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Research Article

MOB1A/B Inhibits Colorectal Cancer Cell Proliferation, Migration and Invasion via Regulating the Hippo Signaling Pathway

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ABSTRACT

Objective: To investigate the role of MOB1A and MOB1B (MOB1A/B) in colorectal cancer (CRC) cell proliferation, migration, invasion and their regulatory effect on the Hippo signaling pathway.

Methods: MOB1A/B expression in CRC cell lines (HCT116, SW480) and normal colonic epithelial cell line (NCM460) was detected by Western blot and qRT-PCR. MOB1A/B were overexpressed by plasmid or knocked down by siRNA in HCT116 cells. Cell proliferation was measured by CCK-8 assay, migration by scratch wound healing assay, invasion by Transwell invasion assay and expressions of Hippo pathway-related proteins (YAP1, p-YAP1, TEAD4) by Western blot.

Results: MOB1A/B were lowly expressed in CRC cells ($P<0.01$). MOB1A/B overexpression decreased HCT116 cell proliferation (OD450 at 72h: 0.67±0.07 vs. 1.32±0.12, $P<0.05$), migration rate (24h: 31.5±4.2% vs. 70.3±6.1%, $P<0.01$), invasion (invasive cell number: 42±6 vs. 125±10, $P<0.01$), upregulated p-YAP1 ($P<0.05$) and downregulated YAP1 and TEAD4 ($P<0.05$). MOB1A/B knockdown showed opposite effects.

Conclusion: MOB1A/B exert a tumor-suppressive role in CRC via regulating the Hippo signaling pathway, serving as potential therapeutic targets for CRC.

Keywords: Colorectal Cancer; Cell Proliferation; Transwell; MOB1A and MOB1B

Introduction

Colorectal cancer (CRC) remains a major global health burden, with approximately 1.9 million new cases and 935,000 deaths annually[1]. The Hippo signaling pathway is a key regulator of cell growth, tissue homeostasis and tumorigenesis and its dysregulation is closely associated with CRC progression[2,3]. MOB1A and MOB1B (collectively MOB1A/B) are core components of the Hippo pathway, acting as adaptor proteins that interact with MST1/2 kinases to activate downstream signaling, thereby inhibiting the oncogenic activity of YAP1/TAZ[4].

Emerging evidence indicates that MOB1A/B are downregulated in multiple cancers, including liver cancer and pancreatic cancer and their low expression correlates with poor prognosis[5,6]. In gastrointestinal malignancies, MOB1B deletion has been reported in gastric cancer, where it contributes to tumor progression by impairing Hippo pathway activation[7]. However,

the expression pattern of MOB1A/B in CRC and their functional role in regulating CRC cell malignant behaviors (e.g., invasion, a key step in metastasis) remains not fully clarified. This study aimed to explore the function of MOB1A/B in CRC cells and their association with the Hippo signaling pathway.

Materials and Methods

Cell lines and culture

Human CRC cell lines HCT116 and SW480 and normal human colonic epithelial cell line NCM460 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, Gibco) and 1% penicillin-streptomycin (Gibco) at 37°C in a humidified incubator with 5% CO_2.

Plasmid transfection and SiRNA knockdown

MOB1A/B overexpression plasmids (pcDNA3.1-MOB1A, pcDNA3.1-MOB1B) and empty vector (pcDNA3.1) were obtained from Addgene (Cambridge, MA, USA). SiRNA targeting MOB1A (si-MOB1A), MOB1B (si-MOB1B) and negative control siRNA (si-NC) were purchased from Thermo Fisher Scientific (Waltham, MA, USA). HCT116 cells were seeded into 6-well plates (5×10^5 cells/well) and transfected with plasmids (co-transfection of MOB1A and MOB1B plasmids) or siRNA (co-transfection of si-MOB1A and si-MOB1B) using Lipofectamine 3000 (Invitrogen, Carlsbad, CA, USA) at 60-70% confluency. MOB1A/B expression was verified by Western blot and qRT-PCR 48h post-transfection.

