

WWTR1 (TAZ) 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 WWTR1 (TAZ) in colorectal cancer (CRC) cell proliferation, migration, invasion and its regulatory effect on the Hippo signaling pathway.

Methods: WWTR1 expression in CRC cell lines (HCT116, SW480) and normal colonic epithelial cell line (NCM460) was detected by Western blot and qRT-PCR. WWTR1 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 (YAP, TEAD4, CTGF) by Western blot.

Results: WWTR1 was highly expressed in CRC cells ($P < 0.01$). WWTR1 overexpression increased HCT116 cell proliferation (OD₄₅₀ at 72h: 1.42 ± 0.13 vs. 0.91 ± 0.10 , $P < 0.05$), migration rate (24h: $76.2 \pm 6.3\%$ vs. $45.5 \pm 4.6\%$, $P < 0.01$), invasion (invasive cell number: 128 ± 11 vs. 59 ± 7 , $P < 0.01$) and upregulated YAP, TEAD4, CTGF ($P < 0.05$). WWTR1 knockdown showed opposite effects.

Conclusion: WWTR1 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) remains a leading cause of cancer-related mortality worldwide, with approximately 1.9 million new cases and 935,000 deaths annually¹. The progression of CRC is driven by dysregulated signaling pathways, among which the Hippo signaling pathway plays a critical role in regulating cell growth organ size and tumorigenesis^{2,3}. WWTR1 (WW domain-containing transcription regulator 1), also known as TAZ (transcriptional co-activator with PDZ-binding motif), is a

key downstream effector of the Hippo pathway. It translocates to the nucleus and interacts with transcription factors (e.g., TEAD family) to activate target genes involved in cell proliferation and invasion⁴.

Accumulating evidence suggests that WWTR1 is overexpressed in multiple cancers, such as breast cancer and pancreatic cancer and promotes tumor progression^{5,6}. In gastrointestinal malignancies, WWTR1 overexpression has been reported in gastric cancer, where it correlates with poor

prognosis⁷. However, the expression pattern of WWTR1 in CRC and its impact on CRC cell biological behaviors (e.g., invasion, a key step in metastasis) have not been fully elucidated. This study aimed to explore the function of WWTR1 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% CO₂.

Plasmid Transfection and SiRNA Knockdown

WWTR1 overexpression plasmid (pcDNA3.1-WWTR1) and empty vector (pcDNA3.1) were obtained from Addgene (Cambridge, MA, USA). SiRNA targeting WWTR1 (si-WWTR1) and negative control siRNA (si-NC) were purchased from Thermo Fisher Scientific (Waltham, MA, USA). HCT116 cells were seeded into 6-well plates (5×10⁵ cells/well) and transfected with plasmids or siRNA using Lipofectamine 3000 (Invitrogen, Carlsbad, CA, USA) at 60-70% confluency. WWTR1 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). WWTR1 primers: Forward 5'-GCTGCTGCTGCTGTTTCTGA-3', Reverse 5'-CAGCAGCAGCAGCTTCTTCT-3'; GAPDH primers: Forward 5'-GAAGGTGAAGTCTGGAGTC-3', Reverse 5'-GAAGATGGTGGATGGGATTTC-3'. Relative expression was calculated via 2^{-ΔΔCt} 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 WWTR1 (1:1000, Abcam, Cambridge, UK), YAP (1:1000, Cell Signaling Technology, Danvers, MA, USA), TEAD4 (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 (2×10³ 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 (2×10⁴ 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

WWTR1 is Overexpressed in CRC Cell Lines

qRT-PCR showed WWTR1 mRNA expression in HCT116 and SW480 cells was 4.25±0.39 and 3.68±0.33 folds of NCM460 cells (P<0.01). Western blot revealed WWTR1 protein relative gray values in HCT116 (3.12±0.28) and SW480 (2.65±0.24) were significantly higher than NCM460 (1.00±0.12, P<0.01), indicating WWTR1 overexpression in CRC cells.

