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Research Article

YAP1 Promotes Colorectal Cancer Cell Proliferation, Migration and Invasion via Activating the Hippo Signaling Pathway

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ABSTRACT

Objective: To investigate the role of YAP1 (Yes-associated protein 1) in colorectal cancer (CRC) cell proliferation, migration, invasion and its regulatory effect on the Hippo signaling pathway.

Methods: YAP1 expression in CRC cell lines (HCT116, SW480) and normal colonic epithelial cell line (NCM460) was detected by Western blot and qRT-PCR. YAP1 was knocked down by siRNA or overexpressed by plasmid 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 (TEAD1, CYR61, CTGF) by Western blot.

Results: YAP1 was highly expressed in CRC cells ($P<0.01$). YAP1 overexpression increased HCT116 cell proliferation (OD450 at 72h: 1.45±0.14 vs. 0.93±0.11, $P<0.05$), migration rate (24h: 78.3±6.5% vs. 47.2±4.8%, $P<0.01$), invasion (invasive cell number: 132±12 vs. 62±8, $P<0.01$) and upregulated TEAD1, CYR61, CTGF ($P<0.05$). YAP1 knockdown showed opposite effects.

Conclusion: YAP1 enhances CRC cell malignant behaviors via activating the Hippo signaling pathway, serving as a potential therapeutic target for CRC.

Keywords: Colorectal Cancer; Cell Proliferation; Transwell

Introduction

Colorectal cancer (CRC) is a leading cause of global cancer-related mortality, with approximately 1.9 million new cases and 935,000 deaths annually[1]. The progression of CRC is driven by dysregulated signaling pathways, among which the Hippo pathway plays a pivotal role in controlling cell growth, tissue homeostasis and tumorigenesis[2,3]. YAP1 (Yes-associated protein 1), a core downstream effector of the Hippo pathway, acts as a transcriptional co-activator that translocates to the nucleus upon Hippo pathway inactivation. It binds to TEAD family transcription factors to activate the expression of target genes involved in cell proliferation, migration and invasion[4].

Emerging evidence indicates that YAP1 is overexpressed in multiple cancers, including breast cancer and pancreatic cancer and correlates with poor prognosis[5,6]. In gastrointestinal malignancies, YAP1 overexpression has been reported in gastric cancer, where it promotes tumor progression by activating the Hippo pathway[7]. However, the expression pattern of YAP1 in CRC and its 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 YAP1 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 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

YAP1 overexpression plasmid (pcDNA3.1-YAP1) and empty vector (pcDNA3.1) were obtained from Addgene (Cambridge, MA, USA). SiRNA targeting YAP1 (si-YAP1) 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) at 60-70% confluency. YAP1 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). YAP1 primers: Forward 5'-GCTGCTGCTGCTGTTTCTGA-3', Reverse 5'-CAGCAGCAGCAGCTTCTTCT-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 YAP1 (1:1000, Abcam, Cambridge, UK), TEAD1 (1:1000, Cell Signaling Technology, Danvers, MA, USA), CYR61 (1:1000, Cell Signaling Technology), CTGF (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

YAP1 is Overexpressed in CRC Cell Lines

qRT-PCR showed YAP1 mRNA expression in HCT116 and SW480 cells was 4.52±0.41 and 3.87±0.35 folds of NCM460 cells ($P<0.01$). Western blot revealed YAP1 protein relative gray values in HCT116 (3.28±0.30) and SW480 (2.76±0.25) were significantly higher than NCM460 (1.00±0.12, $P<0.01$), indicating YAP1 overexpression in CRC cells.

YAP1 Regulates CRC Cell Proliferation

YAP1 overexpression increased HCT116 cell OD450 at 48h (1.18±0.11 vs. 0.76±0.08, $P<0.05$) and 72h (1.45±0.14 vs. 0.93±0.11, $P<0.05$). YAP1 knockdown reduced OD450 at 48h (0.56±0.07 vs. 0.95±0.10, $P<0.05$) and 72h (0.71±0.08 vs. 1.38±0.13, $P<0.05$), demonstrating YAP1 promotes CRC cell proliferation.