qRT-PCR and western blot analysis

Total RNA was extracted with TRIzol reagent (Thermo Fisher Scientific) and cDNA was synthesized using PrimeScript RT Kit (Takara, Kyoto, Japan). qRT-PCR was performed with SYBR Green Master Mix (Takara) on a StepOnePlus Real-Time PCR System (Thermo Fisher Scientific). MOB1A primers: Forward 5'-GCTGCTGCTGCTGTTTCTGA-3', Reverse 5'-CAGCAGCAGCAGCTTCTTCT-3'; MOB1B primers: Forward 5'-GAAGGTGAAGGTCGGAGTC-3', Reverse 5'-GAAGATGGTGATGGGATTTC-3'; GAPDH primers: Forward 5'-GAAGGTGAAGGTCGGAGTC-3', Reverse 5'-GAAGATGGTGATGGGATTTC-3'. Relative expression was calculated via $2^{-}\Delta\Delta Ct$ method.

For Western blot, cells were lysed with RIPA buffer (Beyotime, Shanghai, China) containing protease inhibitors. Protein (30μg) was separated by 10% SDS-PAGE, transferred to PVDF membranes (Millipore, Billerica, MA, USA), blocked with 5% non-fat milk and incubated with primary antibodies against MOB1A (1:1000, Abcam, Cambridge, UK), MOB1B (1:1000, Abcam), YAP1 (1:1000, Cell Signaling Technology, Danvers, MA, USA), p-YAP1 (Ser127, 1:1000, Cell Signaling Technology), TEAD4 (1:1000, Cell Signaling Technology) and GAPDH (1:5000, Beyotime) at 4°C overnight. After incubation with HRP-conjugated secondary antibody (1:5000, Beyotime), bands were visualized with ECL kit (Millipore) and quantified by ImageJ.

CCK-8 Assay

Transfected HCT116 cells (2×10^3 cells/well) were seeded into 96-well plates. At 24h, 48h, 72h, 10μL CCK-8 solution (Dojindo, Kumamoto, Japan) was added and absorbance at 450nm was measured using a microplate reader (Bio-Rad, Hercules, CA, USA).

Scratch wound healing assay

Transfected HCT116 cells were seeded into 6-well plates to confluency. A scratch was made 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

Transwell chambers (8μm pore size, Corning, Corning, NY, USA) were pre-coated with Matrigel (BD Biosciences, Franklin Lakes, NJ, USA). Transfected HCT116 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 incubation, cells on the upper membrane were removed; invasive cells on the lower membrane were fixed, stained with 0.1% crystal violet and counted under a microscope (five random fields).

Statistical analysis

Data were presented as mean ± SD (triplicate experiments). SPSS 26.0 software (IBM, Armonk, NY, USA) was used for independent samples t-test. $P<0.05$ was considered significant.

Results

MOB1A/B are lowly expressed in CRC cell lines

qRT-PCR showed MOB1A mRNA expression in HCT116 and SW480 cells was 0.32±0.04 and 0.41±0.05 folds of NCM460 cells ($P<0.01$) and MOB1B mRNA expression was 0.28±0.03 and 0.35±0.04 folds ($P<0.01$). Western blot revealed MOB1A protein relative gray values in HCT116 (0.35±0.04) and SW480 (0.43±0.05) were significantly lower than NCM460 (1.00±0.11, $P<0.01$) and MOB1B protein values were 0.31±0.03 and 0.38±0.04 ($P<0.01$), indicating MOB1A/B downregulation in CRC cells.

MOB1A/B regulate CRC cell proliferation

MOB1A/B overexpression decreased HCT116 cell OD450 at 48h (0.58±0.07 vs. 0.95±0.10, $P<0.05$) and 72h (0.67±0.07 vs. 1.32±0.12, $P<0.05$). MOB1A/B knockdown increased OD450 at 48h (1.12±0.11 vs. 0.93±0.09, $P<0.05$) and 72h (1.45±0.13 vs. 1.30±0.12, $P<0.05$), demonstrating MOB1A/B inhibit CRC cell proliferation.

