# JAB1-STAT3 activation loop is associated with recurrence following 5-fluorouracil-based adjuvant chemotherapy in human colorectal cancer

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**Abstract.** Jun activation domain-binding protein 1 (JAB1) has been shown to have multiple roles in tumorigenesis, including the degradation of tumor suppressor proteins such as p53, Smad7, Runx3 and the cyclin-dependent kinase inhibitor $p27^{Kip1}$, and the activation of oncogenic transcription factors, such as c-Jun and hypoxia-inducible factor-1α. In addition, our previous study revealed that JAB1 positively regulates signal transducer and activator of transcription 3 (STAT3) DNA-binding activity in human colon cancer cells. In turn, the oncogenic transcription factor STAT3 positively regulates JAB1 expression, indicative of a positive feedback loop. Furthermore, high JAB1 expression is associated with a poor prognosis in numerous malignant carcinomas. However, the association between JAB1 expression and prognosis in colorectal cancer remains unclear. The aim of the present study was to elucidate the association between JAB1 and STAT3 expression and recurrence in colorectal cancer. In the present study, it was found that high *JAB1* expression in primary colorectal cancer tissues is an independent predictor of recurrence following 5-fluorouracil (5-FU)-based adjuvant chemotherapy in colorectal cancer patients, and that high expression of both *JAB1* and *STAT3* in primary colorectal cancer tissues is associated with a lower recurrence-free survival rate following 5-FU-based adjuvant chemotherapy compared to high expression of only *JAB1* or *STAT3*. Overall, these results suggest that *JAB1* is a novel predictive marker of recurrence following 5-FU-based adjuvant chemotherapy in colorectal cancer patients, and that the JAB1-STAT3 activation loop may be a potential therapeutic target in recurrent colorectal cancer following 5-FU-based adjuvant chemotherapy.

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*Key words:* JAB1, STAT3, JAB1-STAT3 activation loop, recurrence, colorectal cancer, 5-FU-based adjuvant chemotherapy

## Introduction

Jun activation domain-binding protein 1 (JAB1) was originally identified as a co-activator of c-Jun that stabilizes its DNA-binding through protein-protein interaction (1). Subsequently, JAB1 was found to interact with numerous other proteins, affecting protein stability and transcriptional activity of its interacting partner proteins, which are involved in the regulation of the cell cycle, signal transduction and DNA repair (2). JAB1 has an important role in tumorigenesis, inactivating tumor suppressor proteins and activating oncogenic transcription factors. JAB1 facilitates the translocation of tumor suppressor proteins, including p53 (3,4), Smad7 (5), Runx3 (6) and the cyclin-dependent kinase inhibitor $p27^{Kip1}$ (7,8), from the nucleus to the cytoplasm, where they are subsequently degraded in the proteasome. JAB1 is also a transcriptional co-activator of c-Jun (1), hypoxia-inducible factor-1α (9,10) and signal transducer and activator of transcription 3 (STAT3) (11). JAB1 is positively regulated by oncogenic transcription factors STAT3 and β-catenin/TCF-4 (12,13). STAT3 positively regulates *JAB1* expression through its binding to the *JAB1* promoter (12), and HER2 increases JAB1 expression through the binding of β-catenin/TCF-4 to the *JAB1* promoter in human breast cancer cells (13). These findings suggest that *JAB1* is a target gene of STAT3 and β-catenin/TCF-4. Overall, with our recent findings that JAB1 positively regulates STAT3 DNA-binding activity in human colon cancer cells (11), the results of these studies suggest that the JAB1-STAT3 activation loop exists in human colorectal cancer cells. Furthermore, high JAB1 expression has been reported to be associated with poor prognosis in numerous malignant carcinomas, including ovarian cancer (14,15), oral squamous cell carcinoma (16), laryngeal squamous cell carcinoma (17), hepatocellular carcinoma (18), glioma (19), soft-tissue sarcoma (20), pancreatic cancer (21), esophageal squamous cell carcinoma (22), lung cancer (23) and non-Hodgkin's lymphoma (24). However, the association between JAB1 expression and prognosis in colorectal cancer remains largely unknown. The objectives of the present study were therefore to elucidate the associations between JAB1 and STAT3 expression and recurrence in colorectal cancer.

