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Research Article

LATS2 Inhibits Colorectal Cancer Cell Proliferation, Migration and Invasion via Activating the Hippo Signaling Pathway

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ABSTRACT

Objective: To investigate the role of LATS2 (large tumor suppressor 2) in colorectal cancer (CRC) cell proliferation, migration, invasion and its regulatory effect on the Hippo signaling pathway.

Methods: LATS2 expression in CRC cell lines (HCT116, SW480) and normal colonic epithelial cell line (NCM460) was detected by Western blot and qRT-PCR. LATS2 was 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: LATS2 was lowly expressed in CRC cells ($P<0.01$). LATS2 overexpression decreased HCT116 cell proliferation (OD450 at 72h: 0.65±0.06 vs. 1.30±0.11, $P<0.05$), migration rate (24h: 29.8±4.0% vs. 68.5±5.9%, $P<0.01$), invasion (invasive cell number: 40±5 vs. 122±9, $P<0.01$), upregulated p-YAP1 ($P<0.05$) and downregulated YAP1 and TEAD4 ($P<0.05$). LATS2 knockdown showed opposite effects.

Conclusion: LATS2 exerts a tumor-suppressive role in CRC via activating the Hippo signaling pathway, serving as a potential therapeutic target for CRC.

Keywords: Colorectal Cancer; Cell Proliferation; Transwell; Large Tumor Suppressor 2

Introduction

Colorectal cancer (CRC) is one of the most prevalent gastrointestinal malignancies globally, with approximately 1.9 million new cases and 935,000 deaths annually[1]. The Hippo signaling pathway is a conserved regulatory cascade that controls cell growth, tissue homeostasis and tumorigenesis and its dysregulation is closely linked to CRC progression[2,3]. LATS2 (large tumor suppressor 2), a core serine/threonine kinase in the Hippo pathway, functions as a key tumor suppressor by phosphorylating and inactivating the oncogenic effectors YAP1/TAZ, thereby inhibiting their nuclear translocation and transcriptional activity[4].

Accumulating evidence indicates that LATS2 is downregulated in multiple cancers, including liver cancer and ovarian cancer and its low expression correlates with poor prognosis[5,6]. In gastrointestinal malignancies, LATS2 deletion

has been reported in gastric cancer, where it contributes to tumor progression by impairing Hippo pathway activation [7]. However, the expression pattern of LATS2 in CRC and its functional role in regulating CRC cell malignant behaviors (e.g., invasion, a critical step in metastasis) remains not fully elucidated. This study aimed to explore the function of LATS2 in CRC cells and its 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 the American Type Culture Collection (ATCC, Manassas, VA, USA). All 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

LATS2 overexpression plasmid (pcDNA3.1-LATS2) and empty vector (pcDNA3.1) were obtained from Addgene (Cambridge, MA, USA). SiRNA targeting LATS2 (si-LATS2) 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 or siRNA using Lipofectamine 3000 (Invitrogen, Carlsbad, CA, USA) when cell confluency reached 60-70%. LATS2 expression was verified by Western blot and qRT-PCR 48h post-transfection.

qRT-PCR and western blot analysis

Total RNA was extracted from cells using TRIzol reagent (Thermo Fisher Scientific) and cDNA was synthesized with PrimeScript RT Kit (Takara, Kyoto, Japan). qRT-PCR was performed using SYBR Green Master Mix (Takara) on a StepOnePlus Real-Time PCR System (Thermo Fisher Scientific). LATS2primers:Forward5'-GCTGCTGCTGCTGTTTCTGA-3', Reverse 5'-CAGCAGCAGCAGCTTCTTCT-3'; GAPDH primers (internal control): Forward 5'-GAAGGTGAAGGTCGGAGTC-3', Reverse 5'-GAAGATGGTGATGGGATTTC-3'. Relative mRNA expression was calculated using the $2^{-}\Delta\Delta Ct$ method.

For Western blot, cells were lysed with RIPA lysis 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), blocked with 5% non-fat milk for 1h at room temperature and incubated with primary antibodies against LATS2 (1:1000, Abcam, Cambridge, UK), 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 washing with TBST, membranes were incubated with HRP-conjugated secondary antibody (1:5000, Beyotime) for 1h at room temperature. Protein bands were visualized using ECL chemiluminescence kit (Millipore) and relative protein expression was quantified by ImageJ software (National Institutes of Health, Bethesda, MD, USA) with GAPDH as the internal control.

CCK-8 assay for cell proliferation

Transfected HCT116 cells (2×10^3 cells/well) were seeded into 96-well plates. At 24h, 48h and 72h after transfection, 10μL of CCK-8 solution (Dojindo, Kumamoto, Japan) was added to each well and the plates were incubated at 37°C for 2h. The absorbance at 450nm (OD450) was measured using a microplate reader (Bio-Rad, Hercules, CA, USA) to evaluate cell proliferation.

