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Expression of CXCR-4 and IDO in human colorectal cancer: An immunohistochemical approach

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Abstract. C-X-C chemokine receptor type 4 (CXCR4), the receptor for the chemokine stromal cell-derived factor (SDF)-1 [also known as C-X-C motif chemokine 12 (CXCL12)], is involved in lymphocyte trafficking. Recent studies have demonstrated that, during pregnancy, a placental enzyme called indoleamine 2, 3-dioxygenase (IDO) exerts a key role in suppressing the maternal T-cell response against the fetus. In the present study, the significance of CXCR4 and IDO expression in human colorectal cancer (CRC) has been investigated by immunohistochemical assay, and their association with survival was analyzed. Tumor specimens (n=60) from patients with different American Joint Committee on Cancer (AJCC) stages of CRC (I or IV) were assessed. In the stage IV group, 23 of 30 cases (77%) stained positive for CXCR4, and 9 of 30 (30%) were positive for IDO. By contrast, in the stage I group, 7 of 30 cases (23%) stained positive for CXCR4, and 15 of 30 cases (50%) were positive for IDO. The 5-year survival rate of those with high CXCR4 expression in tumor specimens (n=30) was significantly worse compared with those with negative CXCR4 expression (16.3 vs. 60.7%, P=0.02). By contrast, the 5-year survival rate of those with high IDO expression in tumor specimens (n=24) was not significantly different compared with those with negative IDO expression (36.4 vs. 56.8%). In the stage I group, 4 patients in the high IDO expression group (n=15) had distant metastases (2 in the liver 1 in the brain, and 1 in the lung). Taken together, CXCR4 appears to be a novel predictive indicator of survival, and IDO expression in the early stage may be a predictor of distant metastasis.

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Introduction

Colorectal cancer (CRC) is the third most common cancer worldwide, and the fourth most common with respect to cancer-associated mortality (1). Surgical excision offers the only potential cure; however, tumor recurrence is comparatively common, even following R0 resection. The 5-year survival rate for patients with stage II CRC is 80-90%, whereas that of those with stage IV is only 10-20% (2). Clinically useful prognostic biomarkers are required to improve the outcome of patients with stage IV CRC. C-X-C chemokine receptor type 4 (CXCR4), a G-protein-coupled chemokine receptor encoded on chromosome 2 (3), exerts its biological effect by binding stromal cell-derived factor 1 (SDF-1) (4); a previous report has also demonstrated that ubiquitin is a natural ligand of CXCR4 (5). Chemokinetic functions in the immune system, chemokines and their receptors also exert critical roles in tumor initiation, progression and metastasis (6). CRC cells have been shown to express the chemokine receptors, CXCR4 and CXCR3 (7-12). In a clinical study, CXCR4 expression in CRC was revealed to increase the recurrence of the cancer, poor survival and liver metastasis (8).

In mammalian organisms, tryptophan is an essential amino acid for cell survival. Tryptophan degradation occurs via the kynurenine pathway, with the initial and rate-limiting catalyzing activity of indoleamine 2,3-dioxygenase (IDO). Initially, the role of IDO was considered to be predominantly antimicrobial in reducing the availability of tryptophan in the inflammatory environment (13-16). IDO was expressed in normal tissues, such as the endothelial cells in the placenta, the epithelial cells in the female genital tract, and the lymphoid tissues in mature dendritic cells (17). IDO occupies a central role in preventing T cell-driven rejection of allogeneic fetuses during pregnancy as the trophoblasts are expressed. IDO was found to induce maternal tolerance to fetal allografts (18). The role of IDO in the immune escape of tumors has been reviewed previously by Zou (19). This suggests that IDO inhibitors may exert antitumor effects by suppressing immune tolerance. IDO expression in human CRC has also been reported (20-22); however, the clinical significance of IDO expression in CRC remains a controversial topic. The aim of the present study was to evaluate the association between CXCR4, IDO and CRC. In addition, the present study also aimed to further investigate a possible prognostic role of IDO and CXCR4 expression

in cases of CRC at stage I and IV of CRC, according to the American Joint Committee on Cancer (AJCC) staging system.

