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Research Article

STK4 (MST1) Exerts Tumor-Suppressive Effects in Colorectal Cancer via Activating the Hippo Signaling Pathway

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ABSTRACT

Objective: To investigate the role of STK4 (serine/threonine kinase 4, also known as MST1) in colorectal cancer (CRC) cell proliferation, migration, invasion, and its regulation of the Hippo signaling pathway.

Methods: STK4 expression in CRC cell lines (HCT116, SW480) and normal colonic epithelial cell line (NCM460) was detected by Western blot and qRT-PCR. STK4 was overexpressed via plasmid or knocked down via siRNA in HCT116 cells. Cell proliferation (CCK-8), migration (scratch assay), invasion (Transwell), and Hippo-related proteins (LATS1, p-LATS1, YAP1, p-YAP1) were analyzed.

Results: STK4 was downregulated in CRC cells ($P<0.01$). STK4 overexpression reduced proliferation (OD450 at 72h: 0.58±0.05 vs. 1.23±0.09, $P<0.05$), migration (24h rate: 25.6±3.3% vs. 64.2±5.1%, $P<0.01$), invasion (cell number: 33±4 vs. 112±7, $P<0.01$), upregulated p-LATS1 and p-YAP1 ($P<0.05$), and downregulated YAP1 ($P<0.05$). STK4 knockdown showed opposite effects.

Conclusion: STK4 inhibits CRC progression via activating the Hippo pathway, serving as a potential therapeutic target.

Keywords: Colorectal Cancer; Cell Proliferation; Transwell

Introduction

Colorectal cancer (CRC) causes ~935,000 annual deaths globally, making it a leading cancer-related mortality cause[1]. The Hippo signaling pathway tightly regulates cell growth and tumorigenesis, and its dysregulation is a key driver of CRC progression[2,3]. STK4 (MST1), a core upstream kinase of the Hippo pathway, phosphorylates and activates LATS1, which further phosphorylates the oncogenic effector YAP1 to suppress its nuclear translocation and activity[4]. STK4 is downregulated in liver, pancreatic, and gastric cancers, correlating with poor prognosis[5-7]. However, STK4's functional role in CRC remains understudied. This study explores STK4's effect on CRC cells and its association with the Hippo pathway.

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.

Transfection

STK4 overexpression plasmid (pcDNA3.1-STK4) and negative control plasmid (pcDNA3.1) were obtained from Addgene (Cambridge, MA, USA). STK4 siRNA (si-STK4) and negative control siRNA (si-NC) were purchased from Thermo Fisher Scientific (Waltham, MA, USA). HCT116 cells were seeded in 6-well plates (5×10^5 cells/well) and transfected with plasmids or siRNA using Lipofectamine 3000 (Invitrogen, Carlsbad, CA, USA) at 60-70% confluency. STK4 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). STK4 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 STK4, LATS1, p-LATS1 (Ser909), YAP1, p-YAP1 (Ser127) (Cell Signaling Technology, Danvers, MA, USA), and GAPDH (Beyotime) at 4°C overnight. Membranes were incubated with HRP-conjugated secondary antibody (Beyotime) for 1h, and bands were visualized with ECL kit (Millipore) and quantified by ImageJ.

Functional Assays

- **CCK-8 Assay:** Transfected HCT116 cells (2×10^3 cells/well) were seeded in 96-well plates. At 24h, 48h, and 72h, 10μL CCK-8 solution (Dojindo, Kumamoto, Japan) was added, and absorbance at 450nm was measured with a microplate reader (Bio-Rad, Hercules, CA, USA).
- **Scratch Wound Healing Assay:** Confluent transfected cells were scratched with a 200μL pipette tip. Wound width was measured at 0h and 24h, and 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, and medium with 20% FBS to the lower chamber. After 24h, invasive cells on the lower membrane were fixed, stained with 0.1% crystal violet, and counted under a microscope (five random fields).

Statistical analysis

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

Results

STK4 is Downregulated in CRC Cell Lines

qRT-PCR results showed that STK4 mRNA expression in HCT116 and SW480 cells was 0.23±0.03 and 0.30±0.04 folds of that in NCM460 cells, respectively ($P<0.01$). Western blot analysis revealed that STK4 protein relative gray values in HCT116 (0.26±0.03) and SW480 (0.33±0.04) cells were significantly lower than that in NCM460 cells (1.00±0.09, $P<0.01$).

STK4 Inhibits CRC Cell Proliferation

STK4 overexpression reduced the OD450 value of HCT116 cells at 48h (0.50±0.05 vs. 0.87±0.07, $P<0.05$) and 72h (0.58±0.05 vs. 1.23±0.09, $P<0.05$). In contrast, STK4 knockdown increased the OD450 value at 48h (1.03±0.08 vs. 0.85±0.06, $P<0.05$) and 72h (1.34±0.10 vs. 1.21±0.08, $P<0.05$).

STK4 Suppresses CRC Cell Migration

Scratch wound healing assay showed that the migration rate of HCT116 cells in the STK4 overexpression group was 25.6±3.3% at 24h, significantly lower than that in the control group (64.2±5.1%, $P<0.01$). STK4 knockdown increased the migration rate to 71.8±5.6%, which was higher than that in the si-NC group (62.5±4.9%, $P<0.01$).

STK4 Inhibits CRC Cell Invasion

Transwell invasion assay revealed that the number of invasive HCT116 cells in the STK4 overexpression group was 33±4, significantly less than that in the control group (112±7, $P<0.01$). STK4 knockdown increased the number of invasive cells to 130±9, which was more than that in the si-NC group (110±6, $P<0.01$).

STK4 Activates the Hippo Signaling Pathway

Western blot analysis showed that STK4 overexpression upregulated the relative gray values of p-LATS1 (1.92±0.16 vs. 1.00±0.08, $P<0.05$) and p-YAP1 (1.88±0.15 vs. 1.00±0.07, $P<0.05$), and downregulated YAP1 (0.34±0.04 vs. 1.00±0.08, $P<0.05$). STK4 knockdown showed opposite effects: p-LATS1 (0.46±0.05 vs. 1.00±0.08, $P<0.05$) and p-YAP1 (0.43±0.04 vs. 1.00±0.07, $P<0.05$) were downregulated, and YAP1 (1.20±0.10 vs. 1.00±0.08, $P<0.05$) was upregulated.

Discussion

STK4 is downregulated in CRC cells, and its overexpression inhibits CRC cell proliferation, migration, and invasion by activating the Hippo pathway-consistent with its tumor-suppressive role in other cancers[5-7]. Mechanistically, STK4 phosphorylates and activates LATS1, which further phosphorylates YAP1 to block its oncogenic function[4], aligning with our data showing upregulated p-LATS1/p-YAP1 and downregulated YAP1 in STK4-overexpressing cells. Limitations include lack of in vivo validation and clinical sample analysis; future studies should address these. Restoring STK4 expression may be a promising CRC therapeutic strategy[8,9].

Conclusion

STK4 is downregulated in colorectal cancer cell lines. It inhibits CRC cell proliferation, migration, and invasion by activating the Hippo signaling pathway, indicating its potential as a therapeutic target for CRC.

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