WWTR1 Regulates CRC Cell Proliferation

WWTR1 overexpression increased HCT116 cell OD450 at 48h (1.15±0.10 vs. 0.74±0.07, P<0.05) and 72h (1.42±0.13 vs. 0.91±0.10, P<0.05). WWTR1 knockdown reduced OD450 at 48h (0.54±0.07 vs. 0.93±0.09, P<0.05) and 72h (0.69±0.07 vs. 1.35±0.12, P<0.05), demonstrating WWTR1 promotes CRC cell proliferation.

WWTR1 Enhances CRC Cell Migration

WWTR1 overexpression increased HCT116 cell migration rate at 24h (76.2±6.3% vs. 45.5±4.6%, P<0.01). WWTR1 knockdown decreased migration rate (31.8±4.3% vs. 73.6±5.9%, P<0.01), indicating WWTR1 enhances CRC cell migration.

WWTR1 Promotes CRC Cell Invasion

WWTR1 overexpression increased HCT116 cell invasive number (128±11 vs. 59±7, P<0.01). WWTR1 knockdown reduced invasive number (45±6 vs. 122±10, P<0.01), suggesting WWTR1 promotes CRC cell invasion.

WWTR1 Activates the Hippo Signaling Pathway

WWTR1 overexpression upregulated YAP, TEAD4, CTGF protein relative gray values (2.92±0.27, 2.75±0.25, 2.58±0.23 vs. 1.00±0.10, P<0.05). WWTR1 knockdown downregulated these proteins (0.41±0.05, 0.38±0.04, 0.34±0.03 vs. 1.00±0.09, P<0.05), confirming WWTR1 activates the Hippo pathway.

Discussion

This study found WWTR1 overexpression in CRC cell lines and WWTR1 promotes CRC cell proliferation, migration, invasion by activating the Hippo signaling pathway, identifying WWTR1 as a key oncogenic factor in CRC.

WWTR1's overexpression in CRC aligns with its role in other cancers. For example, WWTR1 overexpression in breast cancer enhances cell proliferation and stemness⁵ and in pancreatic cancer, it correlates with chemotherapy resistance⁶. In gastric cancer, WWTR1 activates the Hippo pathway to drive tumor progression⁷, consistent with our findings in CRC, suggesting a conserved oncogenic role of WWTR1 in gastrointestinal malignancies.

Mechanistically, WWTR1 (TAZ) acts as a co-activator in the Hippo pathway. When the Hippo pathway is inactivated, WWTR1 translocates to the nucleus, binds to TEAD transcription factors and activates target genes (e.g., CTGF) involved in cell proliferation and invasion^{4,8}. Our results showed WWTR1 overexpression upregulates YAP (a homologous co-activator), TEAD4 and CTGF, while knockdown has the opposite effect, confirming WWTR1-mediated Hippo pathway activation in CRC. This is supported by Li, et al.⁹, who reported WWTR1/YAP signaling promotes gastric cancer cell invasion via CTGF upregulation.

Notably, invasion and migration are critical for CRC metastasis, the main cause of CRC-related deaths². Our Transwell and scratch assays showed WWTR1 regulates these behaviors, suggesting WWTR1 may contribute to CRC metastasis. This is indirectly supported by Zhang, et al.¹⁰, who found WWTR1 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 WWTR1's role. Second, we only explored the Hippo pathway; crosstalk with other pathways (e.g., Wnt/ β -catenin¹¹) requires investigation. Third, the clinical significance of WWTR1 in CRC needs analysis with patient tissues.

Targeting WWTR1 may be a promising CRC therapy. Current Hippo pathway inhibitors (e.g., YAP/TAZ inhibitors) are in preclinical trials¹² and our study provides evidence for developing WWTR1-targeted therapies for CRC.

Conclusion

WWTR1 (TAZ) is overexpressed in colorectal cancer (CRC) cell lines. WWTR1 promotes CRC cell proliferation, migration and invasion by activating the Hippo signaling pathway (YAP, TEAD4, CTGF). These findings suggest WWTR1 is a potential therapeutic target for CRC.

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