In the present study, it was found that high *JAB1* expression in primary colorectal cancer tissues is an independent predictor of recurrence following 5-fluorouracil (5-FU)-based adjuvant chemotherapy in colorectal cancer patients, and high expression of both *JAB1* and *STAT3* in primary colorectal cancer tissues is associated with a lower recurrence-free survival rate compared to high expression of only *JAB1* or *STAT3*.

## Materials and methods

*Patients and clinical samples*. A total of 57 patients with colorectal cancer who underwent surgical treatment at Yamaguchi University Hospital (Ube, Yamaguchi, Japan), Yamaguchi Saiseikai Shimonoseki General Hospital (Shimonoseki, Yamaguchi, Japan) and Yamaguchi Rosai Hospital (Sanyo-Onoda, Yamaguchi, Japan) between April 2012 and December 2013 were enrolled in the present study. All patients had stage II or III colorectal cancer and were treated with FOLFOX, UFT/UZEL or Xeloda following curative surgical operation. Primary colorectal cancer tissues from 50 patients (age range, 48-84 years; 31 males and 19 females) were immediately taken from resected colorectal tissues and kept at -80˚C until total RNA extraction, followed by reverse transcription-quantitative polymerase chain reaction (RT-qPCR). Additionally, 7 paired samples of primary colorectal cancer tissue and liver metastasis from 7 patients (age range, 63-81 years; 6 males and 1 female) who had undergone mainly 5-FU-based chemotherapy were formalin-fixed and paraffin-embedded immunohistochemical staining. Written informed consent was obtained from all patients, and approval was provided by Institutional Review Board of Yamaguchi University Hospital and the affiliated hospitals. The samples were used in accordance with the Declaration of Helsinki.

*Regimens of adjuvant chemotherapy*. The choice of adjuvant chemotherapy was made by the patient in consultation with the surgeon. UFT/LV was given in 5-week cycle consisting of 4 weeks of treatment and 1 week of rest. The cycle was repeated at least 5 times. The UFT dose was 300 mg/m$^2$/day and LV dose 75 mg/day. Xeloda was administered at a dose of 1,250 mg/m$^2$ twice a day for 14 days, followed by 7 days of rest. Standard care included a total of 8 cycles. Modified FOLFOX6 [a modified folinic acid, leucovorin (LV), 5-FU, and oxaliplatin (OX)] regimen was as follows; OX 85 mg/m$^2$, LV 200 mg/m$^2$, 5-FU bolus 400 mg/m$^2$, 5-FU infusion 2,400 mg/m$^2$ over 46 h. The treatment was repeated every 2 weeks. Standard care included a total of 12 cycles.

*Immunohistochemical staining*. Tissue specimens were fixed with 20% formalin for 3-5 days at room temperature and paraffin-embedded. Sections (3-$\mu$m thick) from the tissue specimens were deparaffinized with xylene at room temperature and rehydrated with graded ethanol. The tissue sections were then incubated with 3% hydrogen peroxide ($H_2O_2$) in methanol for 30 min to block endogenous peroxidase activity, followed by antigen retrieval at 95˚C for 20 min in Dako Target Retrieval solution (Agilent Technologies, Inc., Santa Clara, CA, USA). Tissue sections were subsequently incubated in Dako Protein Block Serum-Free Ready-to-Use (Agilent Technologies, Inc.) for 30 min at room temperature to prevent non-specific binding, and were then incubated with anti-rabbit polyclonal JAB1 (FL-334) primary antibody (catalog no. sc-9074; Santa Cruz Biotechnology, Inc., Dallas, TX, USA) diluted at 1:100 overnight at 4˚C. After washing several times with phosphate-buffered saline, the sections were incubated with anti-rabbit immunoglobulin conjugated to horseradish peroxidase (EnVision+ HRP-labeled polymer anti-rabbit system; catalog no. K4002; Dako North America, Inc., Carpinteria, CA, USA) as a secondary antibody for 30 min at room temperature. The sections were treated with 0.2 mg/ml diaminobenzidine for 15 sec and counterstained in Mayer's hematoxylin for 30 sec. Images were captured from at least 10 randomly selected fields per sample using an All-in-One fluorescence microscope (magnification, x40; KEYENCE, Osaka, Japan). Immunoreactivity was independently evaluated by two.

