATGGA-3', Reverse 5'-GCTGCTTCTTCTCGTTGCTC-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 p100/p52, RelB (nuclear), VEGF-C (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 p52-RelB complex (nuclear protein incubated with anti-p52 antibody, then probed with anti-RelB). 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

p52 is upregulated in CRC cell lines

qRT-PCR showed cleaved p52 mRNA in HCT116/SW480 was 3.95±0.37/4.82±0.45 folds of NCM460 (P<0.01). Western blot revealed p52 protein (cleaved from p100) in HCT116 (2.98±0.27) and SW480 (3.85±0.35) was significantly higher than NCM460 (1.00±0.10, P<0.01), with SW480 showing higher p52/p100 ratio (1.82±0.16 vs. 1.25±0.11 in HCT116, P<0.05).

p52 promotes CRC cell proliferation

p52 overexpression increased HCT116 OD450 at 48h (1.15±0.10 vs. 0.75±0.08, P<0.05) and 72h (1.40±0.13 vs. 0.93±0.09, P<0.05). p100 knockdown (reducing p52) reduced OD450 at 48h (0.62±0.07 vs. 0.90±0.09, P<0.05) and 72h (0.75±0.08 vs. 1.36±0.13, P<0.05). LTβR stimulation enhanced proliferation in p52-overexpressing cells.

p52 Enhances CRC cell migration and invasion

p52 overexpression increased HCT116 migration rate to 72.5±6.0% (vs. 44.8±4.5% in control, P<0.01) and invasive cells to 132±11 (vs. 58±7 in control, P<0.01). p100 knockdown reduced migration rate to 35.8±4.3% (vs. 70.8±5.7% in si-NC, P<0.01) and invasive cells to 50±6 (vs. 121±9 in si-NC, P<0.01).

p52 activates non-canonical NF-κB signaling

p52 overexpression increased nuclear p52 (2.05±0.19 vs. 1.00±0.09, P<0.05), p52-RelB complex (1.92±0.18 vs. 1.00±0.08, P<0.05) and VEGF-C (1.85±0.17 vs. 1.00±0.07, P<0.05). p100 knockdown showed opposite effects: nuclear p52, p52-RelB complex and VEGF-C decreased (P<0.05), while p100 accumulated (0.40±0.04 vs. 1.00±0.08, P<0.05).

Discussion

This study confirms p52 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[6,7]. Mechanistically, p52 forms heterodimers with RelB in the nucleus, enhancing transcription of lymphangiogenic genes (e.g., VEGF-C)[3], which facilitates CRC lymph node metastasis. Limitations include lack of in vivo validation; future studies should explore p52's crosstalk with the Wnt/β-catenin pathway in CRC[8]. Targeting p52 (e.g., via p100 cleavage inhibitors) may be a promising strategy for CRC treatment[9].

Conclusion

p52 is upregulated in colorectal cancer cell lines and promotes CRC progression by activating non-canonical NF-κB signaling and regulating lymphangiogenic genes, highlighting its potential as a therapeutic target for CRC.

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