Materials and methods

Patient selection. Patients who underwent surgery for AJCC stage I or IV CRC were selected consecutively at the Department of Surgery, the Jikei University School of Medicine between January and November 2003. A total of 60 specimens were assessed from 60 patients (Table I). No stage I patients in the present cohort received any adjuvant chemotherapy, whereas all the eligible patients with stage IV CRC received fluorouracil- and leucovorin-based chemotherapy.

Immunohistochemical analysis. For the immunohistochemical study, formalin-fixed, paraffin-embedded sections were used. Immunostaining was performed using the labeled, streptavidin-biotin peroxidase complex method associated with the Ventana auto-immunostaining system (Ventana Japan, K.K., Yokohama, Japan), according to the manufacturer's protocol. Murine monoclonal antibodies against human IDO (23) (anti-IDO; dilution, 1:1,000) and against CXCR4 (anti-CXCR4, clone no. 44716; dilution, 1:200; R&D Systems, Inc., Minneapolis, MN, USA) were used. The antigen retrieval procedure was performed in a microwave oven using DAKO® Antigen Retrieval Solution (Dako; Agilent Technologies, Inc., Santa Clara, CA, USA) for 10 min at 95°C to efficiently stain the samples. The sections (Dako Cytomation; Dako; Agilent Technologies, Inc.) were developed with 3,3'-diaminobenzidine with 0.3% hydrogen peroxide, and counterstained with hematoxylin. In all cases, normal epithelial cells were negative for CXCR4 and IDO expression. Neoplastic cells with cytoplasmic and/or membrane-localized immunohistochemical expression of CXCR4 were considered positive cells. Macrophage positivity was used as an adequate internal positive control for each case in order to validate the technical procedure. CXCR4 staining was categorized into four semiquantitative groups based on the rate of stained (positive) tumor cells: Absence of staining; <10% positive cells (low); 10-50% positive cells (moderate); and >50% positive cells (high) (Fig. 1). On the other hand, IDO staining was categorized into two classes: Positive or negative (Fig. 2). Slides were evaluated by two blinded observers (M.O. and M.I.); discordant cases were discussed, and a concordance of opinion was subsequently achieved.

Statistical analysis. Correlations between CXCR4 and IDO expression, baseline patient features, and tumors were studied using contingency tables and the Chi-square test. Overall survival (OS) was defined as the time that had elapsed from the date of the initial diagnosis to mortality, or to the date of the last available information on the patient's vital status. The Kaplan-Meier product limit method was applied to draw the OS curves. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Expression rate. In the stage I group, 7 out of 30 cases (23%) stained positive for CXCR4, and 15 of 30 cases (50%) were positive for IDO. In the stage IV group, 23 of 30 cases (77%)

Table I. Patients' characteristics.

Parameter	Data
Age, years [median (range)]	68 (41-82)
Sex [no. of patients (%)]	
Male	36 (60)
Female	24 (40)
Primary site [no. of patients (%)]	
Colon/rectosigmoid	38 (63.3)
Rectum	22 (26.7)
Stage[a] [no. of patients (%)]	
I	30 (50)
IV	30 (50)

[a]Stages are according to the American Joint Committee on Cancer (AJCC) staging system.

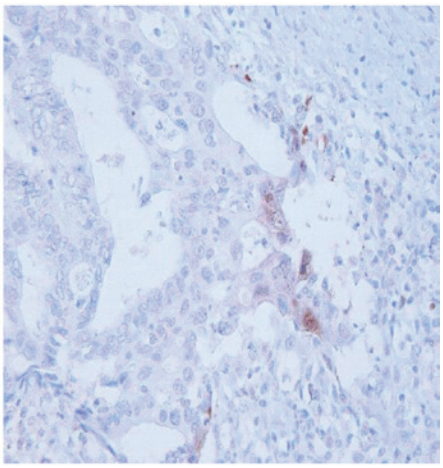

Figure 1. Neoplastic cells with immunohistochemical expression of CXCR4, located in the cytoplasm and/or the membrane, are shown. Cells that revealed immunohistochemical expression of CXCR4 were considered positive. A calculation of 10% positive cells was made, placing the number of positive cells into the 'low' category. CXCR4, C-X-C chemokine receptor type 4.

