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Research Article

Notch3 Promotes Colorectal Cancer Cell Proliferation, Migration and Invasion via Activating the Notch Signaling Pathway

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ABSTRACT

Objective: To explore the role of Notch3 in colorectal cancer (CRC) cell proliferation, migration, invasion, and its regulatory effect on the Notch signaling pathway.

Methods: Notch3 expression in CRC cell lines (SW620, HT-29) and normal colonic epithelial cell line (NCM460) was detected by Western blot and qRT-PCR. Notch3 was knocked down by siRNA or overexpressed by plasmid in SW620 cells. Cell proliferation was measured by CCK-8 assay, migration by scratch wound healing assay, invasion by Transwell invasion assay, and expressions of Notch pathway-related proteins (NICD3, Hes1, Hey2) by Western blot.

Results: Notch3 was highly expressed in CRC cells ($P<0.01$). Notch3 overexpression increased SW620 cell proliferation (OD450 at 72h: 1.41±0.13 vs. 0.92±0.10, $P<0.05$), migration rate (24h: 75.3±6.1% vs. 45.2±4.5%, $P<0.01$), invasion (invasive cell number: 125±10 vs. 58±7, $P<0.01$), and upregulated NICD3, Hes1, Hey2 ($P<0.05$). Notch3 knockdown showed opposite effects.

Conclusion: Notch3 enhances CRC cell malignant behaviors via activating the Notch 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 cancer-related mortality globally, with over 1.9 million new cases and 935,000 deaths annually[1]. The progression of CRC is driven by dysregulated signaling pathways, among which the Notch pathway plays a pivotal role in cell fate regulation, including proliferation, differentiation, and invasion[2,3]. The Notch family comprises four receptors (Notch1-4), and while Notch1 and Notch2 have been extensively studied in CRC, the functional role of Notch3 in CRC remains under investigated.

Notch3 is critical for vascular smooth muscle cell development and has been implicated in multiple cancers, such as lung cancer and pancreatic cancer, where it promotes tumor progression[4,5]. In gastrointestinal malignancies, Notch3 overexpression has been reported in esophageal cancer, correlating with poor prognosis[6]. However, the expression pattern of Notch3 in CRC and its impact on CRC cell biological behaviors (e.g., invasion, a key step in metastasis) have not been fully clarified. This study aimed to investigate the function of Notch3 in CRC cells and its association with the Notch signaling pathway.

Materials and Methods

Cell lines and culture

Human CRC cell lines SW620 and HT-29, 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

Notch3 overexpression plasmid (pcDNA3.1-Notch3) and empty vector (pcDNA3.1) were obtained from Addgene (Cambridge, MA, USA). SiRNA targeting Notch3 (si-Notch3) and negative control siRNA (si-NC) were purchased from Thermo Fisher Scientific (Waltham, MA, USA). SW620 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. Notch3 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). Notch3 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 Notch3 (1:1000, Abcam, Cambridge, UK), NICD3 (1:1000, Cell Signaling Technology, Danvers, MA, USA), Hes1 (1:1000, Cell Signaling Technology), Hey2 (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 SW620 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 SW620 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 SW620 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

Notch3 is Overexpressed in CRC Cell Lines

qRT-PCR showed Notch3 mRNA expression in SW620 and HT-29 cells was 4.12±0.38 and 3.56±0.32 folds of NCM460 cells ($P<0.01$). Western blot revealed Notch3 protein relative gray values in SW620 (3.02±0.27) and HT-29 (2.58±0.23) were significantly higher than NCM460 (1.00±0.12, $P<0.01$), indicating Notch3 overexpression in CRC cells.

Notch3 Regulates CRC Cell Proliferation

Notch3 overexpression increased SW620 cell OD450 at 48h (1.12±0.10 vs. 0.75±0.08, $P<0.05$) and 72h (1.41±0.13 vs. 0.92±0.10, $P<0.05$). Notch3 knockdown reduced OD450 at 48h (0.53±0.07 vs. 0.91±0.09, $P<0.05$) and 72h (0.68±0.07 vs. 1.32±0.11, $P<0.05$), demonstrating Notch3 promotes CRC cell proliferation.

Notch3 Enhances CRC Cell Migration

Notch3 overexpression increased SW620 cell migration rate at 24h (75.3±6.1% vs. 45.2±4.5%, $P<0.01$). Notch3 knockdown decreased migration rate (30.5±4.2% vs. 72.1±5.8%, $P<0.01$), indicating Notch3 enhances CRC cell migration.

Notch3 Promotes CRC Cell Invasion

Notch3 overexpression increased SW620 cell invasive number (125±10 vs. 58±7, $P<0.01$). Notch3 knockdown reduced invasive number (42±6 vs. 118±9, $P<0.01$), suggesting Notch3 promotes CRC cell invasion.

Notch3 Activates the Notch Signaling Pathway

Notch3 overexpression upregulated NICD3, Hes1, Hey2 protein relative gray values (2.85±0.26, 2.63±0.24, 2.45±0.22 vs. 1.00±0.10, $P<0.05$). Notch3 knockdown downregulated these proteins (0.38±0.05, 0.35±0.04, 0.31±0.03 vs. 1.00±0.09, $P<0.05$), confirming Notch3 activates the Notch pathway.

Discussion

This study found Notch3 overexpression in CRC cell lines, and Notch3 promotes CRC cell proliferation, migration, invasion by activating the Notch signaling pathway, identifying Notch3 as a key oncogenic factor in CRC.

Notch3's overexpression in CRC aligns with its role in other cancers. For example, Notch3 overexpression in lung cancer enhances cell proliferation and invasion[4], and in pancreatic cancer, it correlates with chemotherapy resistance[5]. In esophageal cancer, Notch3 activates the Notch pathway to drive tumor progression[6], consistent with our findings in CRC, suggesting

a conserved oncogenic role of Notch3 in gastrointestinal malignancies.

Mechanistically, Notch3 activation involves cleavage to release NICD3, which translocates to the nucleus and forms a complex with CSL to activate target genes (Hes1, Hey2)[7,8]. Our results showed Notch3 overexpression upregulates NICD3, Hes1, Hey2, while knockdown has the opposite effect, confirming Notch3-mediated Notch pathway activation in CRC. This is supported by Li, et al.[9], who reported Notch3/NICD3 signaling promotes gastric cancer cell invasion via Hes1 upregulation.

Notably, invasion and migration are critical for CRC metastasis, the main cause of CRC-related deaths[2]. Our Transwell and scratch assays showed Notch3 regulates these behaviors, suggesting Notch3 may contribute to CRC metastasis. This is indirectly supported by Zhang, et al.[10], who found Notch3 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 Notch3's role. Second, we only explored the Notch pathway; crosstalk with other pathways (e.g., PI3K/Akt[11]) requires investigation. Third, the clinical significance of Notch3 in CRC needs analysis with patient tissues.

Targeting Notch3 may be a promising CRC therapy. Current Notch inhibitors (γ-secretase inhibitors) have off-target effects[12], while Notch3-specific inhibitors could improve specificity. Our study provides evidence for developing Notch3-targeted therapies for CRC.

Conclusion

Notch3 is overexpressed in colorectal cancer (CRC) cell lines. Notch3 promotes CRC cell proliferation, migration, and invasion by activating the Notch signaling pathway (NICD3, Hes1, Hey2). These findings suggest Notch3 is a potential therapeutic target for CRC.

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