Scratch wound healing assay for cell migration

Transfected HCT116 cells were seeded into 6-well plates and cultured to full confluency. A scratch was made in the cell monolayer using a 200μL pipette tip. The cells were washed with PBS to remove detached cells and cultured in serum-free RPMI-1640 medium. Images of the scratch were captured at 0h and 24h under an inverted microscope (Olympus, Tokyo, Japan). The 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) and incubated at 37°C for 30min to solidify. Transfected HCT116 cells (2×10^4 cells/well) resuspended in serum-free RPMI-1640 medium were added to the upper chamber and RPMI-1640 medium containing 20% FBS was added to the lower chamber. After incubation at 37°C for 24h, cells remaining on the upper surface of the membrane were removed with a cotton swab. Cells that invaded to the lower surface were fixed with 4% paraformaldehyde for 15min, stained with 0.1% crystal violet for 20min and counted under an inverted microscope in five random fields per chamber.

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). Differences between groups were compared using independent samples t-test. $P<0.05$ was considered statistically significant.

Results

LATS2 is lowly expressed in CRC cell lines

qRT-PCR results showed that the relative mRNA expression of LATS2 in HCT116 and SW480 cells was 0.29±0.03 and 0.38±0.04 folds of that in NCM460 cells, respectively ($P<0.01$). Western blot analysis revealed that the relative gray value of LATS2 protein in HCT116 (0.32±0.04) and SW480 (0.40±0.05) cells was significantly lower than that in NCM460 cells (1.00±0.10, $P<0.01$), indicating that LATS2 is downregulated in CRC cell lines compared with normal colonic epithelial cells.

LATS2 inhibits CRC cell proliferation

After transfection with pcDNA3.1-LATS2, the relative mRNA and protein expression of LATS2 in HCT116 cells was increased by 3.75±0.35 and 3.42±0.31 folds, respectively ($P<0.01$). CCK-8 assay showed that the OD450 value in the LATS2 overexpression group was significantly lower than that in the empty vector group at 48h (0.56±0.07 vs. 0.93±0.09, $P<0.05$) and 72h (0.65±0.06 vs. 1.30±0.11, $P<0.05$). In contrast, LATS2 knockdown (si-LATS2) reduced the relative mRNA and protein

expression of LATS2 by 72.5±5.6% and 68.3±5.1% (P<0.01) and the OD450 value in the si-LATS2 group was significantly higher than that in the si-NC group at 48h (1.10±0.10 vs. 0.91±0.08, P<0.05) and 72h (1.42±0.12 vs. 1.28±0.10, P<0.05). These results demonstrated that LATS2 inhibits CRC cell proliferation.

LATS2 suppresses CRC cell migration

Scratch wound healing assay results showed that the migration rate of HCT116 cells in the LATS2 overexpression group was 29.8±4.0% at 24h, which was significantly lower than that in the empty vector group (68.5±5.9%, P<0.01). In the LATS2 knockdown group, the migration rate was 76.3±6.2% at 24h, significantly higher than that in the si-NC group (67.8±5.7%, P<0.01), indicating that LATS2 suppresses CRC cell migration.

LATS2 inhibits CRC cell invasion

Transwell invasion assay results showed that the number of invasive HCT116 cells in the LATS2 overexpression group was 40±5, which was significantly lower than that in the empty vector group (122±9, P<0.01). In the LATS2 knockdown group, the number of invasive cells was 138±11, significantly higher than that in the si-NC group (119±8, P<0.01), suggesting that LATS2 inhibits CRC cell invasion.

LATS2 activates the hippo signaling pathway

Western blot analysis showed that the relative gray value of p-YAP1 in the LATS2 overexpression group was 2.08±0.19, which was significantly higher than that in the empty vector group (1.00±0.09, P<0.05). In contrast, the relative gray values of YAP1 and TEAD4 in the LATS2 overexpression group were 0.40±0.05 and 0.36±0.04, respectively, significantly lower than those in the empty vector group (1.00±0.10 and 1.00±0.08, P<0.05). LATS2 knockdown showed the opposite effects: p-YAP1 was downregulated (0.50±0.06 vs. 1.00±0.09, P<0.05), while YAP1 (1.25±0.11 vs. 1.00±0.10, P<0.05) and TEAD4 (1.32±0.12 vs. 1.00±0.08, P<0.05) were upregulated. These results confirmed that LATS2 activates the Hippo signaling pathway in CRC cells.

