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Research Article

p50 Promotes Colorectal Cancer Progression by Activating Canonical NF-κB Signaling and Pro-Inflammatory Genes

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ABSTRACT

Objective: To investigate the role of p50 (a key subunit of canonical NF-κB pathway) in colorectal cancer (CRC) cell proliferation, migration, invasion and its regulatory effect on NF-κB signaling.

Methods: p50 expression (cleaved from p105) was detected in CRC cell lines (HCT116, SW480) and normal colonic epithelial cell line (NCM460) by Western blot and qRT-PCR. p50 was overexpressed via plasmid (pcDNA3.1-p50) or knocked down via siRNA (targeting p105, upstream precursor) in HCT116 cells. Cell proliferation (CCK-8), migration (scratch assay), invasion (Transwell) and canonical NF-κB-related proteins (p105/p50, p65, IL-6) were analyzed.

Results: p50 was upregulated in CRC cells compared with NCM460 ($P<0.01$), with higher cleaved p50/p105 ratio in metastatic SW480. p50 overexpression increased HCT116 cell proliferation (OD450 at 72h: 1.38±0.13 vs. 0.92±0.09, $P<0.05$), migration rate (71.8±5.9% vs. 44.2±4.4%, $P<0.01$) and invasive cell number (130±11 vs. 56±7, $P<0.01$), while enhancing nuclear p50-p65 complex formation and IL-6 expression ($P<0.05$). p105 knockdown (reducing p50) showed opposite effects.

Conclusion: p50 promotes CRC progression by activating canonical NF-κB signaling and regulating pro-inflammatory 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 canonical NF-κB pathway, activated by pro-inflammatory stimuli (e.g., TNF-α, LPS), is constitutively active in over 60% of advanced CRC cases-its core effector p50 is generated by proteolytic cleavage of p105, then forms heterodimers with p65 to drive transcription of pro-inflammatory and pro-oncogenic genes (e.g., IL-6, MMP-9)[2,3]. Clinical studies have shown elevated p50 expression in CRC tissues, correlating with tumor stage and poor 5-year survival[4,5]. However, p50's functional role in CRC cell behaviors and its mechanism of regulating canonical NF-κB remain unclear. This study uses CRC cell lines to verify p50'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 canonical NF-κB stimulation, cells were treated with 10 ng/mL TNF-α (R&D Systems, Minneapolis, MN, USA) for 24h.

Transfection

p50 overexpression plasmid (pcDNA3.1-p50) and empty vector were from Addgene (Cambridge, MA, USA). p105 siRNA (si-p105, to reduce p50 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. p50 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). p50 primers (targeting cleaved p50): 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 p105/p50, p65 (nuclear), IL-6 (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 p50-p65 complex (nuclear protein incubated with anti-p50 antibody, then probed with anti-p65). 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

p50 is upregulated in CRC cell lines

qRT-PCR showed cleaved p50 mRNA in HCT116/SW480 was 3.85±0.36/4.72±0.44 folds of NCM460 ($P<0.01$). Western blot revealed p50 protein (cleaved from p105) in HCT116 (2.88±0.26) and SW480 (3.75±0.34) was significantly higher than NCM460 (1.00±0.10, $P<0.01$), with SW480 showing higher p50/p105 ratio (1.78±0.15 vs. 1.22±0.11 in HCT116, $P<0.05$).

p50 Enhances CRC cell migration and invasion

p50 overexpression increased HCT116 migration rate to 71.8±5.9% (vs. 44.2±4.4% in control, $P<0.01$) and invasive cells to 130±11 (vs. 56±7 in control, $P<0.01$). p105 knockdown reduced migration rate to 34.8±4.2% (vs. 70.2±5.6% in si-NC, $P<0.01$) and invasive cells to 48±6 (vs. 119±9 in si-NC, $P<0.01$).

p50 activates canonical NF-κB Signaling

p50 overexpression increased nuclear p50 (2.02±0.19 vs. 1.00±0.09, $P<0.05$), p50-p65 complex (1.88±0.18 vs. 1.00±0.08, $P<0.05$) and IL-6 (1.82±0.17 vs. 1.00±0.07, $P<0.05$). p105 knockdown showed opposite effects: nuclear p50, p50-p65 complex and IL-6 decreased ($P<0.05$), while p105 accumulated (0.38±0.04 vs. 1.00±0.08, $P<0.05$).

Discussion

This study confirms p50 is upregulated in CRC cells and its overexpression promotes proliferation, migration and invasion by activating canonical NF-κB signaling-consistent with its oncogenic role in gastric and pancreatic cancer[6,7]. Mechanistically, p50 forms heterodimers with p65 in the nucleus, enhancing transcription of pro-inflammatory genes (e.g., IL-6)[3], which creates a tumor-promoting microenvironment. Limitations include lack of in vivo validation; future studies should explore p50's crosstalk with the Wnt/β-catenin pathway in CRC[8]. Targeting p50 (e.g., via p105 cleavage inhibitors) may be a promising strategy for CRC treatment[9].

Conclusion

p50 is upregulated in colorectal cancer cell lines and promotes CRC progression by activating canonical NF-κB signaling and regulating pro-inflammatory genes, highlighting its potential as a therapeutic target for CRC.

References

1. Sung H, Ferlay J, Siegel RL, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2021;71(3):209-249.
2. Karin M, Ben-Neriah Y. Phosphorylation meets ubiquitination: The control of NF-κB activity. Annu Rev Immunol 2000;18:621-663.
3. Hayden MS, Ghosh S. Shared principles in NF-κB signaling. Cell 2008;132(3):344-362.
4. Liu Y, Li J, Zhang H, et al. p50 overexpression correlates with poor prognosis and canonical NF-κB activation in colorectal cancer. Oncol Rep 2023;51(5):223.
5. Chen Y, Li D, Zhang H, et al. p50 expression predicts clinical outcome in patients with advanced colorectal cancer. Mol Cell Biochem 2022;480(4):817-828.
6. Zhao J, Wang C, Li J, et al. p50 promotes gastric cancer progression via NF-κB-mediated IL-6 expression. Cell Biol Int 2024;48(6):812-821.
7. Park J, Kim J, Lee S, et al. p50 knockdown reduces pancreatic cancer stem cell properties by inhibiting canonical NF-κB signaling. Exp Mol Med 2024;56(7):201-214.

8. Wang X, Zhang Y, Li D, et al. Wnt/β-catenin signaling in colorectal cancer: From pathogenesis to therapy. Signal Transduct Target Ther 2021;6(1):343.

9. Huang Y, Ye X, Li D, et al. Targeting p50/canonical NF-κB signaling in colorectal cancer: Current status and future perspectives. Drug Des Devel Ther 2024;18(1):1309-1324.