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# The expression of chemokine receptors CXCR3 and CXCR4 in predicting postoperative tumour progression in stages I-II colon cancer: a retrospective study

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## ABSTRACT

**Objectives:** The prognostic significance of chemokine receptors in stage I/II colon cancer is unclear. We assessed the prognostic value of chemokine receptor CXCR3 and CXCR4 in stage I/II colon cancer.

**Methods:** 145 patients with stage I/II colon cancer who underwent curative surgery alone from 2000 to 2007 were investigated. Chemokine receptor expression was assessed by immunohistochemistry. The associations between CXCR3, CXCR4 and clinicopathological variables were analysed using the $\chi^2$ test, and the relationships between chemokine receptors and a 5-year disease-free survival were analysed by univariate and multivariate analyses.

**Results:** The high-expression rates of CXCR3 and CXCR4 were 17.9% (26/145) and 38.6% (56/145), respectively. There were no significant associations between the expressions of CXCR3, CXCR4 and clinicopathological factors including gender, age, tumour location, histological differentiation, pathological stage, lymphovascular invasion and pretreatment serum carcinoembryonic antigen (CEA). The 5-year disease-free survival was not significantly different between low-expression groups and high-expression groups of CXCR3 and CXCR4. Multivariate analysis revealed that serum CEA and a number of retrieved lymph nodes, rather than chemokine receptors, were independent prognosticators.

**Conclusions:** CXCR3 and CXCR4 are not independent prognosticators for stage I/II colon cancer after curative surgery.

## Strengths and limitations of this study

- This study specially addressed the significance of chemokine receptors in early stage colon cancer, and demonstrated that CXCR3 and CXCR4 could not predict postoperative tumour progression in stage I/II disease.
- However, this study was a retrospective study, all data came from a single cancer center, and it was subject to patient selection biases.

## BACKGROUND

The oncological outcome of patients with stage I/II colon cancer is favourable, with the 5-year survival rate exceeding 70%.[1 2] Although curative surgery is sufficient for the majority of stage I/II patients, those with high-risk factors of recurrence require more treatment to reduce the risk of postoperative progression.[1 3–5] The high-risk criteria are difficult to identify, since the currently acknowledged prognostic clinicopathological features are not optimal in guiding adjuvant therapy.[6 7] Identifying prognostic markers for stage I/II colon cancer remains a significant clinical problem.

An increasing number of recent studies are focusing on the role of chemokines and chemokine receptors (CRs) in tumour progression.[8 9] The interactions of chemokines and CRs are critical in promoting tumour migration and organ-specific metastasis.[10 11] CXCR3 and CXCR4 have been demonstrated to be strongly related to tumour progression in advanced colorectal cancer.[12–14] However, their prognostic value in stage I/II colon cancer is still unclear.

The aim of this study was to investigate whether CXCR3 and CXCR4 can be used as independent prognosticators for predicting the oncological outcome of stage I/II colon cancer.

## PATIENTS AND METHODS

### Patients

From a cohort of 860 consecutive patients with colon cancer who underwent curative

resection at Peking University Cancer Hospital between January 2000 and December 2007, 213 patients were identified as having stage I/II disease. Thirty per cent (n=63) underwent adjuvant chemotherapy and were not included in the cohort. Patients with ulcerative colitis, familial adenomatous polyposis, hereditary non-polyposis colorectal cancer-associated cancers and pure neuroendocrine tumours were excluded. Patients who underwent emergency surgery because of complete bowel obstruction or perforation were also excluded from the cohort. The study finally comprised 145 patients with stage I/II colon cancer who underwent curative surgery without preoperative or postoperative chemotherapy. All clinical and pathological data were available. Follow-up data were obtained from the institutional registration and follow-up database. Patients were followed regularly, with routine physical examination, serum carcinoembryonic antigen (CEA), colonoscopic surveillance, X-ray, ultrasonography and CT scans. We chose the end of the fifth year after surgery as the time-terminus. The primary end point (event) of this study was tumour progression including local recurrence and metachronous distant metastasis. Patients were treated as a censored observation if they outlived closure of follow-up without tumour progression.

All patients provided written, informed consent to agree to donate their tumour tissues for research.

