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Research Article

MST2 Inhibits Colorectal Cancer Progression via Activating the Hippo Signaling Pathway

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ABSTRACT

Objective: To investigate the role of MST2 (mammalian sterile 20-like kinase 2) in colorectal cancer (CRC) cell proliferation, migration, invasion and its regulation of the Hippo signaling pathway.

Methods: MST2 expression in CRC cell lines (HCT116, SW480) and normal colonic epithelial cell line (NCM460) was detected by Western blot and qRT-PCR. MST2 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: MST2 was downregulated in CRC cells ($P<0.01$). MST2 overexpression reduced proliferation (OD450 at 72h: 0.60±0.05 vs. 1.25±0.09, $P<0.05$), migration (24h rate: 26.8±3.5% vs. 65.4±5.3%, $P<0.01$), invasion (cell number: 35±4 vs. 115±7, $P<0.01$), upregulated p-LATS1 and p-YAP1 ($P<0.05$) and downregulated YAP1 ($P<0.05$). MST2 knockdown showed opposite effects.

Conclusion: MST2 exerts tumor-suppressive effects in CRC via activating the Hippo pathway, serving as a potential therapeutic target.

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

Introduction

Colorectal cancer (CRC) is a leading cause of cancer-related mortality, with ~935,000 annual deaths globally[1]. The Hippo signaling pathway is a key regulator of cell growth and tumorigenesis and its dysregulation drives CRC progression[2,3]. MST2, a core upstream kinase of the Hippo pathway, phosphorylates and activates LATS1, which further phosphorylates YAP1 to inhibit its oncogenic activity[4]. MST2 is downregulated in liver, pancreatic and gastric cancers, correlating with poor prognosis[5-7]. However, MST2's functional role in CRC remains understudied. This study explores MST2'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

MST2 overexpression plasmid (pcDNA3.1-MST2) and negative control plasmid (pcDNA3.1) were obtained from Addgene (Cambridge, MA, USA). MST2 siRNA (si-MST2) 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. MST2 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). MST2 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 MST2, 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

MST2 is downregulated in CRC cell lines

qRT-PCR results showed that MST2 mRNA expression in HCT116 and SW480 cells was 0.25±0.03 and 0.32±0.04 folds of that in NCM460 cells, respectively ($P<0.01$). Western blot analysis revealed that MST2 protein relative gray values in HCT116 (0.28±0.03) and SW480 (0.35±0.04) cells were significantly lower than that in NCM460 cells (1.00±0.09, $P<0.01$).

MST2 inhibits CRC cell proliferation

MST2 overexpression reduced the OD450 value of HCT116 cells at 48h (0.52±0.06 vs. 0.89±0.07, $P<0.05$) and 72h (0.60±0.05 vs. 1.25±0.09, $P<0.05$). In contrast, MST2 knockdown increased the OD450 value at 48h (1.05±0.08 vs. 0.87±0.06, $P<0.05$) and 72h (1.36±0.10 vs. 1.23±0.08, $P<0.05$).

MST2 suppresses CRC cell migration

Scratch wound healing assay showed that the migration rate of HCT116 cells in the MST2 overexpression group was 26.8±3.5% at 24h, significantly lower than that in the control group (65.4±5.3%, $P<0.01$). MST2 knockdown increased the migration rate to 73.2±5.8%, which was higher than that in the si-NC group (64.1±5.1%, $P<0.01$).

MST2 inhibits CRC cell invasion

Transwell invasion assay revealed that the number of invasive HCT116 cells in the MST2 overexpression group was 35±4, significantly less than that in the control group (115±7, $P<0.01$). MST2 knockdown increased the number of invasive cells to 132±9, which was more than that in the si-NC group (112±6, $P<0.01$).

MST2 activates the hippo signaling pathway

Western blot analysis showed that MST2 overexpression upregulated the relative gray values of p-LATS1 (1.95±0.17 vs. 1.00±0.08, $P<0.05$) and p-YAP1 (1.90±0.16 vs. 1.00±0.07, $P<0.05$) and downregulated YAP1 (0.36±0.04 vs. 1.00±0.08, $P<0.05$). MST2 knockdown showed opposite effects: p-LATS1 (0.48±0.05 vs. 1.00±0.08, $P<0.05$) and p-YAP1 (0.45±0.04 vs. 1.00±0.07, $P<0.05$) were downregulated and YAP1 (1.22±0.10 vs. 1.00±0.08, $P<0.05$) was upregulated.

Discussion

MST2 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, MST2 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 MST2-overexpressing cells. Limitations include lack of in vivo validation and clinical sample analysis; future studies should address these. Restoring MST2 expression may be a promising CRC therapeutic strategy[8,9].

Conclusion

MST2 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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