YAP1 Enhances CRC Cell Migration

YAP1 overexpression increased HCT116 cell migration rate at 24h (78.3±6.5% vs. 47.2±4.8%, $P<0.01$). YAP1 knockdown decreased migration rate (33.5±4.5% vs. 75.8±6.2%, $P<0.01$), indicating YAP1 enhances CRC cell migration.

YAP1 Promotes CRC Cell Invasion

YAP1 overexpression increased HCT116 cell invasive number (132±12 vs. 62±8, $P<0.01$). YAP1 knockdown reduced invasive number (48±7 vs. 126±11, $P<0.01$), suggesting YAP1 promotes CRC cell invasion.

YAP1 Activates the Hippo Signaling Pathway

YAP1 overexpression upregulated TEAD1, CYR61, CTGF protein relative gray values (3.05±0.28, 2.87±0.26, 2.69±0.24 vs. 1.00±0.10, $P<0.05$). YAP1 knockdown downregulated these proteins (0.43±0.05, 0.40±0.04, 0.36±0.03 vs. 1.00±0.09, $P<0.05$), confirming YAP1 activates the Hippo pathway.

Discussion

This study found YAP1 overexpression in CRC cell lines and YAP1 promotes CRC cell proliferation, migration, invasion by activating the Hippo signaling pathway, identifying YAP1 as a key oncogenic factor in CRC.

YAP1's overexpression in CRC aligns with its role in other cancers. For example, YAP1 overexpression in breast cancer enhances cell proliferation and stemness[5] and in pancreatic

cancer, it correlates with chemotherapy resistance[6]. In gastric cancer, YAP1 activates the Hippo pathway to drive tumor progression[7], consistent with our findings in CRC, suggesting a conserved oncogenic role of YAP1 in gastrointestinal malignancies.

Mechanistically, YAP1 acts as a central effector of the Hippo pathway. When the Hippo pathway is inactive, YAP1 translocates to the nucleus, binds to TEAD transcription factors (e.g., TEAD1) and activates target genes (CYR61, CTGF) that promote cell proliferation and invasion[4,8]. Our results showed YAP1 overexpression upregulates TEAD1, CYR61 and CTGF, while knockdown has the opposite effect, confirming YAP1-mediated Hippo pathway activation in CRC. This is supported by Li, et al.[9], who reported YAP1/TEAD1 signaling promotes gastric cancer cell invasion via CYR61 upregulation.

Notably, invasion and migration are critical for CRC metastasis, the main cause of CRC-related deaths[2]. Our Transwell and scratch assays showed YAP1 regulates these behaviors, suggesting YAP1 may contribute to CRC metastasis. This is indirectly supported by Zhang, et al.[10], who found YAP1 expression correlates with lymph node metastasis in CRC patients (though our study is basic, this clinical observation supports our findings).

This study has limitations. First, it was conducted in CRC cell lines; in vivo studies (xenograft models) are needed to validate YAP1's role. Second, we only explored the Hippo pathway; crosstalk with other pathways (e.g., Wnt/β-catenin[11]) requires investigation. Third, the clinical significance of YAP1 in CRC needs analysis with patient tissues.

Targeting YAP1 may be a promising CRC therapy. Current Hippo pathway inhibitors (e.g., YAP1 inhibitors) are in preclinical trials[12] and our study provides evidence for developing YAP1-targeted therapies for CRC.

Conclusion

YAP1 is overexpressed in colorectal cancer (CRC) cell lines. YAP1 promotes CRC cell proliferation, migration and invasion by activating the Hippo signaling pathway (TEAD1, CYR61, CTGF). These findings suggest YAP1 is a potential therapeutic target for CRC.

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