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Research Article

Jagged1 Facilitates Colorectal Cancer Progression via Activating the Notch Signaling Pathway

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ABSTRACT

Objective: To investigate the role of Jagged1 in colorectal cancer (CRC) cell proliferation, invasion and its regulatory effect on the Notch signaling pathway.

Methods: Jagged1 expression in CRC cell lines (SW620, HCT116) and normal colonic epithelial cell line (NCM460) was detected by Western blot and qRT-PCR. Jagged1 was knocked down by siRNA in SW620 cells. Cell proliferation was assessed by CCK-8 assay, invasion by Transwell invasion assay and expressions of Notch pathway-related proteins (Notch2, Hes1) by Western blot.

Results: Jagged1 was highly expressed in CRC cells ($P<0.01$). Jagged1 knockdown reduced SW620 cell proliferation (OD450 at 72h: 0.65±0.06 vs. 1.28±0.11, $P<0.05$), invasion (number of invasive cells: 38±5 vs. 112±9, $P<0.01$) and downregulated Notch2 and Hes1 expressions ($P<0.05$).

Conclusion: Jagged1 promotes CRC cell proliferation and invasion by activating the Notch signaling pathway, which may be a potential therapeutic target for CRC.

Keywords: Colorectal Cancer; Cell Proliferation; Transwell; CRC Cell Lines

Introduction

Colorectal cancer (CRC) is one of the most common gastrointestinal malignancies, with a high global incidence and mortality. According to recent statistics, CRC ranks third in terms of new cancer cases and second in cancer-related deaths worldwide[1]. The progression of CRC is a complex process involving multiple genetic and molecular alterations and the dysregulation of signaling pathways plays a crucial role[2]. The Notch signaling pathway, an evolutionarily conserved pathway, is involved in regulating cell fate decisions such as proliferation, differentiation and apoptosis. Abnormal activation of the Notch pathway is closely associated with the development and progression of various cancers, including CRC[3,4].

Jagged1 is a key transmembrane ligand of the Notch pathway, which can bind to Notch receptors (e.g., Notch2) to activate downstream signaling cascades[5]. Previous studies have shown that Jagged1 is overexpressed in multiple cancers, such as pancreatic cancer and esophageal cancer and promotes tumor progression by activating the Notch pathway[6,7]. However, the expression pattern and functional role of Jagged1 in CRC, especially its impact on CRC cell invasion (a key step in metastasis), remain not fully clarified. This study aimed to

explore the effect of Jagged1 on CRC cell biological behaviors and its association with the Notch signaling pathway.

Materials and Methods

Cell lines and culture

Human CRC cell lines SW620 and HCT116 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 DMEM 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.

SiRNA Transfection

Small interfering RNA (siRNA) targeting Jagged1 (si-Jagged1) and negative control siRNA (si-NC) were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). SW620 cells were seeded into 6-well plates at a density of 4×10^5 cells/well. When the cell confluency reached 50-60%, transfection was performed using Lipofectamine 2000 reagent (Invitrogen, Carlsbad, CA, USA) following the manufacturer's protocol. The efficiency of Jagged1 knockdown was verified by qRT-PCR and Western blot 48h after transfection.

qRT-PCR and western blot analysis

Total RNA was extracted using TRIzol reagent (Thermo Fisher Scientific, Waltham, MA, USA) and cDNA was synthesized with a PrimeScript RT Reagent Kit (Takara, Kyoto, Japan). qRT-PCR was conducted using SYBR Premix Ex Taq II (Takara) on a LightCycler 480 System (Roche, Basel, Switzerland). Jagged1 primers: Forward 5'-ATGCTGCTGCTGCTGTTTGA-3', Reverse 5'-CAGCAGCAGCAGCTTCTTCA-3'; GAPDH primers: 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 (35μg) were 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 Jagged1 (1:1000, Abcam, Cambridge, UK), Notch2 (1:1000, Cell Signaling Technology, Danvers, MA, USA), Hes1 (1:1000, Cell Signaling Technology) and GAPDH (1:5000, Beyotime) at 4°C overnight. After washing, membranes were incubated with HRP-conjugated secondary antibody (1:5000, Beyotime) for 1h and bands were visualized using an ECL kit (Millipore). Relative protein expression was quantified by ImageJ software.

CCK-8 Assay

Transfected SW620 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 was measured using a microplate reader (Bio-Rad, Hercules, CA, USA) to evaluate cell proliferation.

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) resuspended in serum-free DMEM were added to the upper chamber and DMEM containing 20% FBS was added to the lower chamber. After incubation at 37°C for 24h, cells on the upper surface of the membrane were removed. Cells that invaded to the lower surface were fixed with 4% paraformaldehyde, stained with 0.1% crystal violet and counted under an inverted microscope (Olympus, Tokyo, Japan) in five random fields.

Statistical analysis

All experiments were repeated three times. Data were presented as mean ± standard deviation (SD). Statistical analysis was performed using SPSS 25.0 software (IBM, Armonk, NY, USA). Differences between groups were compared using independent samples t-test. $P<0.05$ was considered statistically significant.

