

## The Clinicopathological Significance of the CXCR2 Ligands, CXCL1, CXCL2, CXCL3, CXCL5, CXCL6, CXCL7, and CXCL8 in Gastric Cancer

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**Abstract.** *Background/Aim:* We have previously reported that chemokine (C-X-C motif) receptor 2 (CXCR2) signaling was associated with the malignant progression of gastric cancer (GC). We thus examined the clinicopathological significance of CXCR2 ligands, CXCL1, CXCL2, CXCL3, CXCL5, CXCL6, CXCL7, and CXCL8, in GC. *Patients and Methods:* The expression of CXCR2 ligands in 590 GC cases was investigated by immunohistochemistry. *Results:* The expression was as follows: CXCL1, 46.2% (257/557); CXCL2, 20.7% (122/590); CXCL3, 17.1% (101/589); CXCL5/CXCL6, 2.9% (17/589); CXCL7, 36.4% (215/590); and CXCL8 1.7% (10/585) of the cases. High invasion depth was correlated with CXCL1 expression. Lymph node metastasis and peritoneal cytology positivity were correlated with high expression of CXCL1 and CXCL7. The prognoses of the CXCL1-positive patients were significantly poorer than those of the CXCL1-negative patients ( $p < 0.001$ ). *Conclusion:* Among the CXCR2 ligands, CXCL7 and especially CXCL1, might play an important role in the malignant progression of GC via CXCR2 signaling.

Cancer progression has been recognized as not only the proliferation of tumor cells, but also the interaction between cancer cells and the surrounding stroma in the tumor

microenvironment (1). Among the tumor stromal cells, fibroblasts (especially cancer-associated fibroblasts) have been reported to be closely associated with tumor development in various solid carcinomas (2-4). We reported that chemokine (C-X-C motif) receptor 2 (CXCR2) signaling might play an important role in the pathogenic construction of tumor fibroblasts in the gastric tumor microenvironment, suggesting that gastric cancer (GC) cells might alter their adjacent stroma to form a permissive environment for tumor progression (5, 6). Following those findings, we reported that C-X-C ligand 1 (CXCL1) from GC cells stimulated the recruitment of bone marrow cells into the GC microenvironment via CXCR2 signaling (7). The reported CXCR2 ligands are not only CXCL1 but also CXCL2, CXCL3, CXCL5, CXCL6, CXCL7, and CXCL8. Until now, there has been only a single *in vitro* study on the significance of these CXCR2 ligands in GC (8).

### Patients and Methods

*Clinical materials.* This study retrospectively analysed 590 GC patients who received gastrectomy at Osaka City University Hospital. GC tissues were obtained from each patient. The pathological diagnoses and classifications were made according to the Japanese classification of gastric carcinoma (15th edition). This study was approved by the Osaka City University Ethics Committee (approval number: 924). Written informed consent for research was obtained from all patients.

*Immunohistochemical determination of CXCL1, CXCL2, CXCL3, CXCL5, CXCL6, CXCL7, and CXCL8.* The immunohistochemical determination of CXCL1, CXCL2, CXCL3, CXCL5, CXCL6, CXCL7, and CXCL8 expression in gastric tumors was performed, as follows: We performed deparaffinization and slides were heated. After blocking endogenous peroxidase activity, the samples were incubated with anti-CXCL1 antibody (1:100; Abcam, Cambridge,

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**Key Words:** CXCR2 ligands, CXCL1, CXCL7, gastric cancer, tumor microenvironment.

