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Jagged-1 Expression Level Is Correlated With Recurrence of Stage III Colorectal Cancer in Patients Receiving Adjuvant Chemotherapy

HONG-BEUM KIM[1*], SEUL-BI LEE[2*], SEONG-JUNG KIM[3], HEE-JEONG LEE[2] and SANG-GON PARK[2]

[1]*Department of Premedical Course, Chosun University School of Medicine, Gwangju, Republic of Korea;*
[2]*Department of Internal Medicine, Hemato-oncology, Chosun University Hospital, Gwangju, Republic of Korea;*
[3]*Department of Internal Medicine, Gastroentelogy, Chosun University Hospital, Gwangju, Republic of Korea*

Abstract. *Background/Aim: Previous reports have indicated that increased expression of Jagged-1 (JAG1) may predict chemotherapy response and poor prognosis for patients with recurrent or metastatic colorectal cancer (CRC). This study aimed to investigate the clinical impact of JAG1 expression level in patients with CRC, including recurrence, especially in those diagnosed with lymph node-positive stage III CRC who underwent complete resection and appropriate adjuvant chemotherapy. Patients and Methods: All patients were enrolled through a retrospective chart review, and only those for whom the clinical course and all clinical information were adequately determined according to the inclusion criteria were selected for retrospective review through medical records. Immunohistochemical staining of JAG1 was performed using paraffin-embedded tissue. JAG1 expression was determined by scoring for staining intensity and percentage of positively stained cells; the final JAG1 score was determined as the sum of both scores. Results: Sixteen patients who experienced relapse and 15 without (for over 3 years) were selected. The protein expression level of JAG1 showed a tendency for being lower in the group without recurrence, although not statistically significantly (p=0.083); however, the mean JAG1 expression score was significantly lower in the group without recurrence (1.53 vs. 3.19; p=0.004). The patients were divided into two groups with low and high JAG1 expression. The results showed that high JAG1 expression was significantly associated with recurrence of stage III CRC (p=0.029). Conclusion: The expression of JAG1 may be a potential novel biomarker for predicting CRC recurrence.*

Standard therapy for early-stage colorectal cancer (CRC) includes surgical resection followed by adjuvant chemotherapy, and systemic adjuvant chemotherapy [5-fluorouracil plus leucovorin] has been globally recommended as standard treatment since the early 1990s (1-3). Recently, many studies have shown that oxaliplatin-based adjuvant chemotherapy is superior to the previously used 5-FU-based chemotherapy regimen for stage III CRC (4-7). However, over 30% of patients with stage III CRC develop recurrence despite the administration of appropriate adjuvant chemotherapy. The tumor, node, metastasis classification is a globally recognized standard prognostic factor; however, this classification is insufficient as a prognostic predictor, especially for recurrence (8-9). Recently, various predictive biomarkers, including target molecules, have been studied to identify high-risk groups for recurrence [reviewed in (10)].

Apurinic/apyrimidinic endodeoxyribonuclease 1 (APEX1)-mediated up-regulation of the Jagged-1 (JAG1)/Notch pathway has been reported to enhance colon cancer progression, and this pathway is also reported to be a major route for chemoresistance in biliary tract cancer, gastric cancer, and colon cancer cell lines (11-14). Furthermore, overexpression of JAG1 was associated with a poor response to chemotherapy in patients with recurrent and metastatic CRC and may be a factor indicating poor prognosis (13).

Based on the aforementioned study, we investigated the clinical relationship between the expression of JAG1 and the recurrence of lymph node-positive stage III CRC in patients who underwent complete resection and adjuvant chemotherapy according to the guidelines.

*These Authors contributed equally to this study as co-first authors.

Correspondence to: Sang-Gon Park, MD, Ph.D., Department of Internal Medicine, Hemato-oncology, Chosun University Hospital, 365 Pilmun-daero, Dong-gu, Gwangju 61453, Republic of Korea. Tel: +82 622203984, Fax: +82 622349653, e-mail: sgpark@chosun.ac.kr

Key Words: Jagged-1, JAG1, biomarker, lymph node-positive stage III CRC, adjuvant chemotherapy, colorectal cancer recurrence.