### Pathological assessment

For each case, sections of formalin-fixed and paraffin-embedded specimens were stained with H&E before optical microscopy examination by one of the authors. Standard pathological analysis was performed on all resection specimens. Each colonic tumour was restaged according to the American Joint Committee on Cancer (AJCC) Cancer Staging Manual, seventh edition. Pathological features including histological differentiation, depth of tumour invasion and lymphovascular invasion were documented and reported. The number of retrieved lymph nodes was also recorded for each case as a potential prognostic factor for analysis. Macroscopic margins were assessed at surgery and microscopic margins were assessed histopathologically to ensure R0 resection.

### Immunohistochemistry

All specimens were immunostained for CXCR3 and CXCR4 (1:100 dilution, R&D Systems, USA). Histological slices of 4 µm thickness were cut from the formalin-fixed, paraffin-embedded tumour tissues and mounted on cationic slides. After dewaxing and rehydration, sections were subjected to heat antigen retrieval in citrate buffer solution (pH 6.0) and heated for 3 min (103 kPa, 120°C). After cooling, 0.5% hydrogen peroxide was applied to block the activation of endogenous peroxidase, and sections were then rinsed five times with phosphate-buffered saline (PBS). The sections were then incubated at 37°C with a primary antibody for CXCR3 or CXCR4 for 1 h. After PBS rinses, sections were incubated at 37°C with a biotinylated secondary antibody (Zymed, USA) for 30 min and with avidin-biotin prediluted biotinylated complex for 10 min at room temperature. The antigenic sites were revealed by adding diaminobenzidine solution. The sections were then washed with distilled water, slightly counterstained with haematoxylin, dehydrated and mounted. The immunohistochemistry images of CXCR3 and CXCR4 are shown in figure 1.

Results were evaluated by one senior pathologist who was blinded to the patient clinical data. Expressions of CXCR3 and CXCR4 were assessed as positive if atleast 1% of the tumour cells were clearly immunostained. The immunostaining intensity of CXCR3 and CXCR4 was classified as previously reported[12 13]: negative, <1% of cells staining positive; weak staining (1–2+), 1–50% of cells staining positive and strong staining (3+), >50% of cells staining positive. The patients were categorised as high expression if the staining intensity was 3+, they were considered as low expression.

### Statistical analysis

Data were analysed using the statistical software SPSS V.16.0 (SPSS Inc., Chicago, Illinois, USA). Differences between categorical variables were assessed using the $\chi^2$ test. Survival curves were generated by the Kaplan–Meier method, and disease-free survival (DFS) rates were compared by the log-rank test. For the multivariate Cox proportional hazards regression analysis, a stepwise backward elimination method was used. p Values in this study were two-sided and $p<0.05$ was considered statistically significant in all univariate analyses, whereas $p<0.1$ has statistical significance in Cox proportional hazards regression for retaining in the equation.

## RESULTS

### Patient baseline characteristics and expression of CXCR3 and CXCR4

Of the 145 patients included, 84 were male and 61 were female. The mean age was 69 years (median 69; range 21–82). Median follow-up was 68.5 months (range 6–120). Ten patients (6.9%) were lost to follow-up and their data were included in the DFS analysis until the date of loss. The percentage of strong, weak and negative immunohistochemistry staining of tumour specimens was 17.9% (26/145), 52.4% (76/145) and 29.7% (43/145), respectively, for CXCR3; and 38.6% (56/145), 44.1% (64/145) and 17.2% (25/145), respectively, for CXCR4. There were no significant differences between patients regarding CXCR3 or CXCR4 expression for gender, age, tumour location, histological differentiation, T-stage, lymphovascular invasion and pretreatment serum CEA. High expression of CXCR4 was significantly more prevalent in patients with <12 retrieved lymph nodes than for patients with ≥12 retrieved lymph nodes (p=0.008), whereas there was no association between CXCR3 and the retrieved lymph node number. The

