Nuclear Expression of Chemokine Receptor CXCR4 Indicates Poorer Prognosis in Gastric Cancer

TAKANOBU MASUDA[1], YUICHIRO NAKASHIMA[1], KOJI ANDO[1], KEIJI YOSHINAGA[1], HIROSHI SAEKI[1], EIJI OKI[1], MASARU MORITA[1], YOSHINAO ODA[2] and YOSHIHIKO MAEHARA[1]

[1]*Department of Surgery and Sciences, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan*
[2]*Department of Anatomic Pathology, Pathological Sciences, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan*

Abstract. *Background: The CXCL12/CXCR4 axis plays a pivotal role in cancer progression and metastases in various epithelial cancer cells. The aim of the present study was to evaluate the localization and correlation between CXCL12/CXCR4 expression and clinicopathological features in gastric cancers. Materials and Methods: This study included 111 Japanese patients with primary gastric cancers, which invade submucosa or more, all of whom underwent gastrectomy between 1992 and 1996. Immunohistochemical analysis was performed. Results: A significant correlation was found in the immunoreactivity of nuclear CXCR4 and poor differentiation (p=0.0026), infiltrated pattern (p<0.0001), larger size (p<0.0001), advanced stage (p=0.0342) and reduced 5-year survival rate (30% vs. 61%, p=0.0012). Multivariate analysis revealed that high nuclear CXCR4 immunoreactivity (RR: 3.077, p=0.0329) retained its strength as an independent prognostic factor for overall survival. Conclusion: High immunoreactivity of nuclear CXCR4 in gastric cancer suggests that CXCL12 binds to its unique receptor CXCR4 at the membrane, translocates to the nucleus and then becomes more invasive, and thus can be considered a prognostic factor.*

Chemokine CXCL12, also called stromal cell-derived factor (SDF)-1α is a member of the CXC sub-family and exerts an effect though its specific receptor CXCR4 (1). Chemokine receptor CXCR4 is a G-protein-coupled receptor, which is characterized by a seven-transmembrane-spanning domain. CXCL12 and CXCR4 play a critical role in the behavior of cancer cells and modulate cell migration, proliferation and survival. CXCL12 and its unique receptor CXCR4 are expressed in various epithelial cancer cells and associated with tumor biology (2-4).

Müller *et al*. (5) first showed that the chemokine receptor CXCR4 was highly expressed in human breast cancer cells and that activation of breast cancer cells with CXCL12 induced chemotaxis and tissue invasion *in vitro*. They also showed that neutralizing the interaction of CXCL12 and CXCR4 significantly impaired metastasis of breast cancer cells to regional lymph nodes and lung *in vivo*, suggesting that chemokines and their receptors have a critical role in determining the metastatic destination of tumor cells. Recently, many reports showed that the CXCL12/CXCR4 axis plays a pivotal role in cancer progression and metastasis in breast (6-8), colorectal (9-13) and lung cancers (14, 15).

As for gastric cancer, the serum or malignant ascitic fluids from patients with advanced disease have been reported to contain high levels of CXCL12 (16, 17). Ishigami *et al*. reported that the CXCL12 immunoreactivity correlated to metastases or poor prognosis (18). However, there have been other controversial reports indicating no correlation to node metastasis or recurrence (19) or reduced expression of CXCL12 mRNA (20). In the same way, CXCR4 positivity correlated with metastases and prognosis (17, 21, 22), whereas sometimes not correlated (19, 23). As many discussion points are left unanswered, we evaluated the CXCL12 and CXCR4 immunoreactivity from the intracellular localization viewpoint.

Materials and Methods

Patients studied. The present study included 111 Japanese patients with primary gastric cancer, which invade submucosa or more, all of whom underwent gastrectomy between 1992 and 1996 at the Department of Surgery and Science (Surgery II), Kyushu University Hospital, Fukuoka. The study group included 83 men and 28 women ranging from 28 to 86 years of age (mean, 62.9 years). No patient

Correspondence to: Takanobu Masuda, MD, Hiroshima Red Cross Hospital & Atomic-bomb Survivors Hospital, 1-9-6, Senda-machi, Naka-ku, Hiroshima, 730-8619, Japan. Tel: +81 822413111, Fax: +81 822460676, e-mail: nbmasuda@surg2.med.kyushu-u.ac.jp

Key Words: CXCL12, immunohistochemistry, translocation.

