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Research Article

IκBα Inhibits Colorectal Cancer Progression by Suppressing Constitutive NF-κB Activation

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ABSTRACT

Objective: To explore the role of IκBα (inhibitor of nuclear factor kappa B alpha) in colorectal cancer (CRC) cell proliferation, migration, invasion and its regulatory effect on the NF-κB signaling pathway.

Methods: IκBα expression was detected in CRC cell lines (HCT116, SW480) and normal colonic epithelial cell line (NCM460) by Western blot and qRT-PCR. IκBα 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: IκBα was downregulated in CRC cells compared with NCM460 ($P<0.01$). IκBα overexpression reduced HCT116 cell proliferation (OD450 at 72h: 0.65±0.06 vs. 1.29±0.12, $P<0.05$), migration rate (28.9±3.6% vs. 67.8±5.5%, $P<0.01$) and invasive cell number (40±5 vs. 123±10, $P<0.01$), while decreasing p-p65 and TNF-α expression ($P<0.05$). IκBα knockdown showed opposite effects.

Conclusion: IκBα functions as a tumor suppressor in CRC by inhibiting NF-κB activation, serving as a potential therapeutic target.

Keywords: Colorectal Cancer; Cell Proliferation; Transwell; CRC Cell Lines

Introduction

Colorectal cancer (CRC) is the second leading cause of cancer-related deaths globally, with ~935,000 annual fatalities[1]. The NF-κB signaling pathway is constitutively activated in over 60% of advanced CRC cases, driving tumor cell survival, invasion and inflammation[2]. IκBα, encoded by the NFKBIA gene, is the primary endogenous inhibitor of NF-κB: it sequesters p65/p50 complexes in the cytoplasm, preventing nuclear translocation and oncogenic gene transcription[3,4]. Clinical studies have shown that IκBα expression is downregulated in CRC tissues, correlating with lymph node metastasis and poor prognosis[5,6]. However, the functional role of IκBα in CRC cell behaviors and its mechanism of regulating NF-κB remain to be fully clarified. This study uses CRC cell lines to verify IκBα'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) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin at 37°C in a 5% CO_2 incubator. For NF-κB stimulation, cells were treated with 10 ng/mL recombinant human TNF-α (R&D Systems, Minneapolis, MN, USA) for 24h.

Transfection

IκBα overexpression plasmid (pcDNA3.1-IκBα) and empty vector were obtained from Addgene (Cambridge, MA, USA). IκBα siRNA (si-IκBα) 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/siRNA using Lipofectamine 3000 (Invitrogen, Carlsbad, CA, USA) at 60-70% confluency. IκBα 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). IκBα primers: Forward 5'-ATGGACTACAGGGACGACCT-3', Reverse 5'-TCAGCTGGGGTTTCTGTTTC-3'; GAPDH primers (internal control): 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 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 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, n=3). 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

IκBα is downregulated in CRC cell lines

qRT-PCR showed IκBα mRNA expression in HCT116 and SW480 cells was 0.26±0.03 and 0.33±0.04 folds of NCM460, respectively ($P<0.01$). Western blot revealed IκBα protein levels in HCT116 (0.29±0.04) and SW480 (0.36±0.05) were significantly lower than NCM460 (1.00±0.10, $P<0.01$), with SW480 showing higher IκBα downregulation than HCT116.

IκBα inhibits CRC cell proliferation

In HCT116 cells, IκBα overexpression reduced OD450 at 48h (0.53±0.06 vs. 0.88±0.08, $P<0.05$) and 72h (0.65±0.06 vs. 1.29±0.12, $P<0.05$). IκBα knockdown increased OD450 at 48h (1.06±0.09 vs. 0.87±0.07, $P<0.05$) and 72h (1.37±0.13 vs. 1.25±0.10, $P<0.05$). TNF-α stimulation failed to rescue proliferation inhibition in IκBα-overexpressing cells.

IκBα suppresses CRC cell migration and invasion

Scratch assay showed IκBα overexpression reduced HCT116 migration rate to 28.9±3.6% (vs. 67.8±5.5% in control, $P<0.01$). Transwell assay revealed IκBα overexpression decreased invasive cell number to 40±5 (vs. 123±10 in control, $P<0.01$). IκBα knockdown showed opposite effects: migration rate increased to 73.5±5.8% (vs. 66.2±5.3% in si-NC, $P<0.01$) and invasive cells increased to 135±12 (vs. 120±9 in si-NC, $P<0.01$).

IκBα inhibits NF-κB signaling activation

IκBα overexpression upregulated total IκBα protein (2.00±0.19 vs. 1.00±0.09, $P<0.05$) and downregulated p-p65 (0.42±0.04 vs. 1.00±0.08, $P<0.05$) and TNF-α (0.39±0.04 vs. 1.00±0.07, $P<0.05$). IκBα knockdown decreased total IκBα (0.45±0.05 vs. 1.00±0.09, $P<0.05$) and increased p-p65 (1.88±0.17 vs. 1.00±0.08, $P<0.05$) and TNF-α (1.82±0.16 vs. 1.00±0.07, $P<0.05$).

Discussion

This study confirms IκBα is downregulated in CRC cells and its overexpression inhibits proliferation, migration and invasion by suppressing NF-κB activation-consistent with its tumor-suppressive role in gastric and pancreatic cancer[7,8]. Mechanistically, IκBα sequesters p65 in the cytoplasm, preventing its nuclear translocation and transcription of pro-oncogenic genes (e.g., TNF-α)[4]. Limitations include lack of in vivo validation; future studies should explore IκBα's crosstalk with Wnt/β-catenin, a key pathway in CRC[9]. Restoring IκBα expression (e.g., via NFKBIA gene delivery) may be a promising strategy for CRC treatment[10].

Conclusion

IκBα is downregulated in colorectal cancer cell lines and inhibits CRC progression by suppressing the NF-κB signaling pathway, highlighting its potential as a therapeutic target for CRC.

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