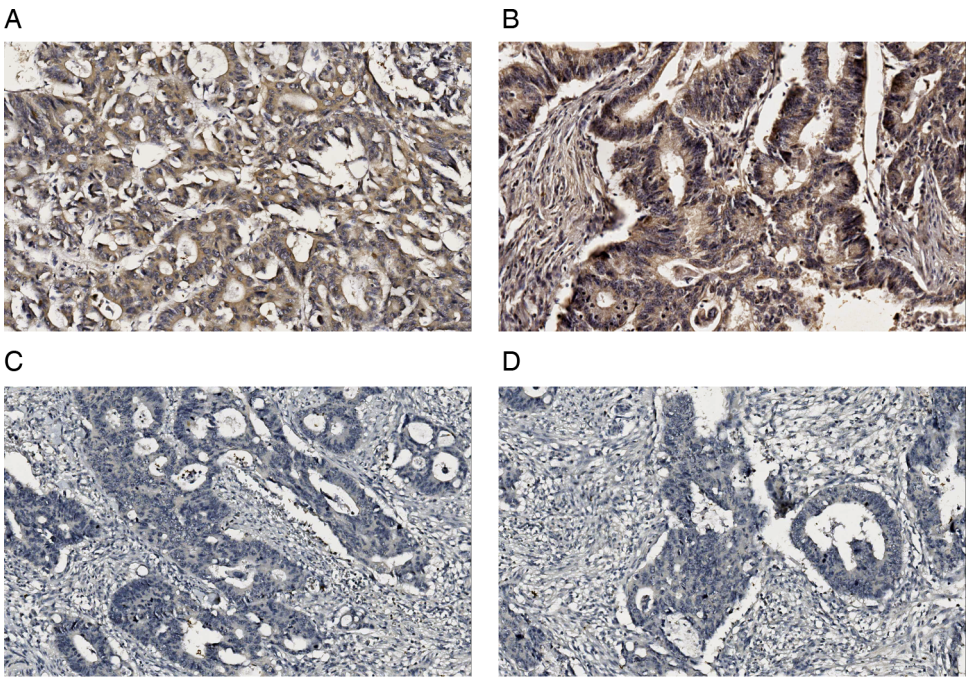


**Figure 1** Immunohistochemical staining of CXCR3 and CXCR4 in primary colon cancer (original magnification ×200). Panel A and B illustrate high expression of CXCR3 and CXCR4, respectively. Panel C and D illustrate low expression of CXCR3 and CXCR4, respectively.

**Table 1** Relationship between CXCR expression and clinicopathological variables

| | | CXCR3 expression | | | CXCR4 expression | | |
|---|---|---|---|---|---|---|---|
| **Variable** | **n** | **High (%) (n=26)** | **Low (%) (n=119)** | **p Value** | **High (%) (n=56)** | **Low (%) (n=89)** | **p Value** |
| Gender | | | | | | | |
| Male | 84 | 18 (69.2) | 66 (55.5) | 0.198 | 33 (58.9) | 51 (57.3) | 0.847 |
| Female | 61 | 8 (30.8) | 53 (44.5) | | 23 (41.1) | 38 (42.7) | |
| Age (year) | | | | | | | |
| <65 | 58 | 7 (26.9) | 51 (42.9) | 0.133 | 19 (33.9) | 39 (43.8) | 0.237 |
| ≥65 | 87 | 19 (73.1) | 68 (57.1) | | 37 (66.1) | 50 (56.2) | |
| Tumour location | | | | | | | |
| Right side | 69 | 13 (50) | 56 (47.1) | 0.786 | 27 (48.2) | 42 (47.2) | 0.904 |
| Left side | 76 | 13 (50) | 63 (52.9) | | 29 (51.8) | 47 (52.8) | |
| Histological differentiation | | | | | | | |
| Well | 23 | 6 (23.1) | 17 (14.3) | 0.554 | 8 (14.3) | 15 (16.9) | 0.802 |
| Moderate | 95 | 14 (53.8) | 81 (68.1) | | 39 (69.6) | 56 (62.9) | |
| Poor | 17 | 4 (15.4) | 13 (10.9) | | 5 (8.9) | 12 (13.5) | |
| Mucinous and signet | 10 | 2 (7.7) | 8 (6.7) | | 4 (7.1) | 6 (6.7) | |
| T-stage | | | | | | | |
| T1-2 | 25 | 5 (19.2) | 20 (16.8) | 0.945 | 9 (16.1) | 16 (18.0) | 0.529 |
| T3 | 93 | 16 (61.5) | 77 (64.7) | | 34 (60.7) | 59 (66.3) | |
| T4 | 27 | 5 (19.2) | 22 (18.5) | | 13 (23.2) | 14 (15.7) | |
| Number of retrieved lymph nodes | | | | | | | |
| <12 | 63 | 14 (53.8) | 49 (41.2) | 0.238 | 32 (57.1) | 31 (34.8) | 0.008 |
| ≥12 | 82 | 12 (46.2) | 70 (58.8) | | 24 (42.9) | 58 (65.2) | |
| Lymphovascular invasion | | | | | | | |
| Yes | 10 | 1 (3.8) | 9 (7.6) | 0.691 | 4 (7.1) | 6 (6.7) | 0.926 |
| No | 135 | 25 (96.2) | 110 (92.4) | | 52 (92.9) | 83 (93.3) | |
| Serum CEA (ng/mL) | | | | | | | |
| <5 | 90 | 15 (57.7) | 75 (63.0) | 0.612 | 35 (62.5) | 55 (61.8) | 0.932 |
| ≥5 | 55 | 11 (42.3) | 44 (37.0) | | 21 (37.5) | 34 (38.2) | |

CEA, carcinoembryonic antigen.