*RT-qPCR*. Resected primary colorectal cancer tissues were disrupted in RLT buffer (Qiagen, Valencia, CA, USA) and homogenized by shaking with stainless steel beads using a Mixer Mill MM300 (both from Qiagen). Total RNA was isolated using an RNeasy Mini kit (Qiagen), according to the manufacturer's protocol. Reverse transcription was performed using PrimeScript RT Master Mix (Perfect Real-Time; Takara Bio, Inc., Otsu, Japan). The template cDNAs were amplified using a QuantiTect SYBR-Green PCR kit (Qiagen) with the specific primers. The sequences of the primers were as follows: *JAB1* forward, 5'-GCAGTGGTGATTGATCCAAC-3' and reverse, 5'-GTCTGGTACTCAGAAGGTCC-3'; *STAT3* forward, 5'-CACTACTAAAGTCAGGTTGCTGGTC-3' and reverse, 5'-AACGTCCCCAGAGTCTTTGTC-3'; *MCL1* forward, 5'-CACAGACGTTCTCGTAAGGAC-3' and reverse, 5'-GATGCCACCTTCTAGGTCCTC-3'; cyclin D1 forward, 5'-CGAGAAGCTGTGCATCTACACC-3' and reverse, 5'-TTCCACTTGAGCTTGTTCACC-3'; and *GAPDH* forward, 5'-TTGGTATCGTGGAAGGACTCA-3' and reverse, 5'-TGTCATCATATTTGGCAGGTT-3'. The PCR thermocycling conditions were 95˚C for 15 min, followed by 50 cycles of 95˚C for 10 sec and 60˚C for 30 sec. *JAB1* and *STAT3* expression was normalized to *GAPDH* expression. RT-qPCR was performed using LightCycler software version 3.5 (Roche Applied Science, Penzberg, Germany), and data were evaluated using the $2^{-\Delta\Delta Cq}$ method (25).

*Statistical analysis*. Statistical analyses were performed using SPSS Statistics 20 for Windows (SPSS, Inc., Chicago, IL, USA). Differences between groups were analyzed using the paired t-test, Mann-Whitney U test or $\chi^2$ test, as appropriate. The association between mRNA expression levels was assessed using Pearson's correlation coefficient. Survival curves were generated using the Kaplan-Meier method and compared using the log-rank test. Receiver operating characteristic (ROC) curve analysis was used to determine the optimum cut-off values for predicting outcome. To determine the cut-off values for *JAB1* and *STAT3* expression, ROC curves were constructed by plotting all possible sensitivity/1-specificity pairs in the training set. The Cox proportional hazards regression model was used to identify the variables associated with recurrence-free survival. $P<0.05$ was considered to indicate a statistically significant difference.

Table I. The association between *JAB1* and *STAT3* expression, and clinicopathological variables in primary colorectal cancer tissues.