Patients and Methods

Patients and tissue samples. This retrospective study was conducted with approval from the Chosun University Hospital's Ethics Committee (IRB number: 2014-12-006-000). Target patients for this study included those with lymph node-positive stage III CRC who underwent complete resection and appropriate adjuvant chemotherapy according to the medical records of Chosun University Hospital. Inclusion criteria were as follows: at least 3 years of observation sufficient to indicate whether relapse occurred; favorable performance status (Eastern Cooperative Oncology Group score of 0-1); normal hepatic, renal, and bone marrow functions; and immunohistochemical staining performed using paraffin-embedded and sectioned patient tissues. However, patients with rectal cancer were limited to those who did not receive preoperative radiotherapy and received adjuvant chemotherapy after being diagnosed with stage III CRC after surgery. These patients were then divided into groups with and without relapse

Immunohistochemistry. Paraffin-embedded and sectioned patient tissues were obtained from the Pathology Tissue Bank of Chosun University Hospital, Gwangju, Republic of Korea. The sections were dewaxed with xylene, rehydrated, and prepared for immunohistochemistry using routine methods. Endogenous peroxidase activity was blocked with 0.03% H_2O_2 for 15 min. Nonspecific binding was suppressed by incubation with 10% normal horse serum (Jackson Immuno Research Laboratories Inc., Suffolk, UK) for 1 h at room temperature (20-25˚C). Thereafter, tissue sections were incubated with the primary antibody mouse anti-JAG1 for 24 h at 4˚C. Tissue sections were stained with mouse anti-JAG1 (sc-390177; 1:300; Santa Cruz Biotechnology, Dallas, TX, USA) antibodies. For immunohistochemistry, a biotinylated goat anti-mouse antibody (Vector Laboratories, Burlingame, CA, USA) followed by horseradish peroxidase-conjugated streptavidin (Vector Laboratories) was used. After immuno-labeling, the specimens were briefly counterstained with hematoxylin. Immunolabeled images were captured using an Olympus C-4040Z digital camera (Olympus Corp., Lake Success, NY, USA) and a BX-50 microscope (Olympus Corp.).

Protein expression was scored in the plasma membrane and cytoplasm for JAG1. JAG1 immunoreactivity was determined according to the score of the staining intensity (0, none; 1, weak; 2, moderate; 3, strong) and the percentage of positively stained cells (0, <5%; 1, 6-25%; 2, 26-50%; 3, 50-75%; 4, >76%) (Figure 1). The final immunoreactivity score was defined as the sum of the staining intensity value score and the estimated value score of the percentage of positively stained cells (total scores: 0-7).

Statistical analysis. Continuous data are presented as means±standard deviation. Statistical comparisons were performed by two-tailed Student's *t*-test and categorical data were analyzed using the chi-square test or Fisher's exact test. The curve for overall survival from initial diagnosis was determined using Kaplan–Meier analyses.

All data were analyzed using SPSS version 21.0 software (IBM, Armonk, NY, USA). Differences and associations with a two-tailed *p*-value of less than 0.05 were considered statistically significant.

Results

Clinical evaluation. According to the aforementioned inclusion criteria, 16 patients with relapse and 15 without were selected, and clinical data were evaluated through retrospective chart review. In each group, clinical analyses of age, sex, tumor location, and tissue differentiation were performed; however, the differences were not statistically significant. The mean disease-free survival was 18.8 months for the group with recurrence and the mean observation period was 80 months for that without. All patients received adjuvant chemotherapy, which was oxaliplatin-based in most cases. Some patients underwent only 5-fluorouracil-based chemotherapy, depending on their status; however, there was no statistically significant difference between the groups (with recurrence, n=4; no recurrence, n=2; p=0.654) (Table I).

For each group, the mean score for JAG1 expression was calculated, and the final score was determined as described above. The distribution of the calculated final score of JAG1 expression demonstrated a tendency for lower expression in the group without recurrence (p=0.024) (Figure 2). Furthermore, the mean final score for JAG1 expression was significantly higher in the group with recurrence (3.38 *vs*. 2.27; p=0.032; Table I).