**Table 2** Association between tumour progression and expression of CXCR3 and CXCR4

| Variable | n | Tumour progression | | p Value |
|---|---|---|---|---|
| | | Yes (%) (n=27) | No (%) (n=118) | |
| CXCR3 expression | | | | |
| High expression | 26 | 6 (22.2) | 20 (16.9) | 0.579 |
| Low expression | 119 | 21 (77.8) | 98 (83.1) | |
| CXCR4 expression | | | | |
| High expression | 56 | 13 (48.1) | 43 (36.4) | 0.260 |
| Low expression | 89 | 14 (51.9) | 75 (63.6) | |

demographic and clinicopathological characteristics are summarised in table 1.

### DFS analysis

Postoperative tumour progression was seen in 27 patients. There were no significant associations between tumour progression and the expression of CXCR3 and CXCR4 (table 2). The 5-year DFS rate for all patients was 81.4%. No significant difference was found in DFS between CXCR3 high-expression groups and low-expression groups (76.9% and 82.4%, respectively; p=0.572; figure 2A), and CXCR4 (76.8% and 84.3%, respectively; p=0.248; figure 2B). Subgroup analysis revealed no significant differences of DFS with regard to CXCR3 expression with respect to tumour location, histological differentiation, tumour, node, metastases (TNM) stage, histological differentiation, lymphovascular invasion and pretreatment serum CEA. With respect to CXCR4, the only statistical difference of DFS in the well-differentiated subgroup was a significantly decreased 5-year DFS rate in patients with CXCR4 high-expression tumours (table 3).

### Multivariate analysis of DFS

Clinicopathological variables were analysed using Cox proportional hazards regression, which involved gender, age, tumour location, histological differentiation, TNM stage, number of retrieved lymph nodes, lymphovascular invasion, pretreatment serum CEA, CXCR3 and CXCR4. Multivariate analysis showed that serum CEA and a number of retrieved lymph nodes were independently associated with DFS (table 4). CXCR3 and CXCR4 were not independent prognosticators for stage I/II colon cancer.

## DISCUSSION

Chemokines are structurally related, small-polypeptide signalling molecules that bind to and activate a family of G-protein-coupled receptors.[9] The interaction of chemokines and CRs are crucial in promoting tumour cell proliferation, angiogenesis and migration.[8 14 15] High expression of CRs in malignant tumours is a strong

