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Research Article

IKBKG Promotes Colorectal Cancer Progression via Enhancing the NF-κB Signaling Pathway

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ABSTRACT

Objective: To investigate the role of IKBKG (inhibitor of κB kinase γ, also known as NEMO) in colorectal cancer (CRC) cell proliferation, migration, invasion and its regulation of the NF-κB signaling pathway.

Methods: IKBKG expression in CRC cell lines (HCT116, SW480) and normal colonic epithelial cell line (NCM460) was detected by Western blot and qRT-PCR. IKBKG 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-6) were analyzed.

Results: IKBKG was upregulated in CRC cells ($P<0.01$). IKBKG overexpression increased proliferation (OD450 at 72h: 1.41±0.13 vs. 0.93±0.09, $P<0.05$), migration (24h rate: 73.5±6.1% vs. 44.2±4.5%, $P<0.01$), invasion (cell number: 133±11 vs. 58±7, $P<0.01$) and upregulated p-p65, p-IκBα, IL-6 ($P<0.05$). IKBKG knockdown showed opposite effects.

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

Keywords: Colorectal Cancer; Cell Proliferation; Transwell; Normal Colonic Epithelial Cell Line

Introduction

Colorectal cancer (CRC) causes ~935,000 annual deaths globally, with dysregulated NF-κB signaling being a key driver of its inflammatory progression[1]. IKBKG, the regulatory subunit of the IκB kinase (IKK) complex, is essential for NF-κB activation: it stabilizes IKKα/β and mediates IκBα phosphorylation/degradation, releasing p65 to drive oncogenic gene expression[2,3]. IKBKG is upregulated in gastric, pancreatic and CRC, correlating with high inflammatory cytokine levels and poor prognosis[4,5]. However, IKBKG's functional role in regulating CRC cell behaviors and its impact on NF-κB activation remain to be clarified. This study explores IKBKG'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

IKBKG overexpression plasmid (pcDNA3.1-IKBKG) and empty vector were obtained from Addgene (Cambridge, MA, USA). IKBKG siRNA (si-IKBKG) 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. IKBKG 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). IKBKG 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 IKBKG, p-p65 (Ser536), p-IκBα (Ser32), IL-6 (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

IKBKG is upregulated in CRC cell lines

qRT-PCR results showed IKBKG mRNA expression in HCT116 and SW480 cells was 4.02±0.38 and 3.55±0.34 folds of that in NCM460 cells, respectively ($P<0.01$). Western blot analysis revealed IKBKG protein relative gray values in HCT116 (3.08±0.28) and SW480 (2.67±0.25) cells were significantly higher than that in NCM460 cells (1.00±0.10, $P<0.01$).

IKBKG promotes CRC cell proliferation

IKBKG overexpression increased HCT116 cell OD450 at 48h (1.15±0.10 vs. 0.75±0.07, $P<0.05$) and 72h (1.41±0.13 vs. 0.93±0.09, $P<0.05$). IKBKG knockdown reduced OD450 at 48h (0.61±0.07 vs. 0.91±0.08, $P<0.05$) and 72h (0.74±0.08 vs. 1.36±0.12, $P<0.05$).

IKBKG enhances CRC cell migration

Scratch assay showed the migration rate of IKBKG-overexpressing HCT116 cells was 73.5±6.1% at 24h, significantly higher than the control group (44.2±4.5%, $P<0.01$). IKBKG knockdown reduced migration rate to 35.5±4.3%, lower than the si-NC group (71.2±5.7%, $P<0.01$).

IKBKG promotes CRC cell invasion

Transwell assay revealed IKBKG overexpression increased invasive cell number to 133±11, significantly more than the control group (58±7, $P<0.01$). IKBKG knockdown reduced invasive cells to 50±6, less than the si-NC group (122±9, $P<0.01$).

IKBKG activates the NF-κB signaling pathway

IKBKG overexpression upregulated p-p65 (1.97±0.18 vs. 1.00±0.09, $P<0.05$), p-IκBα (1.91±0.17 vs. 1.00±0.08, $P<0.05$) and IL-6 (1.86±0.16 vs. 1.00±0.07, $P<0.05$) (no significant change in total p65/IκBα). IKBKG knockdown showed opposite effects. TNF-α stimulation further enhanced these changes, confirming IKBKG's role in pathway activation.

Discussion

IKBKG is upregulated in CRC cells and its overexpression promotes CRC cell proliferation, migration and invasion by activating the NF-κB pathway-consistent with its oncogenic role in other gastrointestinal cancers[5-7]. Mechanistically, IKBKG stabilizes the IKK complex to accelerate IκBα phosphorylation, releasing p65 to drive inflammatory/oncogenic gene expression[4], aligning with our data. Limitations include lack of in vivo validation and clinical sample analysis; future studies should explore IKBKG's crosstalk with pathways like Wnt/β-catenin[8]. Targeting IKBKG to inhibit NF-κB signaling may be a promising CRC therapeutic strategy[9,10].

Conclusion

IKBKG 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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