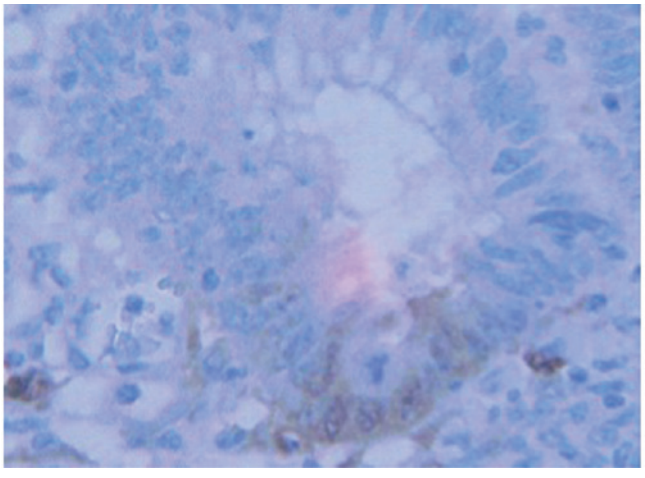

Figure 2. Cells that revealed immunohistochemical expression of IDO were considered positive. The positive cancerous cells were recognized in the submucosal layer. The case illustrated in this figure is T1 case. IDO, indoleamine 2,3-dioxygenase.

Table II. Expression rates of CXCR4 and IDO.

Stage[a]	Protein	Negative (%)	Positive (Low/moderate/high)[b] (%)
Stage I	CXCR4	23 (77)	7 (23) (7/0/0)
	IDO	15 (50)	15 (50)
Stage IV	CXCR4	7 (23)	23 (77) (7/3/13)
	IDO	21 (70)	9 (30)

[a]Stages are according to the American Joint Committee on Cancer (AJCC) staging system. [b]The classification of positive cells as low, moderate and high is only applicable for the CXCR4 data. CXCR4, C-X-C chemokine receptor type 4; IDO, indoleamine 2,3-dioxygenase

stained positive for CXCR4, and 9 of 30 cases (30%) were positive for IDO (Table II).

Association between the 5-year survival rate and the expression levels of CXCR4 and IDO for the tumor specimens. The 5-year survival rate of those patients with a high level of CXCR4 expression in tumor specimens (n=30) was significantly worse compared with those with negative CXCR4 expression (16.3 vs. 60.7%, P=0.02) (Fig. 3). The 5-year survival rate of those with high IDO expression in tumor specimens (n=24) was not significantly different compared with those with negative IDO expression (36.4 vs. 56.8%) (Fig. 4). However, in the stage I group for IDO, although none of the patients in the high IDO expression group (n=15) had negative expression, 4 patients had highly distant metastases (2 in the liver, 1 in the brain, and 1 in the lung) (27%: 4/15). Therefore, CXCR4 appears to be a novel predictive indicator of survival, and high IDO expression in the early stage may be a predictor of distant metastasis.

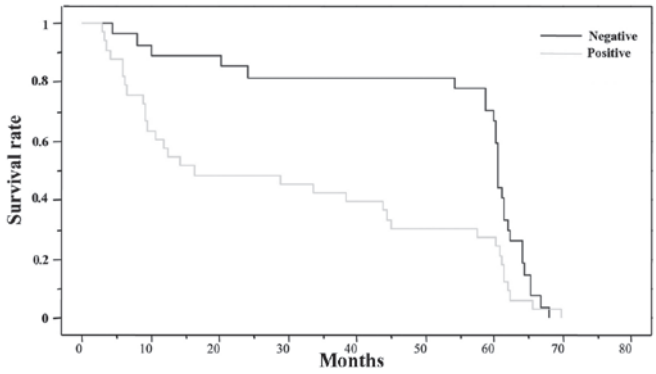

Figure 3. The 5-year survival rate of those patients with high CXCR4 expression in tumor specimens (n=30) was significantly worse compared with those with negative CXCR4 expression (16.3 vs. 60.7%, P=0.02).