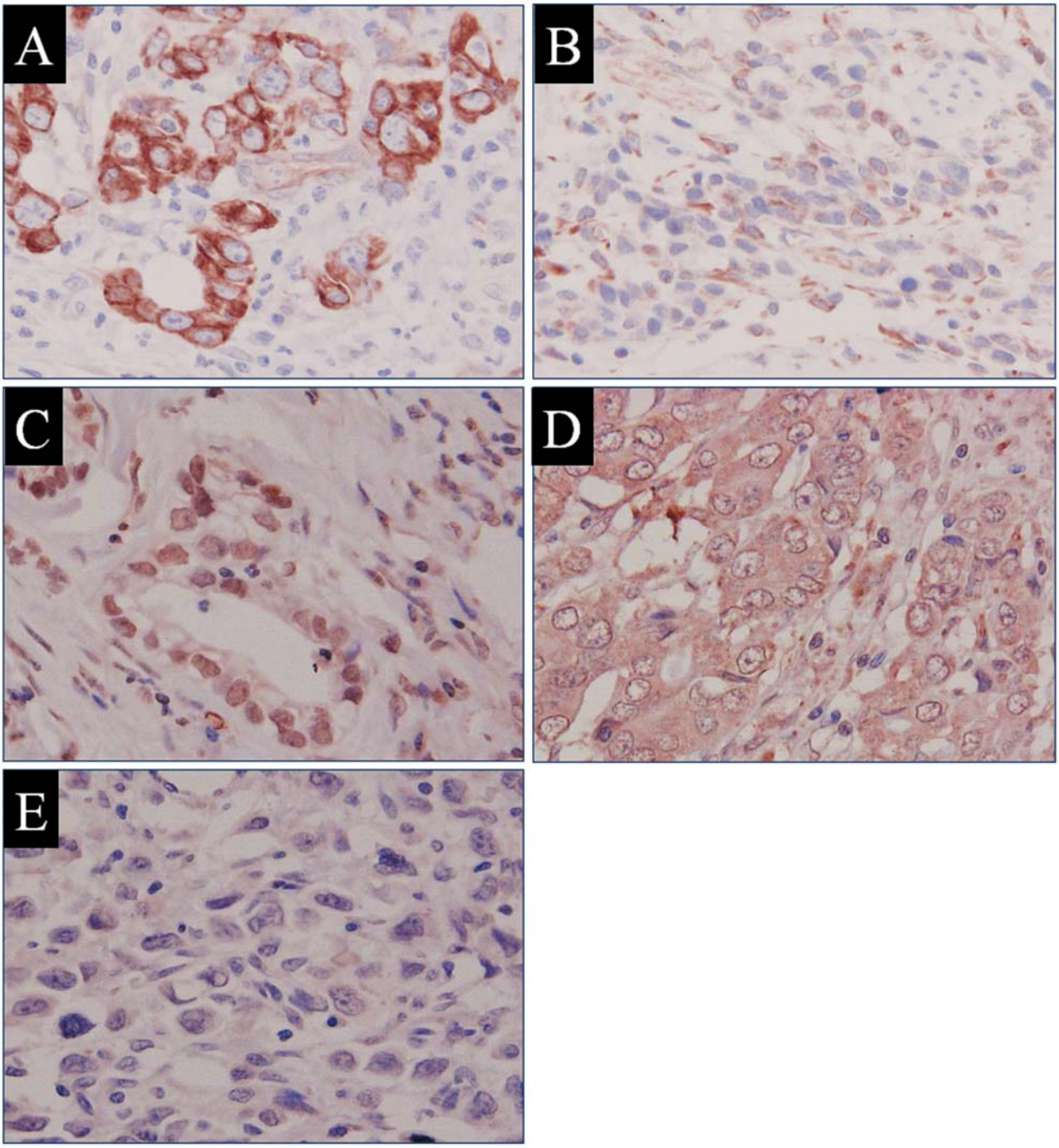

Figure 1. *Immunohistochemistry for CXCL12 (A, B) and CXCR4 (C, D, E) in gastric cancer tissue (magnification ×100). (A) Strong type: CXCL12 is strongly detected in the membrane and cytoplasm of cancer cells. (B) Weak type: CXCL12 is not detected in cancer cells. (C) Nuclear staining: CXCR4 is strongly detected in the nucleus of cancer cells. (D) Cytoplasmic staining: CXCR4 is detected in the cytoplasma of cancer cells. (E) No staining: No CXCR4 is detected in cancer cells.*

treated preoperatively with cytotoxic drugs was included in this study. The median follow-up period was 42.9 months (range, 0.77 to 60). Pathological features are classified based on the Japanese classification of Gastric cancer, 12th edition (24).