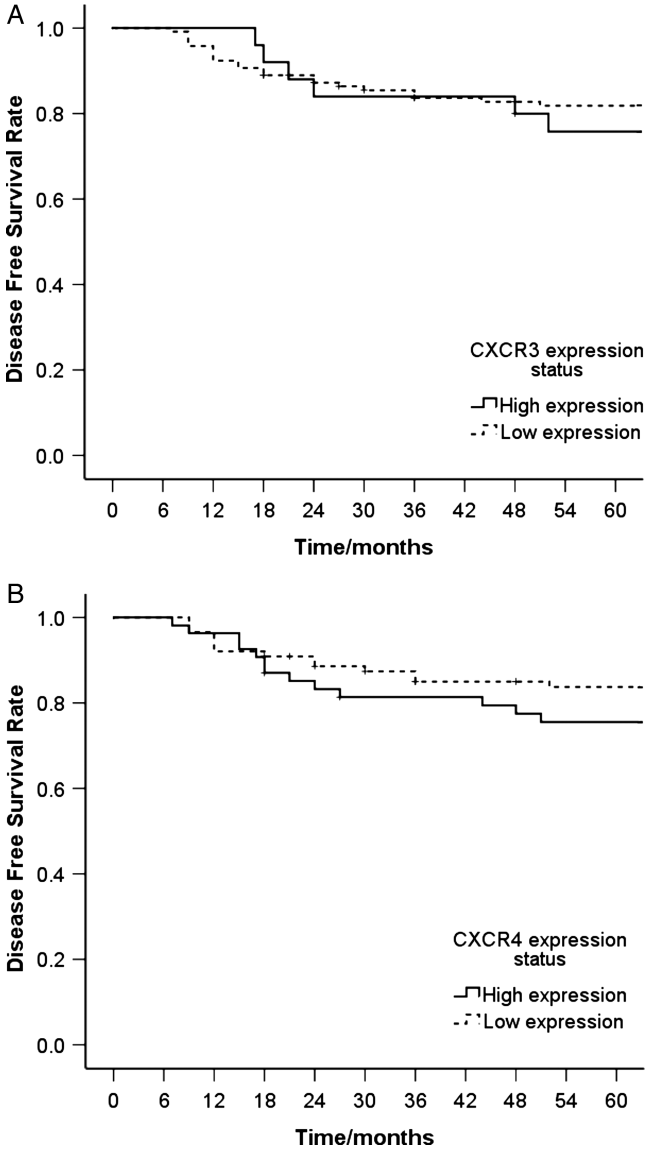


**Figure 2** Disease-free survival (DFS) of included patients with colon cancer related to CXCR3 and CXCR4 expression. Panel A and B present DFS of patients with different expression status of CXCR3 and CXCR4, respectively.

prognosticator for disease progression and poor prognosis.[16–18] With respect to colon cancer, in vitro and in vivo studies conducted within the past decade have confirmed the significance of CXCR3 and CXCR4 in tumour aggression and metastasis.[12 19–21] CXCR3-specific ligands CXCL9 and CXCL10, as well as CXCR4 ligand CXCL 12, secreted by distant organs including the liver and lung facilitate cancer cell metastasis and increase the risk of postoperative recurrence.[12 21 22] However, most studies addressed advanced colon cancer, rather than early stage disease; studies concerning the prognostic significance of CR in stage I/II colon cancer are limited. The prognostic value of CR in stage I/II disease is significantly higher than that in stage III/IV colon cancer; whatever the CR status is, adjuvant chemotherapy would be mandatory for stage III/IV disease, while for stage I/II colon cancer

**Table 3** Subgroup analysis of 5-year disease-free survival according to CXCR expressions for each clinicopathological variable

| Variable | n | CXCR3 expression | | | CXCR4 expression | | |
|---|---|---|---|---|---|---|---|
| | | High (%, n=26) | Low (%, n=119) | p Value | High (%, n=56) | Low (%, n=89) | p Value |
| Tumour location | | | | | | | |
| Right side | 69 | 69.2 | 78.6 | 0.545 | 74.1 | 78.6 | 0.673 |
| Left side | 76 | 84.6 | 85.7 | 1.000 | 79.3 | 89.4 | 0.252 |
| Histological differentiation | | | | | | | |
| Well | 23 | 83.3 | 82.4 | 0.938 | 62.5 | 93.3 | 0.049 |
| Moderate | 95 | 71.4 | 81.5 | 0.485 | 76.9 | 82.1 | 0.611 |
| Poor and Mucinous | 27 | 83.3 | 85.7 | 0.904 | 88.9 | 83.3 | 0.714 |
| TNM stage | | | | | | | |
| I | 25 | 100 | 90 | 0.470 | 88.9 | 93.8 | 0.673 |
| IIA | 93 | 75 | 81.8 | 0.592 | 79.4 | 81.4 | 0.869 |
| IIB | 27 | 60 | 77.3 | 0.573 | 61.5 | 85.7 | 0.150 |
| Lymphovascular invasion | | | | | | | |
| Yes | 10 | 100 | 88.9 | 0.705 | 100 | 83.3 | 0.439 |
| No | 135 | 76 | 81.8 | 0.606 | 75 | 84.3 | 0.203 |
| Serum CEA (ng/mL) | | | | | | | |
| <5 | 90 | 93.3 | 90.7 | 0.687 | 85.7 | 94.5 | 0.149 |
| ≥5 | 55 | 54.5 | 68.2 | 0.477 | 61.9 | 67.6 | 0.768 |