According to the mean scores for JAG1 expression, intensity more than moderately strong or more than 25% positively stained cells was defined as high expression, with a cutoff value greater than 2 points. The patients were classified into low and high expression groups according to this value. High JAG1 expression was significantly associated with recurrence of stage III CRC (p=0.032; Table I).

Considering the intensity and percentage scores, recurrence seemed to be more strongly associated with intensity (1.88 *vs*. 1.13; p=0.005) than the percentage of positively stained cells (1.50 *vs*. 1.13; p=0.149), however, the number of samples was small, so re-analysis in the future seems necessary.

According to the results obtained, again using a cut-off score of 2 points, the low and high expression groups included 14 and 17 patients, respectively. In each group, clinical analyses of age, tumor location, and tissue differentiation were performed; JAG1 expression did not correlate with any factors (Table II).

Chemo-response according to RECIST 1.1 criteria was investigated only in patients with recurrence; Statistical analysis was difficult due to too few numbers, however, there was no case with progressive disease in the group with low JAG1 expression (n=4).

Although the population was small, the survival rate for each group was analyzed. Among the patients with node-positive stage III CRC, the group with low JAG1 expression tended to have longer median overall survival (94.7 *vs*. 42.1 months; p=0.096).

Discussion

Adjuvant chemotherapy is a treatment that increases the cure rate by killing the remaining micro-cancer cells after surgical

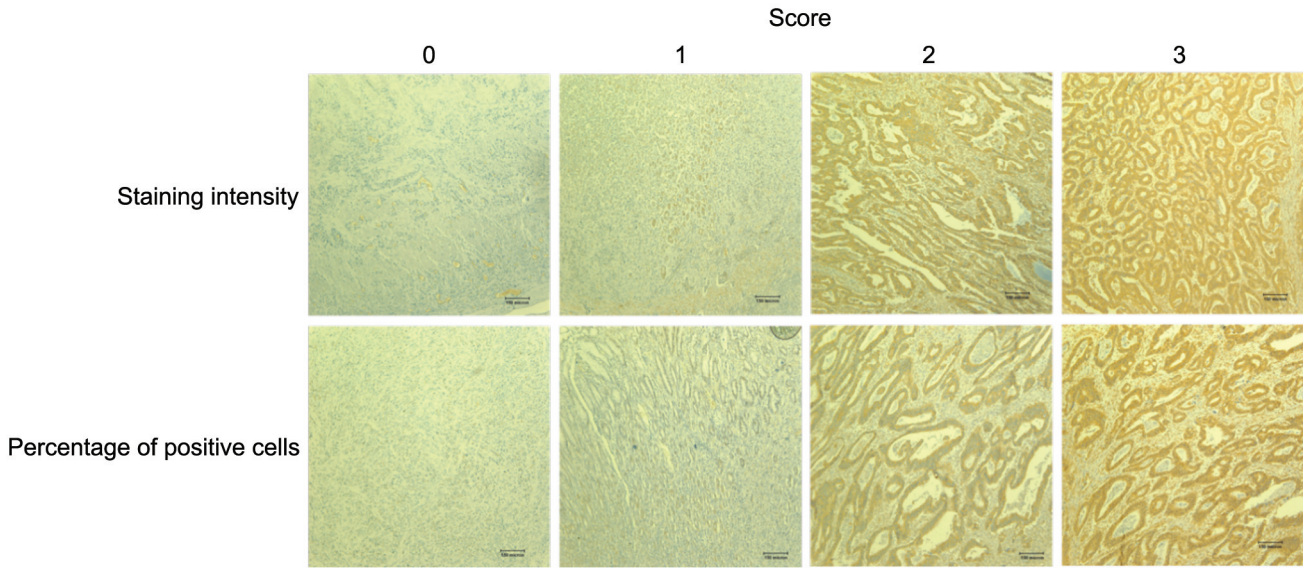

Figure 1. *Representative immunohistochemical scoring of sections of colon cancer specimens from patients. Protein expression was scored in the plasma membrane and cytoplasm for Jagged-1. Scoring for staining intensity: 0, none; 1, weak; 2, moderate; 3, strong; and for the percentage of positively stained cells: 0, <5%; 1, 6-25%; 2, 26-50%; 3, 50-75%; 4: ≥76% (no section scored 4). Bars=150 μm.*