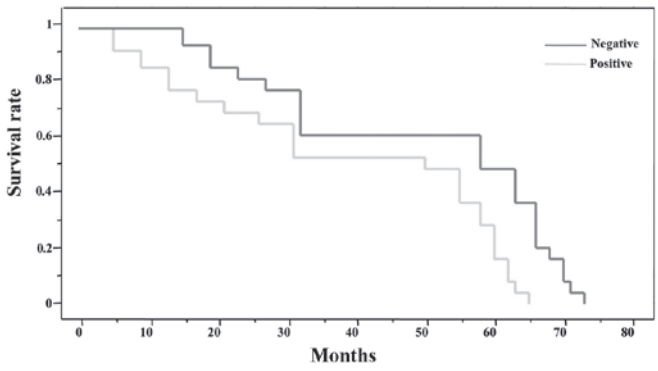

Figure 4. The 5-year survival rate of those patients with high IDO expression in tumor specimens (n=24) was not significantly different compared with those with negative IDO expression (36.4 vs. 56.8%).

Discussion

Chemokines are structurally related, small-polypeptide signaling molecules that bind to and activate a family of G-protein-coupled receptors (22). The interaction of chemokines and CRs is crucial in promoting tumor cell proliferation, angiogenesis and migration (24-26). The chemokine receptor, CXCR4, is the first identified chemokine receptor to exert a critical role in determining the metastatic destination of breast cancer to the bone and lungs, where its ligand, C-X-C motif chemokine 12 (CXCL12), is abundant (27,28). In clinical studies, the expression of CXCR4 in CRC was demonstrated to increase the recurrence of the cancer, poor survival and liver metastasis (8). The CXCR4 ligand, CXCL12, was secreted by distant metastasis, and this increased the risk of postoperative recurrence (29). The present study has demonstrated that CXCR4 was a prognostic factor of postoperative tumor progression. The high expression of CXCR4 presented a significant difference with respect to the decreased survival rate. CXCR4 has been considered as a potential therapeutic target in several studies. A number of *in vitro* studies have reported that inhibiting the interactions of chemokine CXCR4 using antibodies or small molecules markedly reduces the metastasis of colorectal cancer cells (30,31); this approach could become a promising therapy for colon cancer.

Uyttenhove *et al* (32) provided the first evidence for a tumor-immune resistance mechanism, based on tryptophan degradation in a murine model in which these authors demonstrated that the immunomodulatory enzyme, IDO, reduced anti-tumor T cell attack. Muller *et al* (33) stated that IDO activity in tumor cells is the relevant target for inhibition to overcome immune escape (33). Thus, it appears plausible that

the identity of IDO-expressing cells may depend on the type of tumor. Okamoto *et al* (34) reported a marked association between IDO-staining patterns within tumor cells and OS in patients with serous ovarian cancer. According to the data in the present study, IDO expression significantly correlated with the distant metastases in early-stage CRC. In early-stage CRC, within the patients who expressed a high level of IDO, the initial immunological response of the host towards the primary tumor may be able to generate tolerizing conditions. As a result, IDO-expressing tumor cells may be able to stave off an immune attack. Thus, if the expression of IDO was demonstrated to be a risk factor for early-stage colon cancer, this population of cells might become a potential indicator for postoperative chemotherapy. Additional, large cohort studies in a multicenter setting are necessary to validate these findings, and to examine the potential mechanisms for ligand interactions concerning IDO and CXCR4 in CRC metastasis.

In conclusion, CXCR4 appears to be a novel predictive indicator of survival, and IDO expression in the early stages of colon cancer may be a predictor of distant metastasis.

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