| | *JAB1* expression | | | *STAT3* expression | | |
|---|---|---|---|---|---|---|
| Clinicopathological variables | Low | High | P-value | Low | High | P-value |
| Total | 33 | 17 | | 33 | 17 | |
| Gender | | | 0.23 | | | 0.23 |
| Male | 20 | 11 | | 20 | 11 | |
| Female | 13 | 6 | | 13 | 6 | |
| Age (years) | 70.0±10.6 | 72.1±6.6 | 0.48 | 70.5±9.8 | 71.2±8.9 | 0.80 |
| Location | | | 0.84 | | | 0.84 |
| Right | 13 | 8 | | 13 | 8 | |
| Left | 10 | 4 | | 10 | 4 | |
| Rectum | 10 | 5 | | 10 | 5 | |
| Histological grade | | | 0.77 | | | 0.77 |
| Well | 3 | 2 | | 4 | 1 | |
| Moderate | 26 | 14 | | 26 | 14 | |
| Poor | 4 | 1 | | 3 | 2 | |
| Invasion depth | | | 0.40 | | | 0.40 |
| T2 | 1 | 1 | | 1 | 1 | |
| T3 | 22 | 8 | | 22 | 8 | |
| T4 | 10 | 8 | | 10 | 8 | |
| Lymphatic metastasis | | | 0.87 | | | 0.05 |
| + | 21 | 11 | | 18 | 14 | |
| - | 12 | 6 | | 15 | 3 | |
| Lymphatic invasion | | | 0.96 | | | 0.96 |
| + | 30 | 16 | | 30 | 16 | |
| - | 3 | 1 | | 3 | 1 | |
| Venous invasion | | | 0.20 | | | 0.49 |
| + | 15 | 11 | | 16 | 10 | |
| - | 18 | 6 | | 17 | 7 | |
| Stage | | | 0.13 | | | 0.04 |
| 2a | 9 | 1 | | 10 | 0 | |
| 2b | 3 | 5 | | 5 | 3 | |
| 3a | 1 | 0 | | 1 | 0 | |
| 3b | 13 | 5 | | 12 | 6 | |
| 3c | 7 | 6 | | 5 | 8 | |

STAT3, signal transducer and activator of transcription 3; JAB1, Jun activation domain-binding protein 1.

## Results

*Association between JAB1 and STAT3 expression, and clinicopathological parameters in primary colorectal cancer tissues.* To investigate *JAB1* and *STAT3* expression level in 50 primary colorectal cancer tissues, RT-qPCR was performed. ROC curve analysis was used to obtain the optimal cut-off values of *JAB1* and *STAT3* expression, and this was used to classify 50 primary colorectal cancer tissues into high or low expression group of *JAB1* or *STAT3*. The association between JAB1 and STAT3 expression, and clinicopathological parameters was investigated. As shown in Table I, high *JAB1* expression was not associated with any of the investigated clinicopathological parameters, including age, sex, tumor location, histological grade, invasion depth, lymphatic metastasis, lymphatic invasion, venous invasion and tumor-node-metastasis (TNM) stage. However, high *STAT3* expression was significantly associated with advanced TNM stage (P=0.04).

*Association between JAB1 and STAT3 expression in primary colorectal cancer tissues.* RT-qPCR followed by scatter plot analysis showed that *JAB1* expression significantly correlated with *STAT3* expression in primary colorectal cancer tissues (Fig. 1A, P=0.001, r=0.755), and *JAB1* expression in tumors with high *STAT3* expression was significantly increased compared with that in tumors with low *STAT3* expression (Fig. 1B, P=0.007).

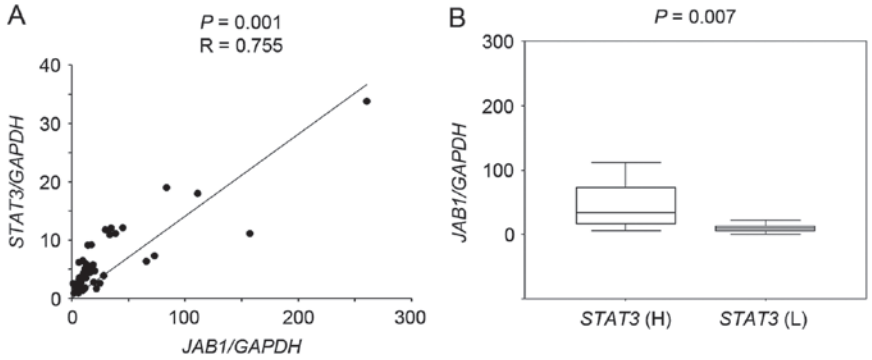


Figure 1. Association between *JAB1* and *STAT3* expression in 50 primary colorectal cancer tissues. Reverse transcription-quantitative polymerase chain reaction was performed to determine the *JAB1*, *STAT3* and *GAPDH* expression level. (A) The ratio of *JAB1* or *STAT3* expression to *GAPDH* expression was normalized to the lowest value and is presented as a scatter plot. (B) *JAB1* expression in tumors with high or low expression of *STAT3*. *STAT3* (H), high *STAT3* expression group; *STAT3* (L), low *STAT3* expression group. JAB1, Jun activation domain-binding protein 1; STAT3, signal transducer and activator of transcription 3.