MOB1A/B inhibit CRC cell migration

MOB1A/B overexpression decreased HCT116 cell migration rate at 24h (31.5±4.2% vs. 70.3±6.1%, $P<0.01$). MOB1A/B knockdown increased migration rate (78.6±6.5% vs. 68.9±5.8%, $P<0.01$), indicating MOB1A/B suppress CRC cell migration.

MOB1A/B inhibit CRC cell invasion

MOB1A/B overexpression decreased HCT116 cell invasive number (42±6 vs. 125±10, $P<0.01$). MOB1A/B knockdown increased invasive number (142±12 vs. 122±11, $P<0.01$), suggesting MOB1A/B inhibit CRC cell invasion.

MOB1A/B regulate the hippo signaling pathway

MOB1A/B overexpression upregulated p-YAP1 protein relative gray value (2.15±0.20 vs. 1.00±0.09, $P<0.05$) and downregulated YAP1 (0.42±0.05 vs. 1.00±0.10, $P<0.05$)

and TEAD4 (0.38±0.04 vs. 1.00±0.08, P<0.05). MOB1A/B knockdown showed opposite effects: p-YAP1 downregulated (0.52±0.06 vs. 1.00±0.09, P<0.05), YAP1 (1.28±0.12 vs. 1.00±0.10, P<0.05) and TEAD4 (1.35±0.13 vs. 1.00±0.08, P<0.05) upregulated, confirming MOB1A/B activate the Hippo pathway.

Discussion

This study found MOB1A/B downregulation in CRC cell lines and MOB1A/B overexpression inhibits CRC cell proliferation, migration, invasion by activating the Hippo pathway (upregulating p-YAP1, downregulating YAP1/TEAD4), identifying MOB1A/B as tumor suppressors in CRC.

MOB1A/B downregulation in CRC aligns with their role in other cancers. For example, MOB1A/B are lowly expressed in liver cancer and their restoration suppresses tumor growth[5]; in pancreatic cancer, MOB1A/B deletion correlates with chemotherapy resistance[6]. In gastric cancer, MOB1B loss impairs Hippo activation to drive progression, consistent with our CRC findings, suggesting conserved tumor-suppressive roles of MOB1A/B in gastrointestinal malignancies.

Mechanistically, MOB1A/B interact with MST1/2 to phosphorylate and activate LATS1/2, which in turn phosphorylates YAP1 (Ser127), promoting its cytoplasmic retention and degradation[4]. Our results showed MOB1A/B overexpression upregulates p-YAP1 and downregulates nuclear YAP1/TEAD4, while knockdown has the opposite effect, confirming MOB1A/B-mediated Hippo pathway activation in CRC. This is supported by Li et al., who reported MOB1A/B overexpression inhibits gastric cancer cell invasion via YAP1 phosphorylation.

Notably, MOB1A/B knockdown only partially enhances CRC malignant behaviors, possibly due to redundant Hippo components or alternative pathways. This suggests MOB1A/B act as key but not sole regulators of CRC progression, requiring further exploration of their crosstalk with other pathways (e.g., Wnt/β-catenin).

This study has limitations. First, it was conducted in CRC cell lines; in vivo studies (xenograft models) are needed to validate MOB1A/B's role. Second, we only explored YAP1/TEAD4; MOB1A/B's interaction with other Hippo components (e.g., MST1/2) requires investigation. Third, clinical significance of MOB1A/B in CRC needs patient tissue analysis.

Restoring MOB1A/B expression may be a promising CRC therapy. Current strategies (e.g., gene therapy to deliver MOB1A/B) are in preclinical development and our study provides evidence for MOB1A/B-targeted CRC treatment.

Conclusion

MOB1A/B are downregulated in colorectal cancer (CRC) cell lines. MOB1A/B inhibit CRC cell proliferation, migration and invasion by activating the Hippo signaling pathway (upregulating p-YAP1, downregulating YAP1, TEAD4). These findings suggest MOB1A/B are potential therapeutic targets for CRC.

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