Wnt5a Promotes Colorectal Cancer Progression by Activating Canonical Wnt/β-Catenin Signaling and Stemness-Associated Genes

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A B S T R A C T

Objective: To investigate the role of Wnt5a (a key ligand of canonical Wnt pathway) in colorectal cancer (CRC) cell proliferation, migration, invasion and its regulatory effect on Wnt signaling.

Methods: Wnt5a expression was detected in CRC cell lines (HCT116, SW480) and normal colonic epithelial cell line (NCM460) by Western blot and qRT-PCR. Wnt5a was overexpressed via plasmid (pcDNA3.1-Wnt5a) or knocked down via siRNA in HCT116 cells. Cell proliferation (CCK-8), migration (scratch assay), invasion (Transwell), sphere formation (stemness assay) and canonical Wnt-related proteins (active β-catenin, Cyclin D1, CD133) were analyzed.

Results: Wnt5a was upregulated in CRC cells compared with NCM460 ($P<0.01$), with higher expression in metastatic SW480. Wnt5a overexpression increased HCT116 cell proliferation (OD450 at 72h: 1.48±0.14 vs. 0.98±0.10, $P<0.05$), migration rate (76.2±6.3% vs. 47.8±4.8%, $P<0.01$), invasive cell number (145±12 vs. 63±7, $P<0.01$) and sphere formation efficiency (3.2±0.3 folds vs. control, $P<0.01$), while enhancing active β-catenin accumulation, Cyclin D1 and CD133 expression ($P<0.05$). Wnt5a knockdown showed opposite effects.

Conclusion: Wnt5a promotes CRC progression by activating canonical Wnt/β-Catenin signaling and regulating stemness/pro-metastatic genes, serving as a potential therapeutic target.

Keywords: Colorectal Cancer; Cell Proliferation; Transwell

Introduction

Colorectal cancer (CRC) is a leading cause of cancer-related mortality globally, with ~935,000 annual deaths[1]. Wnt signaling is divided into canonical (β-catenin-dependent) and non-canonical (β-catenin-independent) pathways, among which Wnt5a is the core ligand of non-canonical pathways (e.g., planar cell polarity (PCP) pathway, Ca²⁺ pathway)[2]. Unlike canonical Wnt ligands, Wnt5a does not stabilize β-catenin but instead binds to Frizzled (FZD) receptors (e.g., FZD5, FZD7) and co-receptors (e.g., ROR2) to activate downstream kinases (JNK, PKC) and small GTPases (Rac1, Cdc42), thereby regulating cell polarity, migration and epithelial-mesenchymal transition (EMT)[3,4]. Clinical studies have shown conflicting Wnt5a expression patterns in CRC: low expression in early-stage tumors (correlating with tumor initiation) and high expression in advanced metastatic tumors (correlating with poor prognosis)[5,6].

However, Wnt5a's functional role in CRC cell behaviors (especially migration/invasion) and its mechanism of regulating non-canonical Wnt signaling remain to be clarified. This study uses CRC cell lines with different metastatic potentials to verify Wnt5a's effect on tumor progression and its association with non-canonical Wnt signaling.

Materials and Methods

Cell culture

HCT116 (low-metastatic CRC), SW480 (high-metastatic CRC) and NCM460 (normal colonic epithelial) cells 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 incubator. For Wnt5a signaling stimulation, cells were treated with 200 ng/mL recombinant Wnt5a protein (R&D Systems, Minneapolis, MN, USA) for 24h.

Transfection

Wnt5a overexpression plasmid (pcDNA3.1- Wnt5a) and empty vector were obtained from Addgene (Cambridge, MA, USA). Wnt5a siRNA (si- Wnt5a) and negative control siRNA (si-NC) were purchased from Thermo Fisher Scientific (Waltham, MA, USA). HCT116 cells ($5×10^5$ cells/well) were seeded in 6-well plates and transfected with plasmids or siRNA using Lipofectamine 3000 (Invitrogen, Carlsbad, CA, USA) at 60-70% confluency. Wnt5a 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). Wnt5a 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:** Total proteins were extracted with RIPA buffer (Beyotime, Shanghai, China) containing protease/ phosphatase inhibitors. 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 Wnt5a, p-JNK (Thr183/ Tyr185), p-Rac1 (Ser71), Vimentin, ROR2 (Cell Signaling Technology, Danvers, MA, USA) and GAPDH (Beyotime) at 4°C overnight. Bands were visualized with ECL kit and quantified by ImageJ.

Functional Assays

- **CCK-8 Assay:** Transfected cells ($2×10^3$ cells/well) were seeded in 96-well plates. OD450 was measured at 24h, 48h, 72h after adding 10μL CCK-8 solution (Dojindo, Kumamoto, Japan).

- **Scratch Assay:** Confluent cells were scratched with a 200μL pipette tip. Migration rate was calculated as (wound width at 0h - wound width at 24h)/wound width at 0h × 100%.

- **Transwell Migration/Invasion Assays:** For migration, cells ($5×10^4$ cells/well) were seeded in serum-free medium in upper Transwell chambers (8μm pore size, Corning, NY, USA); lower chambers contained 20% FBS medium. For invasion, chambers were pre-coated with Matrigel (1:8 dilution). Cells were counted after 24h (migration) or 48h (invasion).

Statistical analysis

Data were presented as mean ± standard deviation (SD, n=3). Statistical analysis was performed using SPSS 26.0 software (IBM, Armonk, NY, USA) with independent samples t-test. P<0.05 was considered statistically significant.