Immunohistochemistry. The avidin-biotin complex method was used for CXCL12 and CXCR4 immunohistochemical staining. Tumor specimens were collected and fixed in 10% formalin. Sections, 5-μm-thick from paraffin-embedded blocks, were de-paraffinized in

xylene and rehydrated in a graded series of ethanols. These sections were heated in 0.01 M citrate buffer (pH6) for 20 minutes at 99˚C for CXCL12 and 10 minutes at 99˚C for CXCR4 to retrieve antigen activity and then cooled at room temperature.

After quenching the endogenous peroxidase activity in methanol containing 3% hydrogen peroxidase for 30 min, the endogenous biotin-avidin was blocked using an endogenous biotin-avidin blocking kit (Nichirei Corp., Tokyo, Japan). Then the sections were incubated with 10% normal rabbit serum for 10 min to block any nonspecific binding of the immunoreagents. The sections were first incubated with mouse anti-human CXCL12 monoclonal antibody (8 μg/ml; clone 79018; R&D system, Minneapolis, MN, USA) for CXCL12 and mouse anti-human CD184 (CXCR4) monoclonal antibody (5 μg/ml; clone 12G5; BD Pharmingen, Franklin Lakes, NJ, USA) for CXCR4 at 4˚C overnight. A Histofine Simple stain PO (M) kit (Nichirei Corp., Tokyo, Japan) was used. The sections were incubated with biotinylated rabbit anti-mouse IgG+IgA+IgM antibody (Nichirei Corp.) for 20 min and subsequently treated with peroxidase labeled streptavidin for 20 min. The sections were developed with diaminobenzidine (DAB) and lightly counterstained with Mayer's hematoxylin and mounted. Careful rinses were performed with several changes of phosphate-buffered saline between each stage of the procedure. Negative controls were obtained by substituting the primary antibody with phosphate-buffered saline.

The evaluation of CXCL12 and CXCR4 expression was performed by two pathologists (Y.N, T.M) without knowledge of the patients' clinicopathological features using light microscopy. Therefore, since the role of these chemokines, CXCL12 and its receptor CXCR4, is invasion and proliferation, we evaluated the invasive front of the tumor by immunostaining . The evaluation of CXCL12 staining was categorized into strong and weak types: staining intensity was stronger or equivalent than positive control, weaker or no staining. CXCR4 staining was evaluated in terms of nuclear expression and cytoplasmic expression.

Statistical analysis. The association of CXCL12 and CXCR4 expression with clinicopathological features was assessed using the Fisher's exact test and Student's *t*-test. Survival rates were visualized by applying Kaplan-Meier curves, and *p*-values were determined by the log-lank test. Multivariate analysis was performed by applying the logistic regression method for the determination of prognostic factors. A *p*-value of less than 0.05 was considered significant. All statistical analyses were conducted using the StatView 5.0 software (SAS institute Inc., San Francisco, CA, USA).

Results

Immunohistochemical staining of CXCL12 in gastric cancer tissue sections revealed predominance in membrane over the cytoplasm (Figure 1A, B). The positive CXCL12 staining was recognized in 98 out of 111 cases (88%). There were no significant differences in histology, depth of invasion, lymph node metastasis, lymphatic invasion, venous invasion, infiltration pattern and stage between high and low CXCL12 expression groups (Table I). Overall survival remained also unaffected (Figure 2A).

