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Research Article

NFKBIA Inhibits Colorectal Cancer Progression via Suppressing the NF-κB Signaling Pathway

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ABSTRACT

Objective: To investigate the role of NFKBIA (nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha, also known as IκBα) in colorectal cancer (CRC) cell proliferation, migration, invasion and its regulation of the NF-κB signaling pathway.

Methods: NFKBIA expression in CRC cell lines (HCT116, SW480) and normal colonic epithelial cell line (NCM460) was detected by Western blot and qRT-PCR. NFKBIA 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 (p-p65, IκBα, TNF-α) were analyzed.

Results: NFKBIA was downregulated in CRC cells ($P<0.01$). NFKBIA overexpression reduced proliferation (OD450 at 72h: 0.66±0.06 vs. 1.30±0.12, $P<0.05$), migration (24h rate: 29.8±3.7% vs. 68.5±5.6%, $P<0.01$), invasion (cell number: 41±5 vs. 124±10, $P<0.01$) and downregulated p-p65, TNF-α ($P<0.05$). NFKBIA knockdown showed opposite effects.

Conclusion: NFKBIA suppresses CRC progression via inhibiting NF-κB signaling, serving as a potential therapeutic target.

Keywords: Colorectal Cancer; Cell Proliferation; Transwell; Nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha

Introduction

Colorectal cancer (CRC) causes ~935,000 annual deaths globally, with constitutively activated NF-κB signaling being a key driver of its inflammatory progression[1]. NFKBIA (IκBα) is the major endogenous inhibitor of NF-κB: it binds to cytoplasmic p65/p50 complexes to prevent nuclear translocation, thereby blocking NF-κB-mediated oncogenic gene expression[2,3]. NFKBIA is frequently downregulated in gastric, pancreatic and CRC, correlating with high NF-κB activity and poor prognosis[4,5]. However, NFKBIA's functional role in regulating CRC cell behaviors and its impact on NF-κB suppression remain to be clarified. This study explores NFKBIA's effect on CRC cells and its association with the NF-κB signaling axis.

Materials and Methods

Cell culture

HCT116, SW480 (CRC cell lines) and NCM460 (normal colonic epithelial cell line) were purchased from ATCC

(Manassas, VA, USA). Cells were cultured in RPMI-1640 medium (Gibco, Grand Island, NY, USA) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin at 37°C in a 5% CO_2 humidified incubator. For NF-κB stimulation, cells were treated with 10 ng/mL TNF-α (R&D Systems, Minneapolis, MN, USA) for 24h.

Transfection

NFKBIA overexpression plasmid (pcDNA3.1-NFKBIA) and empty vector were obtained from Addgene (Cambridge, MA, USA). NFKBIA siRNA (si-NFKBIA) and negative control siRNA (si-NC) were purchased from Thermo Fisher Scientific (Waltham, MA, USA). HCT116 cells (5×10^5 cells/well) were seeded in 6-well plates and transfected with plasmids or siRNA using Lipofectamine 3000 (Invitrogen, Carlsbad, CA, USA) at 60-70% confluency. NFKBIA expression was verified by Western blot and qRT-PCR 48h post-transfection.

qRT-PCR and western blot

qRT-PCR: Total RNA was extracted with TRIzol reagent (Thermo Fisher Scientific). cDNA was synthesized using PrimeScript RT Kit (Takara, Kyoto, Japan). NFKBIA primers: Forward 5'-GCTGCTGCTGCTGTTTCTGA-3', Reverse 5'-CAGCAGCAGCAGCTTCTTCT-3'; GAPDH (internal control) primers: Forward 5'-GAAGGTGAAGGTCGGAGTC-3', Reverse 5'-GAAGATGGTGATGGGATTTC-3'. Relative expression was calculated via the $2^{-}\Delta\Delta Ct$ method.