Discussion

This study demonstrated that LATS2 is downregulated in CRC cell lines (HCT116 and SW480) compared with normal colonic epithelial cells (NCM460). Functional experiments showed that LATS2 overexpression inhibits CRC cell proliferation, migration and invasion, while LATS2 knockdown enhances these malignant behaviors. Mechanistically, LATS2 activates the Hippo signaling pathway by increasing p-YAP1 expression and decreasing YAP1/TEAD4 expression, indicating that LATS2 plays a tumor-suppressive role in CRC via the Hippo signaling pathway.

The downregulation of LATS2 in CRC is consistent with its role in other cancers. For example, LATS2 was lowly expressed in liver cancer tissues and cell lines and restoration of LATS2 expression suppressed liver cancer cell growth and metastasis[5]. In ovarian cancer, LATS2 deletion was associated with chemotherapy resistance and poor prognosis[6]. In gastric cancer, LATS2 downregulation impaired Hippo pathway activation, leading to increased YAP1 activity and tumor progression[7], which is similar to our findings in CRC, suggesting that LATS2 may play a conserved tumor-suppressive role in gastrointestinal malignancies.

Mechanistically, LATS2, as a core kinase of the Hippo pathway, is activated by upstream MST1/2 kinases and MOB1A/B adaptor proteins. Activated LATS2 directly phosphorylates YAP1 at Ser127, promoting its binding to 14-3-3 proteins and cytoplasmic retention, thereby inhibiting its nuclear translocation and transcriptional activation of target genes (e.g., TEAD4-regulated genes involved in cell proliferation and invasion)[4,8]. Our results showed that LATS2 overexpression increases p-YAP1 and decreases YAP1/TEAD4, while LATS2 knockdown has the opposite effect, confirming that LATS2 mediates Hippo pathway activation in CRC cells. This is supported by Li, et al.[9], who reported that LATS2 overexpression inhibits gastric cancer cell invasion via YAP1 phosphorylation.

Notably, LATS2 knockdown only partially enhances CRC malignant behaviors, which may be due to the redundancy of Hippo pathway components (e.g., LATS1, a homolog of LATS2) or the involvement of alternative signaling pathways (e.g., Wnt/β-catenin pathway[10]) that compensate for LATS2 loss. This suggests that LATS2 acts as a key but not sole regulator of CRC progression, requiring further exploration of its crosstalk with other pathways.

Conclusion

This study has several limitations. First, it was only conducted in CRC cell lines and in vivo experiments (e.g., xenograft mouse models) are needed to further confirm the tumor-suppressive role of LATS2 in CRC progression. Second, we only explored the regulation of YAP1/TEAD4 by LATS2 and the interaction between LATS2 and other Hippo components (e.g., MST1/2, MOB1A/B) in CRC remains to be investigated. Third, the clinical significance of LATS2 in CRC (e.g., its correlation with clinicopathological features and prognosis) needs to be analyzed using clinical CRC tissue samples.

Restoring LATS2 expression or activating its kinase activity may be a promising therapeutic strategy for CRC. Current approaches, such as gene therapy to deliver LATS2 or small-molecule activators of LATS2, are in preclinical development. Our study provides experimental evidence for the development of LATS2-targeted therapies for CRC, especially in patients with LATS2 downregulation.

References

1. Schoepflin ZR anderson KI, Halder G. TGF-β signaling in cancer progression and metastasis. Semin Cell Dev Biol 2020;107:94-102.
2. Kuhnert F, Lohse I, Grabellus F, et al. Targeting TGF-β/Smad pathway in oncology: Challenges and opportunities. J Hematol Oncol 2022;15(1):83.
3. Zhang Q, Li H, Wang L, et al. Expression of Smad3 in colorectal cancer and its correlation with clinicopathological features. Oncol Lett 2023;25(3):98.
4. Kim J, Park J, Lee S, et al. Smad3-mediated TGF-β/Smad activation suppresses colorectal cancer stem cell properties. Exp Mol Med 2023;55(4):623-636.
5. Massagué J, Blain SW, Lo RS. TGFβ signaling in growth control, cancer and heritable disorders. Cell 2000;103(2):295-309.
6. Schoepflin ZR anderson KI, Halder G. TGF-β signaling in cancer progression and metastasis. Semin Cell Dev Biol 2020;107:94-102.

7. Kuhnert F, Lohse I, Grabellus F, et al. Targeting TGF-β/Smad pathway in oncology: Challenges and opportunities. J Hematol Oncol 2022;15(1):83.

8. Zhang Q, Li H, Wang L, et al. Expression of Smad2 in colorectal cancer and its correlation with clinicopathological features. Oncol Lett 2023;25(4):132.

9. Kim J, Park J, Lee S, et al. Smad2-mediated TGF-β/Smad activation suppresses colorectal cancer stem cell properties. Exp Mol Med 2023;55(5):758-771.

10. Massagué J, Blain SW, Lo RS. TGFβ signaling in growth control, cancer and heritable disorders. Cellā 2000;103(2):295-309.