CXCR4 staining was seen in the cytoplasm and/or nucleus (Figure 1C, D, E). The respective positive expression rate for

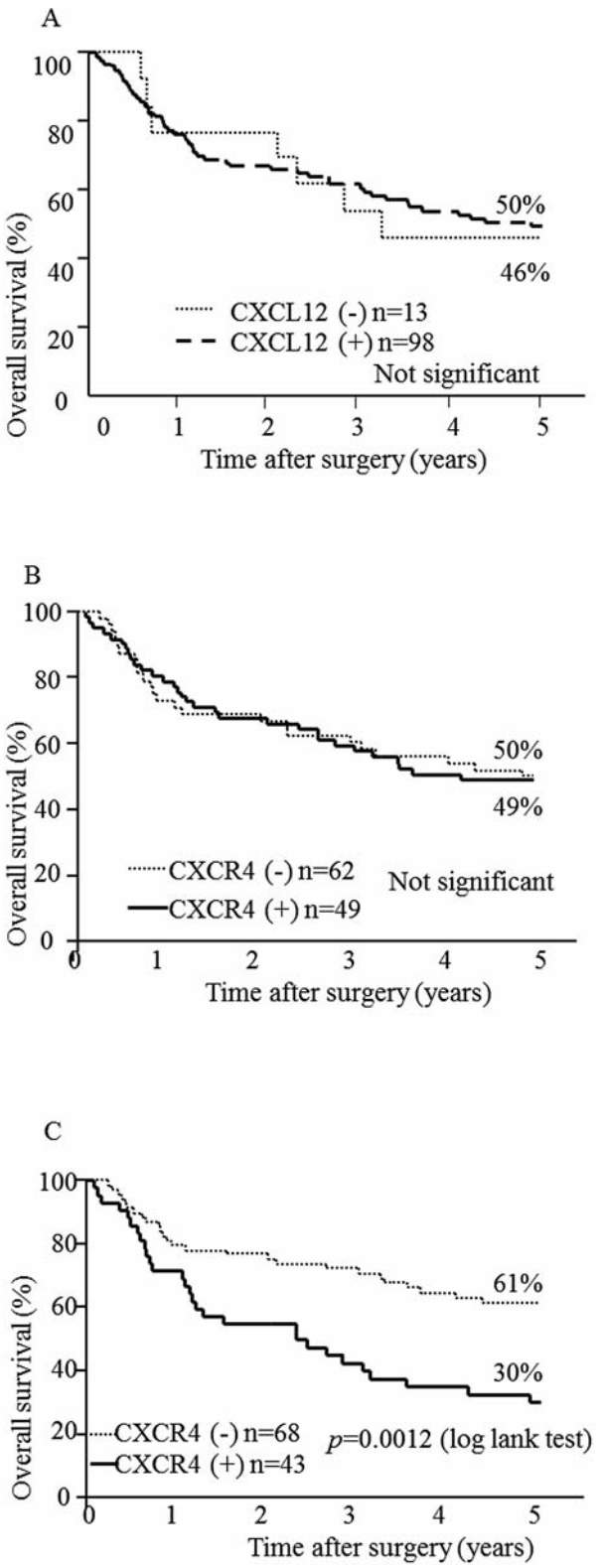

Figure 2. *Overall survival according to CXCL12 and CXCR4 immunoreactivity. (A) CXCL12 immunoreactivity. (B) Cytoplasmic CXCR4 immunoreactivity. (C) Nuclear CXCR4 immunoreactivity.*

Table I. *Correlation between CXCL12 immunoreactivity and clinicopathological features.*

Factor	CXCL12 immunoreactivity		
	Low (n=13)	High (n=98)	*p*-Value
Age			
mean (SD)	59.6 (9.3)	63.3 (12.6)	ns
Gender			
male	10	73	
female	3	25	ns
Tumor location			
Esophagus	0	1	
Upper third	4	23	
Middle third	6	33	
Lower third	3	35	
Whole	0	1	
Tumor location			
Anterior	2	14	
Posterior	4	15	
Greater	1	12	
Lesser	1	28	
Circle	5	24	
Macroscopic type			
0	1	27	
I	0	3	
II	3	11	
III	4	27	
IV	1	20	
V	4	5	
Differentiation			
well/mode	4	39	
por/sig	9	59	ns
Depth of invasion			
SM, MP, SS	5	53	
SE, Si	8	45	ns
Size			
mean (SD)	9.6 (3.7)	7.0 (4.8)	0.0594
Lymphatic invasion			
absent	6	55	
present	7	43	ns
Venous invasion			
absent	10	65	
present	3	33	ns
Infiltration pattern			
alpha	0	15	
beta	7	41	
gamma	6	42	ns
Lymph node metastasis			
absent	3	39	
present	10	59	ns
Liver metastasis			
absent	12	94	
present	1	4	ns
Peritoneal dissemination			
absent	11	93	
present	2	5	ns
Stage			
I, II	5	48	
III, IV	8	50	ns