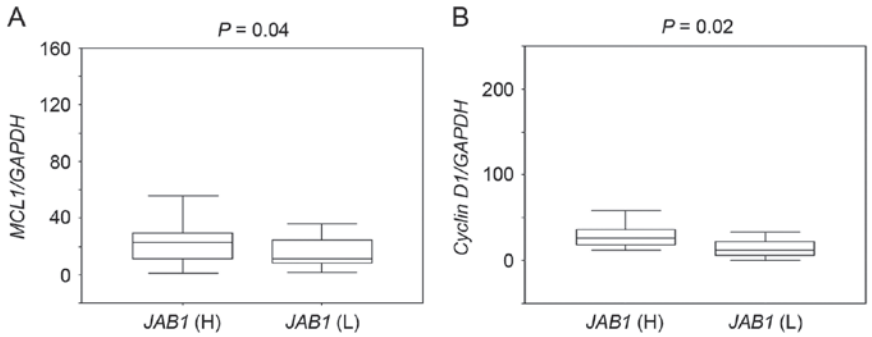


Figure 2. Comparison of the expression of the signal transducer and activator of transcription 3 target genes *MCL1* and cyclin D1 in primary colorectal cancer tissues with high or low expression of *JAB1*. (A) Comparison of *MCL1* expression in tumors with high or low *JAB1* expression. (B) Comparison of cyclin D1 expression in tumors with high or low *JAB1* expression. The ratio of *MCL1* or cyclin D1 expression to *GAPDH* expression was normalized to the lowest value. *JAB1* (H), high *JAB1* expression group; *JAB1* (L), low *JAB1* expression group. JAB1, Jun activation domain-binding protein 1.

*Expression of the STAT3 target genes MCL1 and cyclin D1 is associated with JAB1 expression*. The association between *JAB1* expression and the expression of the STAT3 target genes *MCL1* and cyclin D1 was then investigated. *MCL1* and cyclin D1 expression in tumors with high *JAB1* expression was significantly higher than that in tumors with low *JAB1* expression (Fig. 2, P=0.04 and P=0.02, respectively).

*High JAB1 expression in primary colorectal cancer tissues is associated with a lower recurrence-free survival rate following 5-FU-based adjuvant chemotherapy*. The present study determined whether clinicopathological variables, including *JAB1* and *STAT3* expression, in primary colorectal cancer tissues were associated with recurrence following 5-FU-based adjuvant chemotherapy. The median follow-up period for all patients was 21.4 months (range, 5.6-38.3 months). Histological grade (poor), invasion depth (T4) and *JAB1* expression (high) were significantly associated with recurrence (Table II, P=0.03, 0.01 and 0.01, respectively). To further examine the associations between *JAB1* and *STAT3* expression and recurrence-free survival rate following 5-FU-based adjuvant chemotherapy, Kaplan-Meier analyses were performed. Patients with high *JAB1* expression had a significantly decreased recurrence-free survival rate following 5-FU-based adjuvant chemotherapy compared to those with low *JAB1* expression (Fig. 3A, P=0.017), whilst there was no significant difference in survival with respect to *STAT3* expression (Fig. 3B, P=0.068). Patients with high expression of both *JAB1* and *STAT3* had a significantly decreased recurrence-free survival rate following 5-FU-based adjuvant chemotherapy compared to all other patients (Fig. 3C, P=0.012), and compared to patients with high expression of only *JAB1* or *STAT3*.