Results

Jagged1 is overexpressed in CRC cell lines

qRT-PCR results showed that the relative mRNA expression of Jagged1 in SW620 and HCT116 cells was 3.52±0.33 and 2.98±0.27 folds of that in NCM460 cells, respectively ($P<0.01$). Western blot analysis revealed that the relative gray value of Jagged1 protein in SW620 (2.71±0.24) and HCT116 (2.25±0.20) cells was significantly higher than that in NCM460 cells (1.00±0.10, $P<0.01$), indicating that Jagged1 is overexpressed in CRC cell lines.

Knockdown of jagged1 inhibits CRC cell proliferation

After transfection with si-Jagged1, the relative mRNA and protein expression of Jagged1 in SW620 cells was reduced by 75.6±6.2% and 70.3±5.8%, respectively ($P<0.01$), confirming efficient knockdown. CCK-8 assay showed that there was no significant difference in OD450 between the si-Jagged1 group and si-NC group at 24h (0.43±0.04 vs. 0.46±0.05, $P>0.05$). At 48h, the OD450 in the si-Jagged1 group was 0.51±0.06, which was significantly lower than that in the si-NC group (0.95±0.08, $P<0.05$). At 72h, the OD450 in the si-Jagged1 group further decreased to 0.65±0.06, significantly lower than that in the si-NC group (1.28±0.11, $P<0.05$), suggesting that Jagged1 knockdown inhibits CRC cell proliferation.

Knockdown of jagged1 Suppresses CRC cell invasion

Transwell invasion assay results showed that the number of invasive SW620 cells in the si-Jagged1 group was 38±5, which was significantly less than that in the si-NC group (112±9, $P<0.01$), indicating that Jagged1 silencing reduces CRC cell invasion ability.

Knockdown of jagged1 downregulates notch signaling pathway-related proteins

Western blot analysis showed that the relative gray value of Notch2 in the si-Jagged1 group was 0.39±0.05, significantly lower than that in the si-NC group (1.00±0.08, $P<0.05$). The relative gray value of Hes1 in the si-Jagged1 group was 0.36±0.04, also significantly lower than that in the si-NC group (1.00±0.06, $P<0.05$), suggesting that Jagged1 regulates CRC cell biological behaviors by activating the Notch signaling pathway.

Discussion

This study demonstrated that Jagged1 is overexpressed in CRC cell lines (SW620 and HCT116) compared with normal

colonic epithelial cells (NCM460). Functional experiments showed that knockdown of Jagged1 significantly inhibits the proliferation and invasion of SW620 cells and downregulates the expression of Notch2 and Hes1 (key molecules of the Notch signaling pathway). These results indicate that Jagged1 promotes CRC progression by activating the Notch signaling pathway.

The overexpression of Jagged1 in CRC is consistent with previous studies in other gastrointestinal cancers. For example, Jagged1 was overexpressed in pancreatic cancer tissues and cell lines and its high expression was associated with poor prognosis of patients[6]. In esophageal cancer, Jagged1 promoted cancer cell invasion and metastasis by activating the Notch pathway[7]. In CRC, previous studies have shown that the Notch pathway is abnormally activated[3,4] and our study further identified Jagged1 as an important upstream activator of the Notch pathway in CRC.

Mechanistically, Jagged1, as a ligand of the Notch pathway, binds to Notch receptors (such as Notch2) on the cell surface, triggering the cleavage of the Notch intracellular domain (NICD). The released NICD translocates to the nucleus and forms a complex with CSL transcription factors, thereby activating the transcription of downstream target genes such as Hes1[5,8]. Our results showed that knockdown of Jagged1 reduced the expression of Notch2 and Hes1, confirming that Jagged1 mediates the activation of the Notch pathway in CRC cells. This is consistent with the findings of Li, et al.[9], who reported that Jagged1/Notch signaling promotes the proliferation and invasion of gastric cancer cells.

Notably, invasion is a key step in CRC metastasis, which is the main cause of death in CRC patients[2]. Our Transwell invasion assay showed that Jagged1 knockdown significantly reduced CRC cell invasion, suggesting that Jagged1 may play a crucial role in CRC metastasis. This is supported by Zhang, et al.[10], who found that Jagged1 expression was positively correlated with lymph node metastasis in CRC patients (though our study is a basic experiment, this clinical observation indirectly supports our findings).

This study has some limitations. First, it was only conducted in CRC cell lines and in vivo experiments (such as xenograft mouse models) are needed to further confirm the role of Jagged1 in CRC progression. Second, we only explored the association between Jagged1 and the Notch pathway and the potential crosstalk between Jagged1 and other signaling pathways (e.g., Wnt/β-catenin pathway[11]) in CRC remains to be investigated. Third, the specific mechanism by which Jagged1 regulates Notch2 (rather than other Notch receptors) in CRC needs to be further clarified.

Targeting Jagged1 may provide a new strategy for CRC treatment. Currently, some Notch pathway inhibitors are in preclinical or clinical trials, but targeting Notch ligands (such as Jagged1) may have higher specificity and fewer side effects. Our study provides experimental evidence for the development of Jagged1-targeted therapies for CRC.

Conclusion

Jagged1 is overexpressed in colorectal cancer (CRC) cell lines. Knockdown of Jagged1 inhibits CRC cell proliferation and invasion by downregulating the Notch signaling pathway (Notch2, Hes1). These findings suggest that Jagged1 is a potential therapeutic target for CRC.

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