SD: Standard Deviation, ns: not significant, well: well differentiated adenocarcinoma, mode: moderately differentiated adenocarcinoma, por: poorly differentiated adenocarcinoma, sig: signet-ring cell carcinoma, SM: submucosa, MP: muscularis propria, SS: subserosa, Si: tumor invasion of adjacent structures.

cytoplasmic CXCR4 was 44% (49/111) and 39% (43/111) for the nuclear CXCR4 one. There was no correlation between cytoplasmic and nuclear CXCR4 expression. There were no significant differences between high and low cytoplasmic CXCR4 expression in histology, depth of invasion, size, lymphatic invasion, venous invasion, infiltration pattern, lymph node metastasis, stage (Table II) and survival (Figure 2B). Notably, in contrast to cytoplasmic CXCR4 staining, nuclear CXCR4 staining was significantly higher in the undifferentiated cancer ($p=0.0026$), larger in size ($p<0.0001$), infiltrated pattern ($p<0.0001$) and advanced stage ($p=0.0342$) (Table II). Patients with high nuclear CXCR4 expression showed a reduced 5-year survival rate compared to that in patients with low nuclear CXCR4 expression (30% *vs.* 61%), as depicted in the survival curve ($p=0.0012$) shown in Figure 2C. Using logistic regression analysis, high nuclear CXCR4 immunoreactivity (RR: 3.077, $p=0.0329$) retained its strength as an independent prognostic factor for overall survival, as lymph node metastasis (RR: 7.299, $p=0.0002$) and lymphatic invasion (RR: 4.202, $p=0.0054$) did (Table III).

Discussion

The CXCL12/CXCR4 axis was initially found to be stimulated by the homing of lymphocytes to inflammatory tissues and has recently been found to be involved in many areas of immunology and human development, including organogenesis, vascularisation, haematopoiesis and embryogenesis (25). Within hypoxic areas of tumors, both CXCL12 expression by carcinoma-associated fibroblasts (CAFs) and CXCR4 expression on tumor cells increase, which stimulates tumor cell motility and invasiveness (2). Fibroblast-derived CXCL12 promotes tumorigenesis by two major mechanisms. First, CXCL12 promotes tumor cell growth by directly stimulating tumor cell growth *via* CXCR4 in a paracrine fashion. Secondly, CXCL12 from CAFs induces recruitment of endothelial progenitors, which allow for tumor angiogenesis in an endocrine fashion. Targeted metastasis to the marrow or other sites of high CXCL12 expression involves CXCR4 activation on circulating tumor cells that "hijack" the CXCR4/CXCL12 axis for homing to microenvironments that normally are restricted to haematopoietic progenitor cells (HPCs) (2).

In gastric cancer, Ishigami *et al.* reported that CXCL12 in the cellular membrane of cancer cells may give cancer cells themselves more aggressive behavior in an autocrine fashion (18). High concentration of CXCL12 in serum (16) or malignant ascitic fluids (17) from patients with gastric cancer has also been reported. However, our study could not show any correlation between CXCL12 expression both in cancer cells and fibroblasts. Shibuta *et al.* reported reduced expression of *CXCR4* mRNA in gastric cancer (20). From these findings, immunohistochemistry of CXCL12 in cancer

Table II. *Correlation between cytoplasmic and nuclear CXCR4 immunoreactivity and clinicopathological features.*