Cox proportional hazard regression analysis was then performed to identify independent prognostic factors. The recurrence-free survival rate following 5-FU-based adjuvant chemotherapy was strongly associated with histological grade (poor), invasion depth (T3) and *JAB1* expression (high) on univariate analysis (Table III, P=0.02, 0.01 and 0.03, respectively). In multivariate analysis, high *JAB1* expression was the only independent predictor of recurrence-free survival following 5-FU-based adjuvant chemotherapy (Table III, P=0.04).

*JAB1 protein expression in primary colorectal cancer tissues and liver metastases following mainly 5-FU-based chemotherapy*. Seven paired samples of primary colorectal cancer tissue and liver metastasis from 7 patients who had undergone mainly 5-FU-based chemotherapy were prepared. Clinical variables, including the chemotherapy regimen and treatment effects, are shown in Table IV. Nuclear and cytoplasmic JAB1 protein expressions were detected in both primary colorectal

Table II. Association between recurrence following fluorouracil-based adjuvant chemotherapy and clinicopathological variables, including *JAB1* and *STAT3* expression, in primary colorectal cancer tissues.

| Clinicopathological variables | No recurrence | Recurrence | P-value |
|---|---|---|---|
| Total | 35 | 15 | |
| Gender | | | 0.98 |
| Male | 23 | 8 | |
| Female | 12 | 7 | |
| Age (years) | 71.2±8.3 | 69.6±11.7 | 0.60 |
| Location | | | 0.29 |
| Right | 14 | 7 | |
| Left | 12 | 2 | |
| Rectum | 9 | 6 | |
| Histological grade | | | 0.03 |
| Well | 3 | 2 | |
| Moderate | 31 | 9 | |
| Poor | 1 | 4 | |
| Invasion depth | | | 0.01 |
| T2 | 2 | 0 | |
| T3 | 26 | 4 | |
| T4 | 7 | 11 | |
| Lymphatic metastasis | | | 0.73 |
| + | 21 | 11 | |
| - | 14 | 4 | |
| Lymphatic invasion | | | 0.45 |
| + | 33 | 13 | |
| - | 2 | 2 | |
| Venous invasion | | | 0.16 |
| + | 18 | 8 | |
| - | 17 | 7 | |
| Stage | | | 0.10 |
| 2a | 10 | 0 | |
| 2b | 4 | 4 | |
| 3a | 1 | 0 | |
| 3b | 13 | 5 | |
| 3c | 7 | 6 | |
| Oxaliplatin | | | 1.00 |
| + | 7 | 3 | |
| - | 28 | 12 | |
| *JAB1* | | | 0.01 |
| High | 8 | 9 | |
| Low | 27 | 6 | |
| *STAT3* | | | 0.06 |
| High | 9 | 8 | |
| Low | 26 | 7 | |

STAT3, signal transducer and activator of transcription 3; JAB1, Jun activation domain-binding protein 1.

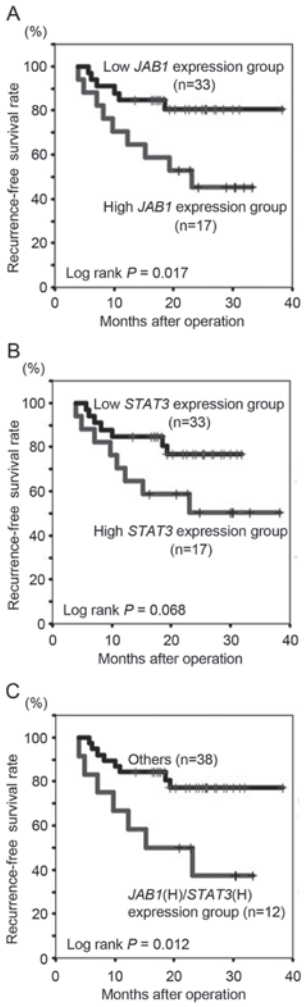


Figure 3. Kaplan-Meier curves for recurrence-free survival following 5-FU-based adjuvant chemotherapy. (A) Recurrence-free survival curve following 5-FU-based adjuvant chemotherapy with respect to *JAB1* expression. (B) Recurrence-free survival curve following 5-FU-based adjuvant chemotherapy with respect to *STAT3* expression. (C) Recurrence-free survival curve following 5-FU-based adjuvant chemotherapy with respect to combined *JAB1* and *STAT3* expression. *JAB1* (H)/*STAT3* (H) expression group, high expression group of both *JAB1* and *STAT3*. JAB1, Jun activation domain-binding protein 1; STAT3, signal transducer and activator of transcription 3; 5-FU, 5-fluorouracil.