resection to reduce the likelihood of recurrence. Therefore, the effectiveness of adjuvant chemotherapy is related to the response to chemotherapy; this has been shown in various major cancer types including breast, lung, and gastric (15). In CRC, fluoropyrimidine-based adjuvant chemotherapy has demonstrated efficacy since the 1990s (2, 3). Ten years ago, fluoropyrimidine-based chemotherapy with oxaliplatin was shown to be effective, and for patients with lymph node-positive stage III CRC, recent clinical guidelines recommend oxaliplatin-based adjuvant chemotherapy after complete surgical resection to prevent recurrence or metastasis within 6 months (4-7).

Moreover, with the development of surgical techniques, the rate of curative resections is gradually increasing, even among elderly patients. The neurotoxicity and nephrotoxicity of oxaliplatin are controversial regarding the exact dose and duration of oxaliplatin administration according to the guidelines, and several clinical studies are still ongoing to identify the most appropriate regimen (8-10, 16-18). However, more than 30% of patients with CRC show local or metastatic recurrence despite appropriate adjuvant chemotherapy. Therefore, it is important to identify high-risk factors for recurrence in patients with CRC undergoing adjuvant chemotherapy, and many molecular biomarkers are being studied for this purpose. Various molecular markers or genomic profiling have been studied to identify high-risk groups for recurrence when adjuvant chemotherapy is administered in CRC (10, 19).

Mutations in representative genes, including KRAS proto-oncogene, GTPase and B-Raf proto-oncogene serine/threonine kinase, as well as defective DNA mismatch repair genes have been established as prognostic biomarkers for the recurrence of stage II or III CRC in patients receiving appropriate adjuvant chemotherapy (19-22). In addition, according to recent studies on Oncotype DX, a recurrence score method using a 12-gene expression level, in patients with stage II and III CRC receiving oxaliplatin-based adjuvant chemotherapy has been reported as a very useful tool in predicting recurrence, and has been verified through several large-scale studies (23-25). This predicts poor prognosis for CRC receiving adjuvant chemotherapy; however, these scoring methods using Oncotype DX are not specific for detecting recurrence in patients with stage III CRC. Additionally, many genes other than the representative ones are reported to be related to recurrence (23-28). Therefore, new predictive molecular markers of recurrence need to be investigated, specifically in patients with CRC who are receiving adjuvant therapy after curative resection.

The Notch signaling pathway has gained attention as a field of research, and recent reports have implicated it in the development and progression of various malignant solid tumors (29, 30). JAG1 is one of five Notch receptor ligands and has been reported to stimulate the proliferation and metastasis of cancer cells by improving the survival of cancer stem cells (14, 31, 32). APEX1-driven activation of the JAG1 pathway has been demonstrated to enhance colon cancer progression (11) and is reported as a chemoresistance factor in biliary tract, colon, and gastric cancer cell lines (11-14). Furthermore, high expression of JAG1 was associated with factors predictive of low efficacy of chemotherapy in

Table I. *Clinical characteristic of patients with (n=16) and without recurrence (n=15) in node-positive stage III colorectal cancer.*

	Recurrence (n=16)	No recurrence (n=15)	*p*-Value
Age, years			
Mean±SD	63±10.4	60±10.2	0.502
Gender, n			
Male	11	11	>0.99
Female	5	4	
Tumor location, n			
Ascending colon	3	4	0.809
Transverse colon	0	1	
Descending colon	2	2	
Sigmoid colon	8	6	
Rectum	3	2	
Histology (differentiation), n			
Well/moderate	13	13	>0.99
Poor/mucinous	3	2	
Adjuvant chemotherapy			
5-FU-based	4	2	
Oxaliplatin-based	12	13	0.654
Total JAG1 score, n			
0	0	2	
1	1	1	
2	3	7	
3	4	1	
4	5	4	
5	3	0	0.083
Mean±SD	3.38±1.2	2.27±1.3	**0.022**
JAG1 expression, n			
Low (score ≤2)	4	10	
High (score >2)	12	5	**0.032**

5-FU: 5-Fluorouracil; JAG1: Jagged-1; SD: standard deviation. Statistically significant *p*-values are shown in bold.