Factor	Cytoplasmic immunoreactivity			Nuclear immunoreactivity		
	Low (n=62)	High (n=49)	*p*-Value	Low (n=68)	High (n=43)	*p*-Value
Age						
mean(SD)	64.7 (11.8)	60.6 (12.6)	0.0426	64.1 (10.6)	60.9 (14.4)	ns
Gender						
male	44	39		56	27	
female	18	10	ns	12	16	0.0259
Tumor location						
Esophagus	0	1		0	1	
Upper third	14	13		16	11	
Middle third	22	17		23	16	
Lower third	24	14		25	13	
Whole	1	0		0	1	
Tumor location						
Anterior	8	8		9	7	
Posterior	12	7		14	5	
Greater	8	5		11	2	
Lesser	14	15		20	9	
Circle	19	10		10	19	
Macroscopic type						
0	16	12		21	7	
I	0	3		2	1	
II	10	4		11	3	
III	18	13		19	12	
IV	11	10		5	16	
V	6	3		6	3	
Differentiation						
well/mode	22	21		34	9	
por/sig	40	28	ns	34	34	0.0026
Depth of invasion						
SM, MP, SS	35	23		41	17	
SE, Si	27	26	ns	27	26	0.0506
Size						
mean(SD)	7.2 (5.1)	7.4 (4.3)	ns	5.8 (3.3)	9.6 (5.8)	<0.0001
Lymphatic invasion						
absent	31	30		41	20	
present	31	19	ns	27	23	ns
Venous invasion						
absent	45	30		46	29	
present	17	19	ns	22	14	ns
Infiltration pattern						
alpha	7	8		14	1	
beta	29	19		36	12	
gamma	26	22	ns	18	30	<0.0001
Lymph node metastasis						
absent	25	17		29	13	
present	37	32	ns	39	30	ns
Liver metastasis						
absent	60	46		65	41	
present	2	3	ns	3	2	ns
Peritoneal dissemination						
absent	57	47		65	39	
present	5	2	ns	3	4	ns
Stage						
I, II	34	19		38	15	
III, IV	28	30	ns	30	28	0.0342

SD: Standard Deviation, ns: not significant, well: well differentiated adenocarcinoma, mode: moderately differentiated adenocarcinoma, por: poorly differentiated adenocarcinoma, sig: signet-ring cell carcinoma, SM: submucosa, MP: muscularis propria, SS: subserosa, Si: tumor invasion of adjacent structures.

Table III. *Multivariate analysis for overall survival.*

Factors	Status	RR	95%CI	*p*-Value
Differentiation	well/mode *vs.* por/sig	1.164	0.407-3.333	0.7764
Depth of invasion	SM, MP, SS *vs.* SE, Si	1.297	0.476-2.100	0.6104
Lymphatic invasion	absent *vs.* present	4.202	1.527-11.628	0.0054
Venous invasion	absent *vs.* present	1.021	0.351-2.976	0.9688
Lymph node metastasis	absent *vs.* present	7.299	2.564-20.833	0.0002
Nuclear CXCR4 immunoreactivity	low *vs.* high	3.077	1.095-8.621	0.0329

RR: relative risk, CI: Confidence interval, well: well differentiated adenocarcinoma, mode: moderately differentiated adenocarcinoma, por: poorly differentiated adenocarcinoma, sig: signet-ring cell carcinoma, SM: submucosa, MP: muscularis propria, SS: subserosa, Si: tumor invasion of adjacent structures.

cells may show fibroblast-derived CXCL12 binding to its receptor in cancer cells, while CXCR4-positive cancer cells are reported to be associated with lymphatic (21) or haematogenous (22) metastases and peritoneal dissemination (17). All these reports have evaluated the expression of CXCR4 in the cellular membrane or cytoplasm of cancer cells. We evaluated the localization of CXCR4 expression in both cytoplasm and nucleus. The nuclear CXCR4 positivity is often recognized in poorly differentiated adenocarcinoma, which tended to be large and of the infiltrative type, resulting with patients' poor prognosis.

Binding of CXCL12 to CXCR4 results in receptor dimerization and endosomal internalization of the receptor–ligand complex (26). CXCR4 found in cancer cell was not limited to the cell membrane but was also observed frequently in the cytoplasm and, occasionally, in the nucleus. Some recent studies reported that CXCL12/SDF-1α stimulation could trigger CXCR4 internalization and subsequently CXCR4 endocytosis in renal cell carcinoma (27) and colon cancer (13) cell lines. CXCR4 nuclear localization in renal cell carcinoma (A-498) cells was found associated with increased Matrigel matrix invasion, a metastatic trait (27). In clinical settings, nuclear CXCR4 significantly correlated with lymph node metastasis in breast cancer (28) and colorectal cancer (11), suggesting that nuclear expression of CXCR4 may play a role in the progression of cancer. From our data in this study, nuclear CXCR4 expression in the primary gastric cancer may reflect increased potential for infiltration and poor outcome. Further investigation is necessary to elucidate the functional mechanism of CXCL12/CXCR4 axis for its effective application in cancer treatment.

Conclusion

We investigated the immunoreactivity and intracellular localization of CXCL12 and CXCR4 in gastric cancer tissue in an immunohistochemical manner. In gastric cancer microenvironment, it is suggested that CXCL12 binds to its unique receptor CXCR4 at the membrane level and then translocates to the nucleus becoming more invasive resulting, thus, as a prognostic factor.

