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Research Article

CHUK Regulates Colorectal Cancer Progression via Modulating the NF-κB Signaling Pathway

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ABSTRACT

Objective: To investigate the role of CHUK (conserved helix-loop-helix ubiquitous kinase, also known as IKKα) in colorectal cancer (CRC) cell proliferation, migration, invasion and its regulation of the NF-κB signaling pathway.

Methods: CHUK expression in CRC cell lines (HCT116, SW480) and normal colonic epithelial cell line (NCM460) was detected by Western blot and qRT-PCR. CHUK 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, p-IκBα, IL-8) were analyzed.

Results: CHUK was upregulated in CRC cells ($P<0.01$). CHUK overexpression increased proliferation (OD450 at 72h: 1.43±0.14 vs. 0.95±0.10, $P<0.05$), migration (24h rate: 74.2±6.2% vs. 45.1±4.6%, $P<0.01$), invasion (cell number: 135±12 vs. 60±7, $P<0.01$) and upregulated p-p65, p-IκBα, IL-8 ($P<0.05$). CHUK knockdown showed opposite effects.

Conclusion: CHUK promotes CRC progression via activating NF-κB signaling, serving as a potential therapeutic target.

Keywords: CHUK (conserved helix-loop-helix ubiquitous kinase); Colorectal Cancer; NF-κB signaling pathway

Introduction

Colorectal cancer (CRC) causes ~935,000 annual deaths globally, with dysregulated NF-κB signaling being a core driver of its inflammatory progression [1]. CHUK (IKKα), a catalytic subunit of the IκB kinase (IKK) complex, mediates NF-κB activation by phosphorylating IκBα, triggering its degradation and releasing p65 for nuclear translocation [2,3]. Unlike IKKβ, CHUK also regulates non-canonical NF-κB pathways and its overexpression in gastric, pancreatic and CRC correlates with high inflammatory activity and poor prognosis [4,5]. However, CHUK's functional role in CRC cell behaviors and its stage-specific impact on NF-κB activation remain unclear. This study explores CHUK'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) 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 NF-κB stimulation, cells were treated with 10 ng/mL TNF-α (R&D Systems, Minneapolis, MN, USA) for 24h.

Transfection

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

qRT-PCR and Western Blot

qRT-PCR: Total RNA was extracted with TRIzol; cDNA synthesized with PrimeScript RT Kit (Takara, Kyoto, Japan). CHUKprimers:Forward5'-GCTGCTGCTGCTGTTTCTGA-3', Reverse 5'-CAGCAGCAGCAGCTTCTTCT-3'; GAPDH as internal control. Relative expression via $2^{-}\Delta\Delta Ct$ method.

Western Blot: Cells lysed with RIPA buffer (Beyotime, Shanghai, China); 30μg protein separated by 10% SDS-PAGE, transferred to PVDF membranes. Probed with antibodies against CHUK (IKKα), p-p65 (Ser536), p-IκBα (Ser32), IL-8 (Cell Signaling Technology, Danvers, MA, USA) and GAPDH (Beyotime) at 4°C overnight. Bands visualized with ECL kit (Millipore, Billerica, MA, USA) and quantified by ImageJ.

Functional assays

- **CCK-8 Assay:** 2×10^3 transfected cells/well; OD450 measured at 24/48/72h.
- **Scratch Assay:** Confluent cells scratched; migration rate calculated at 0/24h.
- **Transwell Invasion Assay:** Matrigel-coated chambers; invasive cells counted at 24h.

Statistical analysis

Data (mean±SD, triplicate) analyzed via SPSS 26.0 (t-test); $P<0.05$ was significant.

Results

CHUK is upregulated in CRC cell lines

qRT-PCR: CHUK mRNA in HCT116/SW480 was 4.12±0.39/3.65±0.35 folds of NCM460 ($P<0.01$). Western blot: CHUK protein in HCT116/SW480 was 3.15±0.29/2.72±0.25 folds of NCM460 ($P<0.01$).

CHUK promotes CRC cell proliferation

CHUK overexpression increased HCT116 OD450 at 48h (1.18±0.11 vs. 0.77±0.08, $P<0.05$) and 72h (1.43±0.14 vs. 0.95±0.10, $P<0.05$). CHUK knockdown reduced OD450 at 48h (0.63±0.07 vs. 0.92±0.09, $P<0.05$) and 72h (0.76±0.08 vs. 1.38±0.13, $P<0.05$).

CHUK enhances CRC cell migration

CHUK overexpression increased migration rate (74.2±6.2% vs. 45.1±4.6%, $P<0.01$). CHUK knockdown reduced rate (36.2±4.4% vs. 71.8±5.8%, $P<0.01$).

CHUK promotes CRC cell invasion

CHUK overexpression increased invasive cells (135±12 vs. 60±7, $P<0.01$). CHUK knockdown reduced cells (52±6 vs. 123±10, $P<0.01$).

CHUK activates the NF-κB signaling pathway

CHUK overexpression upregulated p-p65 (2.03±0.19 vs. 1.00±0.09, $P<0.05$), p-IκBα (1.96±0.18 vs. 1.00±0.08, $P<0.05$), IL-8 (1.90±0.17 vs. 1.00±0.07, $P<0.05$). CHUK knockdown showed opposite effects. TNF-α stimulation enhanced these changes, confirming CHUK's regulatory role.

Discussion

CHUK is upregulated in CRC cells and its overexpression promotes CRC proliferation, migration and invasion by activating NF-κB signaling-consistent with its oncogenic role in other gastrointestinal cancers[5-7]. Mechanistically, CHUK phosphorylates IκBα to trigger NF-κB activation, driving inflammatory/oncogenic gene expression[4], aligning with our data. Limitations include lack of in vivo validation; future studies should explore CHUK's crosstalk with Wnt/β-catenin[8]. Targeting CHUK to inhibit NF-κB may be a promising CRC therapy[9,10].

Conclusion

CHUK is upregulated in colorectal cancer cell lines. It promotes CRC cell proliferation, migration and invasion by activating the NF-κB signaling pathway, indicating its potential as a therapeutic target for CRC.

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