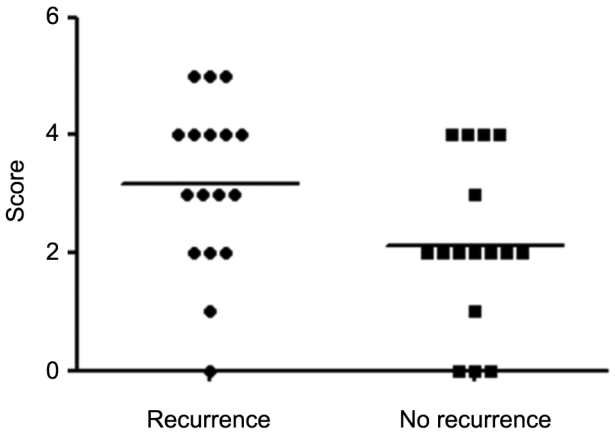

Figure 2. *Scatter plot of Jagged-1 expression in stage III colorectal cancer. Scatter plots show the distribution of total scores determined by immunohistochemical analysis of tissue sections from patients with (n=16) and without recurrence (n=15).*

Table II. *Clinical characteristic of patients according to Jagged-1 (JAG1) expression. Patients were divided into groups with low and high JAG1 expression according to score.*

	JAG1 expression		
	Low (score ≤2) (n=14)	High (score >2) (n=17)	*p*-Value
Age, years			
Mean±standard deviation	63.2±10.4	60±10.3	0.547
Gender, n			
Male	10	12	
Female	4	5	>0.999
Tumor location, n			
Ascending colon	4	3	
Transverse colon	0	1	
Descending colon	0	2	
Sigmoid colon		8	
Rectum	4	3	0.975
Colon	10	14	
Rectum	4	3	0.671
Histological differentiation, n			
Well/moderate	11	15	
Poor/mucinous	3	2	0.636
Response to chemotherapy			
PR	3	2	
SD+PD	1	10	0.093
Disease control by chemotherapy			
PR+SD	4	6	
PD	0	6	0.234
Overall survival, months			
Median (95% CI)	94.7 (85.6-103.8)	42.1 (20.2-63.9)	0.096

CI: Confidence interval; PD: progressive disease; PR: partial response; SD: stable disease.

patients with recurrent and metastatic CRC; this low efficacy has an adverse effect on survival (13). The activation of cancer stem cells through the overexpression of JAG1 is one of the causes of chemoresistance in gastric cancer (14). High expression of Notch ligand JAG1 was also associated with poor prognosis and high recurrence after curative surgery in patients with CRC (33). However, that report included patients with all stages of CRC who underwent surgical resection, and it was not clear whether they received adequate adjuvant chemotherapy. Therefore, our research aimed to determine whether the expression level of JAG1 can be used as a new molecular biomarker of recurrence in patients with lymph node-positive stage III CRC receiving appropriate adjuvant chemotherapy.

The results of this retrospective study may be explained by the hypothesis of our previous studies, wherein the high expression of JAG1 was related to increased cancer stem cell proliferation, increasing the chemoresistance rate. Increased expression of JAG1 is also associated with chemoresistance

to adjuvant chemotherapy. Moreover, recurrence occurs more often because of failure to eradicate the remaining micro-residual tumor in stage III CRC with high expression of JAG1. The recurrence rate was significantly lower in the group with low JAG1 expression when patients with stage III CRC with lymph node metastasis received appropriate chemotherapy because of their positive response to adjuvant chemotherapy.

In conclusion, our study revealed an association between the recurrence rate of stage III CRC and the expression level of JAG1. The results showed that high JAG1 expression is associated with a high recurrence rate in patients with stage III CRC who received appropriate adjuvant chemotherapy. JAG1 expression can potentially be used as a novel biomarker to predict CRC recurrence.

Conflicts of Interest

The Authors declare that they have no conflicts of interest.

Authors' Contributions

KIM HB and Lee SB were the major contributors in writing the article; LEE HJ and KIM SJ advised on article writing and drafting; Park SG were involved in drafting, writing and editing the manuscript, and reviewed the article as corresponding author. All Authors read and approved the final article.

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