Acknowledgements

We appreciate Y. Kubota for the paraffin sections and advice for immunohistochemistry.

References

1 Tachibana K, Hirota S, Iizasa H, Yoshida H, Kawabata K, Kataoka Y, Kitamura Y, Matsushima K, Yoshida N, Nishikawa S, Kishimoto T and Nagasawa T: The chemokine receptor CXCR4 is essential for vascularization of the gastrointestinal tract. Nature *393(6685)*: 591-594, 1998.

2 Burger JA and Kipps TJ: CXCR4: a key receptor in the crosstalk between tumor cells and their microenvironment. Blood *107(5)*: 1761-1767, 2006.

3 Ratajczak MZ, Zuba-Surma E, Kucia M, Reca R, Wojakowski W and Ratajczak J: The pleiotropic effects of the SDF-1-CXCR4 axis in organogenesis, regeneration and tumorigenesis. Leukemia *20(11)*: 1915-1924, 2006.

4 Arya M, Patel HR and Williamson M: Chemokines: key players in cancer. Curr Med Res Opin *19(6)*: 557-564, 2003.

5 Müller A, Homey B, Soto H, Ge N, Catron D, Buchanan ME, McClanahan T, Murphy E, Yuan W, Wagner SN, Barrera JL, Mohar A, Verástegui E and Zlotnik A: Involvement of chemokine receptors in breast cancer metastasis. Nature *410(6824)*: 50-56, 2001.

6 Salvucci O, Bouchard A, Baccarelli A, Deschênes J, Sauter G, Simon R, Bianchi R and Basik M: The role of CXCR4 receptor expression in breast cancer: a large tissue microarray study. Breast Cancer Res Treat *97(3)*: 275-283, 2006.

7 Mirisola V, Zuccarino A, Bachmeier BE, Sormani MP, Falter J, Nerlich A and Pfeffer U: CXCL12/SDF1 expression by breast cancers is an independent prognostic marker of disease-free and overall survival. Eur J Cancer *45(14)*: 2579-2587, 2009.

8 Woo SU, Bae JW, Kim CH, Lee JB and Koo BW: A significant correlation between nuclear CXCR4 expression and axillary lymph node metastasis in hormonal receptor negative breast cancer. Ann Surg Oncol *15(1)*: 281-285, 2008.