cancer tissues and liver metastases. Nuclear and cytoplasmic JAB1 protein expressions in liver metastases appeared to be increased compared with those in primary colorectal cancer tissues (Fig. 4A-D), and the proportion of cells with positive nuclear JAB1 expression in liver metastases was significantly increased compared with that in primary colorectal cancer tissues (Fig. 4E, P=0.018).

## Discussion

It has been reported that *JAB1* expression is transcriptionally regulated through STAT3 binding to the *JAB1* promoter in human breast cancer cells (12). Furthermore, our previous study revealed that JAB1 positively regulates STAT3 DNA-binding activity in human colorectal cancer cells (11). Consistent with these findings, in the present study, it was found that *JAB1* expression was increased in tumors with high *STAT3* expression compared with that in tumors with low *STAT3* expression, and

Table III. Univariate analysis and multivariate analysis for clinicopathological variables affecting the recurrence-free survival rate following fluorouracil-based chemotherapy.

| | Univariate analysis | | Multivariate analysis | |
|---|---|---|---|---|
| Clinicopathological variables | HR (95% CI) | P-value | HR (95% CI) | P-value |
| Gender (male vs. female) | 1.55 (0.56-4.28) | 0.40 | | |
| Age (years) | 0.99 (0.94-1.04) | 0.63 | | |
| Location (rectum) | 1.83 (0.65-5.15) | 0.25 | | |
| Histological grade (poor) | 4.07 (1.29-12.9) | 0.02 | 3.10 (0.90-10.65) | 0.07 |
| Invasion depth (≥T3) | 4.10 (1.40-12.0) | 0.01 | 2.80 (0.89-8.87) | 0.08 |
| Lymphatic metastasis (+) | 1.66 (0.53-5.20) | 0.39 | | |
| Lymphatic invasion (+) | 0.45 (0.10-2.02) | 0.30 | | |
| Venous invasion (+) | 0.96 (0.35-2.65) | 0.94 | | |
| Stage (≥3a) | 1.85 (0.59-5.80) | 0.29 | | |
| *JAB1* (high) | 3.27 (1.16-9.20) | 0.03 | 2.80 (1.07-8.78) | 0.04 |
| *STAT3* (high) | 2.45 (0.90-6.86) | 0.08 | | |

HR, hazard ratio; CI, confidence interval; STAT3, signal transducer and activator of transcription 3; JAB1, Jun activation domain-binding protein 1.

Table IV. Clinicopathological characteristics of colorectal patients with liver metastasis.

| Case no. | Regimen | Cycle | Effect | Invasion depth | Histological grade | Lymph invasion | Venous invasion |
|---|---|---|---|---|---|---|---|
| 1 | Pmab + FOLFOX | 6 | PR | SE | mod | 1 | 1 |
| 2 | Bmab + Xelox | 11 | PD | SS | mod | 1 | 0 |
| 3 | Bmab + FOLFIRI | 6 | SD | SS | mod | 1 | 1 |
| 4 | Pmab + FOLFOX | 6 | PR | SS | pap | 1 | 1 |
| 5 | Bmab + FOLFOX | 10 | SD | SS | mod | 2 | 2 |
| 6 | Bmab + Xeloda | 4 | PR | SS | mod | 0 | 0 |
| 7 | Bmab + FOLFIRI | 20 | SD | SS | mod | 1 | 2 |

Pmab, panitumumab; Bmab, bevacizumab; FOLFOX, folinic acid + fluorouracil + oxaliplatin; FOLFIRI, folinic acid + fluorouracil + irinotecan; Xelox, capecitabine + oxaliplatin; PR, partial response; PD, progressive disease; SD, stable disease; SE, serosa; SS, subserosa; mod, moderately differentiated carcinoma; pap, papillary adenocarcinoma.