CEA, carcinoembryonic antigen; TNM, tumour, node, metastases

radical surgery may be the unique therapy since adjuvant chemotherapy could only benefit those patients with high-risk factors of tumour progression.[4 5] Thus, if the expressions of CXCR3 or CXCR4 were proved to be risk factors for early stage colon cancer, they might become potential indicators for postoperative chemotherapy. Wu *et al*[12] once reported that although CXCR3 expression was strongly associated with distant metastasis and postoperative overall survival of colon cancer' it failed to demonstrate the prognostic value of CXCR3 for stage I/II disease. Kim *et al*[21] investigated CXCR4 expression in 125 patients including 57 with stage I/II disease. Although the study reported a correlation of the high expression of CXCR4 with increased tumour progression in stage I/II cancer, no definite conclusion was possible for several reasons. First, the sample size was too small to gain a reliable result. Second, this study examined rectal cancer and colon, whereas the treatment and prognosis of stage II rectal cancer are completely different with stage II colon cancer.[1 23] The same problems also existed in other studies concerning CR.[13]

The present study is the first to investigate the prognostic value of CXCR3 and CXCR4 in stage I/II colon cancer. A similar published study by Zhang *et al*[24] once mentioned that CXCR4 expression was closely associated with overall survival in stages II-III colon cancer, and it suggested that the coexpression of CXCR4 and CD133 was an efficient prognosticator. However, this study did not provide a further subgroup analysis to eliminate the prognostic value of CXCR4 in stage II patients. Our study demonstrated that CXCR3 and CXCR4 were not prognostic factors of postoperative tumour progression in stages I-II colon cancer. Although in the subgroup of well-differentiated tumour, patients with high expression of CXCR4 presented a tendency of decreased DFS, a

**Table 4** Multivariate analysis of disease-free survival by Cox proportional hazards regression (backward method)

| Variable | HR | 95% CI | p Value* |
|---|---|---|---|
| Gender | | | |
| Male | 1 | | |
| Female | 0.614 | 0.263 to 1.433 | 0.259 |
| Age | | | |
| <65 | 1 | | |
| ≥65 | 1.009 | 0.974 to 1.046 | 0.603 |
| Tumour location | | | |
| Right side | 1 | | |
| Left side | 0.622 | 0.279 to 1.390 | 0.247 |
| Histological differentiation | | | |
| Well | 1 | | |
| Moderate | 2.19 | 0.338 to 14.191 | 0.411 |
| Poor and Mucinous | 0.909 | 0.117 to 7.085 | 0.927 |
| TNM stage | | | |
| I | 1 | | |
| IIA | 1.246 | 0.768 to 1.587 | 0.163 |
| IIB | 2.431 | 0.862 to 2.982 | 0.331 |
| Lymphovascular invasion | | | |
| No | 1 | | |
| Yes | 3.398 | 0.453 to 25.497 | 0.234 |
| Number of retrieved lymph nodes | | | |
| <12 | 1 | | |
| ≥12 | 0.955 | 0.907 to 1.006 | 0.085 |
| Serum CEA | | | |
| <5 | 1 | | |
| ≥5 | 4.516 | 1.975 to 10.330 | <0.001 |
| CXCR3 | | | |
| High expression | 1 | | |
| Low expression | 1.1 | 0.422 to 2.869 | 0.845 |
| CXCR4 | | | |
| High expression | 1 | | |
| Low expression | 0.813 | 0.362 to 1.830 | 0.618 |

*p<0.1 indicates statistical significance.
CEA, carcinoembryonic antigen; TNM, tumour, node, metastases.

definitive conclusion was not possible due to the ambiguous p value.

CXCR3 and CXCR4 have been considered as potential therapeutic targets in many studies. Several in vitro studies reported that inhibiting the interactions of chemokine and CXCR3 or CXCR4 using antibodies or small molecules significantly reduced the metastasis of colorectal cancer cells[24 25]; this approach could become a promising therapy for colon cancer. However, the present results suggest that the antagonisms for CXCR3 and CXCR4 may not work on stage I/II disease, since the expressions of CXCR3 and CXCR4 did not affect postoperative DFS. On the other hand, the positive rates of these two CRs were significantly lower in stage I/II disease compared with stage III/IV disease.[12 13] Admittedly, the present study has its own limitations of retrospective studies and is subject to patient selection biases. However, it provides important information that targeted treatment of CXCR3 or CXCR4 positive patients may be more appropriate for patients with advanced colon cancer.

In conclusion, CXCR3 and CXCR4 do not predict postoperative tumour progression in stage I/II colon cancer.

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**competing interests** None.

**Ethics approval** This study was approved by Peking University Cancer Hospital ethics.

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