9 Kim J, Takeuchi H, Lam ST, Turner RR, Wang HJ, Kuo C, Foshag L, Bilchik AJ and Hoon DS: Chemokine receptor CXCR4 expression in colorectal cancer patients increases the risk for recurrence and for poor survival. J Clin Oncol *23(12)*: 2744-2753, 2005.
10 Ottaiano A, Franco R, Aiello Talamanca A, Liguori G, Tatangelo F, Delrio P, Nasti G, Barletta E, Facchini G, Daniele B, Di Blasi A, Napolitano M, Ieranò C, Calemma R, Leonardi E, Albino V, De Angelis V, Falanga M, Boccia V, Capuozzo M, Parisi V, Botti G, Castello G, Vincenzo Iaffaioli R and Scala S: Overexpression of both CXC chemokine receptor 4 and vascular endothelial growth factor proteins predicts early distant relapse in stage II-III colorectal cancer patients. Clin Cancer Res *12(9)*: 2795-2803, 2006.
11 Yoshitake N, Fukui H, Yamagishi H, Sekikawa A, Fujii S, Tomita S, Ichikawa K, Imura J, Hiraishi H and Fujimori T: Expression of SDF-1 alpha and nuclear CXCR4 predicts lymph node metastasis in colorectal cancer. Br J Cancer *98(10)*: 1682-1689, 2008.
12 Speetjens FM, Liefers GJ, Korbee CJ, Mesker WE, van de Velde CJ, van Vlierberghe RL, Morreau H, Tollenaar RA and Kuppen PJ: Nuclear localization of CXCR4 determines prognosis for colorectal cancer patients. Cancer Microenviron *2(1)*: 1-7, 2009.
13 Wang SC, Lin JK, Wang HS, Yang SH, Li AF and Chang SC: Nuclear expression of CXCR4 is associated with advanced colorectal cancer. Int J Colorectal Dis *25(10)*: 1185-1191, 2010.
14 Na IK, Scheibenbogen C, Adam CStroux A, Ghadjar P, Thiel E, Keilholz U and Coupland SE: Nuclear expression of CXCR4 in tumor cells of non-small cell lung cancer is correlated with lymph node metastasis. Hum Pathol *39(12)*: 1751-1755, 2008.
15 Wagner PL, Hyjek E, Vazquez MF, Meherally D, Liu YF, Chadwick PA, Rengifo T, Sica GL, Port JL, Lee PC, Paul S, Altorki NK and Saqi A: CXCL12 and CXCR4 in adenocarcinoma of the lung: association with metastasis and survival. J Thorac Cardiovasc Surg *137(3)*: 615-621, 2009.
16 Woo IS, Hong SH, Byun JH, Kang JH, Jeon HM and Choi MG: Circulating stromal cell derived factor-1alpha (SDF-1alpha) is predictive of distant metastasis in gastric carcinoma. Cancer Invest *26(3)*: 256-261, 2008.
17 Yasumoto K, Koizumi K, Kawashima A, Saitoh Y, Arita Y, Shinohara K, Minami T, Nakayama T, Sakurai H, Takahashi Y, Yoshie O and Saiki I: Role of the CXCL12/CXCR4 axis in peritoneal carcinomatosis of gastric cancer. Cancer Res *66(4)*: 2181-2187, 2006.
18 Ishigami S, Natsugoe S, Okumura H, Matsumoto M, Nakajo A, Uenosono Y, Arigami T, Uchikado Y, Setoyama T, Arima H, Hokita S and Aikou T: Clinical implication of CXCL12 expression in gastric cancer. Ann Surg Oncol *14(11)*: 3154-3158, 2007.
19 Tsuboi K, Kodera Y, Nakanishi H, Ito S, Mochizuki Y, Nakayama G, Koike M, Fujiwara M, Yamamura Y and Nakao A: Expression of CXCL12 and CXCR4 in pT3-stage gastric cancer does not correlate with peritoneal metastasis. Oncol Rep *20(5)*: 1117-1123, 2008.
20 Shibuta K, Begum NA, Mori M, Shimoda K, Akiyoshi T and Barnard GF: Reduced expression of the CXC chemokine hIRH/SDF-1alpha mRNA in hepatoma and digestive tract cancer. Int J Cancer *73(5)*: 656-662, 1997.
21 Lee HJ, Kim SW, Kim HY, Li S, Yun HJ, Song KS, Kim S and Jo DY: Chemokine receptor CXCR4 expression, function, and clinical implications in gastric cancer. Int J Oncol *34(2)*: 473-480, 2009.
22 Iwasa S, Yanagawa T, Fan J and Katoh R: Expression of CXCR4 and its ligand SDF-1 in intestinal-type gastric cancer is associated with lymph node and liver metastasis. Anticancer Res. *29(11)*: 4751-4758, 2009.
23 Kwak MK, Hur K, Park DJ, Lee HJ, Lee HS, Kim WH, Lee KU, Choe KJ and Yang HK: Expression of chemokine receptors in human gastric cancer. Tumour Biol *26(2)*: 65-70, 2005.
24 Japanese Gastric Cancer Association. Japanese classification of gastric carcinoma, 12th ed. Tokyo: Kanehara & Co., Ltd; 1993.
25 Murdoch C: CXCR4: chemokine receptor extraordinaire. Immunol Rev *177*: 175-184, 2000.
26 Signoret N, Rosenkilde MM, Klasse PJ, Schwartz TW, Malim MH, Hoxie JA and Marsh M: Differential regulation of CXCR4 and CCR5 endocytosis. J Cell Sci *111(Pt 18)*: 2819-2830, 1998.
27 Wang L, Wang Z, Yang B, Yang Q, Wang L and Sun Y: CXCR4 nuclear localization follows binding of its ligand SDF-1 and occurs in metastatic but not primary renal cell carcinoma. Oncol Rep *22(6)*: 1333-1339, 2009.
28 Kato M, Kitayama J, Kazama S and Nagawa H: Expression pattern of CXC chemokine receptor-4 is correlated with lymph node metastasis in human invasive ductal carcinoma. Breast Cancer Res *5(5)*: R144-150, 2003.

Received July 8, 2014
Revised August 6, 2014
Accepted August 8, 2014