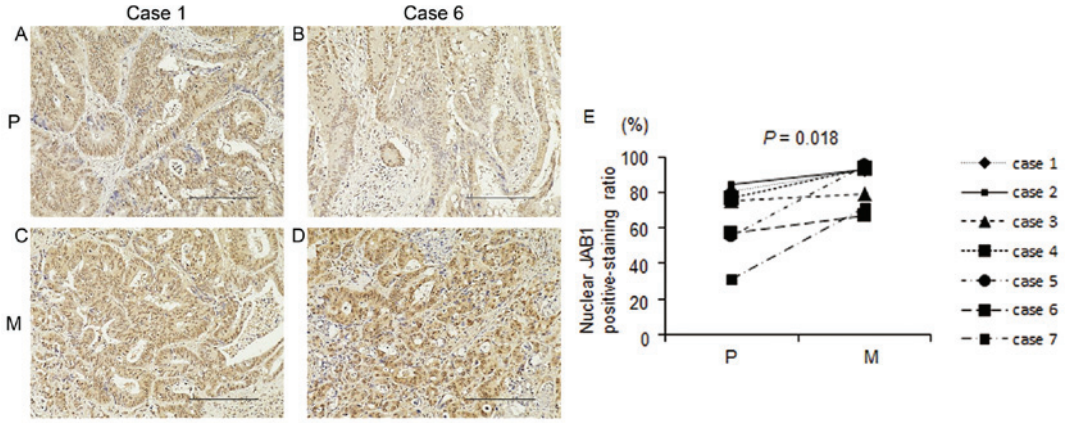


Figure 4. Immunohistochemical analysis of JAB1 expression in primary colorectal cancer tissues and liver metastases following mainly 5-FU-based chemotherapy. (A-D) Representative images of JAB1 staining in primary colorectal cancer tissues (A and B) and in liver metastases (C and D). Primary colorectal cancer tissue (A) and liver metastasis (C) are derived from the same patient (case 1). Primary colorectal cancer tissue (B) and liver metastasis (D) are also paired (case 6). Magnification, x40. Scale bar, 200 $\mu$m. (E) The proportion of cells positive for nuclear JAB1 expression in primary colorectal cancer tissues and liver metastases following mainly 5-FU-based chemotherapy (n=7). P, primary colorectal cancer tissue; M, liver metastasis. JAB1, Jun activation domain-binding protein 1; 5-FU, 5-fluorouracil.

that the STAT3 target genes *MCL1* and cyclin D1 had increased expressed in tumors with high *JAB1* expression compared to those with low *JAB1* expression. These findings suggest that JAB1 cooperates with STAT3, and that the JAB1-STAT3 activation loop is present in human colorectal cancer cells. Notably, patients with tumors that highly expressed both *JAB1* and *STAT3* had a lower recurrence-free survival rate following 5-FU-based adjuvant chemotherapy than those patients with tumors that highly expressed only *JAB1* or *STAT3*, suggesting that the JAB1-STAT3 activation loop has an important role in recurrence following 5-FU-based adjuvant chemotherapy. Furthermore, the present findings indicate that high *JAB1* expression in primary colorectal cancer is a significant predictor of recurrence following this treatment. It was also found that the proportion of tumor cells with positive nuclear JAB1 expression was significantly increased in liver metastases following mainly 5-FU-based chemotherapy compared with that in primary colorectal cancer tissues. Notably, a recent study found that nuclear JAB1 expression was increased in recurrent nasopharyngeal carcinoma following radiotherapy compared with primary nasopharyngeal carcinoma (26). Together with the present findings, this suggests that nuclear JAB1 has a role in recurrence. Additional studies are required to elucidate the association between nuclear JAB1 and recurrence.

Overall, the present findings suggest that the JAB1-STAT3 activation loop can confer resistance to 5-FU-based adjuvant chemotherapy and is thus a potential therapeutic target in recurrent colorectal cancer following 5-FU-based adjuvant chemotherapy.

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