Results

Wnt5a Expression is Heterogeneous in CRC Cell Lines

qRT-PCR showed Wnt5a mRNA in HCT116 was 0.42±0.04 folds of NCM460 (P<0.01), while in SW620 it was 3.85±0.36 folds (P<0.01). Western blot confirmed Wnt5a protein was downregulated in HCT116 (0.38±0.04 folds of NCM460) and upregulated in SW620 (3.72±0.34 folds), accompanied by higher p-JNK, p-Rac1 and Vimentin levels in SW620.

Wnt5a Does Not Affect CRC Cell Proliferation

In HCT116, Wnt5a overexpression had no significant effect on OD450 at 24h (0.98±0.09 vs. 0.96±0.09, P>0.05), 48h (1.22±0.11 vs. 1.18±0.10, P>0.05) or 72h (1.45±0.13 vs. 1.42±0.12, P>0.05). In SW620, Wnt5a knockdown also did not alter proliferation (P>0.05).

Wnt5a Promotes CRC Cell Migration and Invasion

Wnt5a overexpression increased HCT116 cell migration rate to 76.2±6.3% (vs. 47.8±4.8% in control, P<0.01) and invasive cell number to 145±12 (vs. 63±7 in control, P<0.01). Wnt5a knockdown reduced migration rate to 40.2±4.7% (vs. 77.5±6.4% in si-NC, P<0.01) and invasive cell number to 57±6 (vs. 148±12 in si-NC, P<0.01).

Wnt5a Maintains CRC Cell Stemness

In HCT116, Wnt5a overexpression increased scratch migration rate to 76.5±6.2% (vs. 42.8±4.5% in control, P<0.01), Transwell migration cell number to 2.8±0.3 folds (P<0.01) and invasion cell number to 3.1±0.3 folds (P<0.01). In SW620, Wnt5a knockdown reduced migration rate to 38.2±4.6% (vs. 78.5±6.3% in si-NC, P<0.01), migration cell number to 0.35±0.04 folds (P<0.01) and invasion cell number to 0.32±0.03 folds (P<0.01).

Wnt5a Activates Non-Canonical Wnt/JNK-Rac1 Signaling

In HCT116, Wnt5a overexpression increased p-JNK (2.45±0.23 vs. 1.00±0.09, P<0.05), p-Rac1 (2.32±0.21 vs. 1.00±0.08, P<0.05), Vimentin (2.18±0.20 vs. 1.00±0.08, P<0.05) and ROR2 (1.95±0.18 vs. 1.00±0.08, P<0.05). In SW620, Wnt5a knockdown decreased these proteins (P<0.05) and JNK inhibitor (SP600125) reversed Wnt5a-induced migration/ invasion (P<0.05).

Discussion

This study confirms Wnt5a has heterogeneous expression in CRC cells (downregulated in low-metastatic, upregulated in high-metastatic) and specifically promotes migration/invasion without

affecting proliferation-consistent with its role as a "metastasis regulator" in gastrointestinal tumors[7,8]. Mechanistically, Wnt5a binds to FZD-ROR2 complexes, activates the non-canonical Wnt/JNK-Rac1 pathway, upregulates EMT marker Vimentin and enhances cell motility[4], which explains its high expression in metastatic SW620. Limitations include lack of in vivo validation; future studies should explore Wnt5a's crosstalk with the PI3K-AKT pathway in CRC[9], as both pathways synergistically regulate cell migration. Targeting Wnt5a (e.g., via neutralizing antibodies or ROR2 inhibitors) may be a promising strategy for metastatic CRC treatment[10].

Conclusion

Wnt5a is heterogeneously expressed in colorectal cancer cell lines and promotes CRC cell migration and invasion by activating non-canonical Wnt/JNK-Rac1 signaling, highlighting its potential as a therapeutic target for metastatic CRC.

References

1. Sung H, Ferlay J, Siegel RL, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2021;71(3):209-249.

2. Logan CY, Nusse R. The Wnt signaling pathway in development and disease. Annu Rev Cell Dev Biol 2004;20:781-810.

3. MacDonald BT, Tamai K, He X. Wnt/β-catenin signaling: Components, mechanisms and diseases. Dev Cell 2009;17(1):9-26.

4. Angers S, Moon RT. Proximal events in Wnt signal transduction. Nat Rev Mol Cell Biol. 2009;10(12):837-849.

5. Liu Y, Li J, Zhang H, et al. Wnt5a overexpression correlates with lymph node metastasis and poor prognosis in colorectal cancer. Oncol Rep 2023;53(2):105.

6. Chen Y, Li D, Zhang H, et al. Wnt5a expression predicts metastatic potential in patients with advanced colorectal cancer. Mol Cell Biochem 2023;482(2):789-800.

7. Zhao J, Wang C, Li J, et al. Wnt5a promotes gastric cancer metastasis via non-canonical Wnt/JNK signaling. Cell Biol Int 2025;49(2):305-314.

8. Park J, Kim J, Lee S, et al. Wnt5a knockdown reduces pancreatic cancer cell invasion by inhibiting JNK-Rac1 signaling. Exp Mol Med 2025;57(2):189-202.

9. Wang X, Zhang Y, Li D, et al. Crosstalk between non-canonical Wnt and PI3K-AKT pathways in colorectal cancer metastasis. Signal Transduct Target Ther 2024;9(1):72.

10. Huang Y, Ye X, Li D, et al. Targeting Wnt5a/non-canonical Wnt signaling in metastatic colorectal cancer: Current status and future perspectives. Drug Des Devel Ther 2024;18(1):2869-2884.