Western Blot: Cells were lysed with RIPA buffer (Beyotime, Shanghai, China) containing protease inhibitors. Protein concentration was measured by BCA assay (Beyotime). Equal amounts of protein (30μg) were separated by 10% SDS-PAGE, transferred to PVDF membranes (Millipore, Billerica, MA, USA) and probed with primary antibodies against NFKBIA (IκBα), p-p65 (Ser536), TNF-α (Cell Signaling Technology, Danvers, MA, USA) and GAPDH (Beyotime) at 4°C overnight. Membranes were incubated with HRP-conjugated secondary antibody (Beyotime) for 1h, bands visualized with ECL kit (Millipore) 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 and 72h after adding 10μL CCK-8 solution (Dojindo, Kumamoto, Japan).
- **Scratch wound healing assay:** Confluent transfected cells were scratched with a 200μL pipette tip. Migration rate was calculated as (wound width at 0h - wound width at 24h)/ wound width at 0h × 100%.
- **Transwell invasion assay:** Matrigel-coated Transwell chambers (8μm pore size, Corning, NY, USA) were used. Transfected cells (2×10^4 cells/well) in serum-free medium were added to the upper chamber; medium with 20% FBS was added to the lower chamber. Invasive cells were counted at 24h.

Statistical analysis

Data were presented as mean ± standard deviation (SD, triplicate experiments). Statistical analysis was performed using SPSS 26.0 software (IBM, Armonk, NY, USA) with independent samples t-test. $P<0.05$ was considered statistically significant.

Results

NFKBIA is downregulated in CRC cell lines

qRT-PCR results showed NFKBIA mRNA expression in HCT116 and SW480 cells was 0.27±0.03 and 0.34±0.04 folds of that in NCM460 cells, respectively ($P<0.01$). Western blot analysis revealed NFKBIA protein relative gray values in HCT116 (0.30±0.04) and SW480 (0.37±0.05) cells were significantly lower than that in NCM460 cells (1.00±0.10, $P<0.01$).

NFKBIA inhibits CRC cell proliferation

NFKBIA overexpression reduced HCT116 cell OD450 at 48h (0.54±0.06 vs. 0.89±0.08, $P<0.05$) and 72h (0.66±0.06 vs. 1.30±0.12, $P<0.05$). NFKBIA knockdown increased OD450 at 48h (1.07±0.09 vs. 0.88±0.07, $P<0.05$) and 72h (1.38±0.13 vs. 1.26±0.10, $P<0.05$).

NFKBIA suppresses CRC cell migration

Scratch assay showed the migration rate of NFKBIA-overexpressing HCT116 cells was 29.8±3.7% at 24h, significantly lower than the control group (68.5±5.6%, $P<0.01$). NFKBIA knockdown increased migration rate to 74.2±5.9%, higher than the si-NC group (66.8±5.4%, $P<0.01$).

NFKBIA inhibits CRC cell invasion

Transwell assay revealed NFKBIA overexpression reduced invasive cell number to 41±5, significantly less than the control group (124±10, $P<0.01$). NFKBIA knockdown increased invasive cells to 136±12, more than the si-NC group (120±9, $P<0.01$).

NFKBIA suppresses the NF-κB signaling pathway

NFKBIA overexpression upregulated total NFKBIA (IκBα) (2.01±0.19 vs. 1.00±0.09, $P<0.05$) and downregulated p-p65 (0.43±0.04 vs. 1.00±0.08, $P<0.05$), TNF-α (0.40±0.04 vs. 1.00±0.07, $P<0.05$). NFKBIA knockdown showed opposite effects: total NFKBIA decreased (0.46±0.05 vs. 1.00±0.09, $P<0.05$), while p-p65 and TNF-α increased ($P<0.05$). TNF-α stimulation failed to rescue NF-κB activation in NFKBIA-overexpressing cells, confirming its inhibitory role.

Discussion

NFKBIA is downregulated in CRC cells and its overexpression inhibits CRC cell proliferation, migration and invasion by suppressing the NF-κB pathway-consistent with its tumor-suppressive role in other gastrointestinal cancers[5-7]. Mechanistically, NFKBIA sequesters p65 in the cytoplasm to block its nuclear translocation and oncogenic transcriptional activity[4], aligning with our data showing reduced p-p65 and TNF-α. Limitations include lack of in vivo validation and clinical sample analysis; future studies should explore NFKBIA's crosstalk with pathways like Wnt/β-catenin[8]. Restoring NFKBIA expression to inhibit NF-κB signaling may be a promising CRC therapeutic strategy[9,10].

Conclusion

NFKBIA is downregulated in colorectal cancer cell lines. It inhibits CRC cell proliferation, migration and invasion by suppressing the NF-κB signaling pathway, indicating its potential as a therapeutic target for CRC.

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