UK), anti-CXCL2 antibody (1:200; Cloud-Clone Co., Katy, TX, USA), anti-CXCL3 antibody (1:200; Cloud-Clone Co.), anti-CXCL5/CXCL6 antibody (1:100; Santa Cruz, Dallas, TX, USA), anti-CXCL7 antibody (1:250; Santa Cruz), and anti-CXCL8 antibody (1:250; R&D systems, Minneapolis, MN, USA) for 1 h at room temperature. The samples were then incubated with biotinylated secondary antibody, followed by treatment with streptavidin-peroxidase reagent, and counterstaining with Mayer's hematoxylin. The expression levels of CXCL families were analyzed by both intensity of staining and percentage of stained cancer cells at the invading tumor front. CXCL1, CXCL2, CXCL3, CXCL5/CXCL6, CXCL7, and CXCL8 expression levels were evaluated as follows: the intensity was given scores 0-3 (0=no, 1=weak, 2=moderate, 3=intense), and the percentage of immune-positive cells was given scores 0-3 (CXCL1: 0=0%-10%, 1=11%-50%, 2=51%-80%, 3=81%-100%, CXCL2: 0=0%, 1=1%-20%, 2=21%-50%, 3=51%-100%, CXCL3: 0=0%, 1=1%-20%, 2=21%-60%, 3=61%-100%, CXCL5/6: 0=0%, 1=1%-10%, 2=11%-40%, 3=41%-100%, CXCL7: 0=0%-20%, 1=21%-50%, 2=51%-70%, 3=71%-100%, and CXCL8: 0=0%-20%, 1=21%-30%, 2=31%-50%, 3=51%-100%). The two scores multiplied to gain the final result of 0-9. Expression was considered positive when scores were CXCL1  $\geq 3$ , CXCL2  $\geq 3$ , CXCL3  $\geq 3$ , CXCL5/CXCL6  $\geq 6$ , CXCL7  $\geq 6$ , and CXCL8  $\geq 2$ . Evaluation was made by two double-blinded independent observers who were unaware of clinical data and outcome. When a different evaluation between the two independent observers was found, the evaluation was rechecked and discussed.

**Statistical analysis.** The chi-square test of Fisher's exact was used to decide the significance of difference between covariates. Survival periods were calculated with the Kaplan-Meier method and analyzed by log-rank test to compare cumulative survival durations among patient groups. In addition, the Cox proportional hazards model was used to compute univariate hazards ratios for the study parameters. The SPSS software program (SPSS Japan, Tokyo, Japan) was used for the analyses. A *p*-value  $<0.05$  indicated a statistically significant difference.

## Results

**Immunostaining of CXCL1, CXCL2, CXCL3, CXCL5/CXCL6, CXCL7, and CXCL8.** Staining of the members of the CXCL family was mainly at the cytoplasm and partially at the nuclei of cancer cells (Figure 1). In the 590 gastric tumors, the rates of positive expression were as follows: CXCL1, 258 (46.2%); CXCL2, 122 (20.7%); CXCL3, 101 (17.1%); CXCL5/6, 17 (2.9%); CXCL7, 215 (36.4%); and CXCL8, 10 (1.7%).

**The relationship between clinicopathological features and the expression of CXCL1, CXCL2, CXCL3, CXCL5/CXCL6, CXCL7, and CXCL8 in gastric cancer.** The clinicopathological characteristics of all 590 cases of GC according to their CXCL expression are summarized in Table I. The CXCL1-positive tumor group was significantly correlated with high age ( $p<0.001$ ), high T-stage ( $p<0.001$ ), lymph node metastasis ( $p<0.001$ ), lymphatic invasion ( $p<0.001$ ), and venous invasion ( $p<0.001$ ). The expression of CXCL2 in cancer cells was significantly correlated with low T-stage ( $p=0.013$ ), no lymph

node metastasis ( $p=0.033$ ), and no peritoneal cytology ( $p=0.01$ ). The expression of CXCL3 in the cancer cells was significantly correlated with low infiltration ( $p=0.002$ ). The expression of CXCL5/CXCL6 in the cancer cells was not significantly correlated with any clinicopathological features. The expression of CXCL7 in the cancer cells was significantly correlated with high age ( $p<0.001$ ), diffuse type ( $p=0.001$ ), lymph node metastasis ( $p=0.026$ ), lymphatic invasion ( $p=0.005$ ), venous invasion ( $p=0.023$ ), infiltration pattern ( $p<0.001$ ), and peritoneal cytology negativity ( $p=0.034$ ). The expression of CXCL8 in the cancer cells was not correlated with any clinicopathological features.

**Survival.** The 5-year overall survival rate of the patients in the CXCL1-positive expression group was significantly worse compared to that of the CXCL1-negative group ( $p<0.001$ , Figure 2A). CXCL1 expression did not predict the prognosis of the GC patients at any stage (data not shown). The Kaplan-Meier survival curve indicated no significant difference in the 5-year overall survival rate between the positive and negative patients in CXCL2, CXCL3, CXCL5/CXCL6, CXCL7, and CXCL8 expression (Figure 2B).

**Univariate and multivariate analyses of overall survival after surgery.** Table II provides the results of the univariate and multivariate analyses of the patients' overall survival. The univariate analysis revealed that poor survival was significantly correlated with CXCL1-positive expression ( $p<0.001$ ), macroscopic type 4 ( $p<0.001$ ), diffuse type ( $p<0.001$ ), high T invasion ( $p<0.001$ ), lymph node metastasis ( $p<0.001$ ), venous invasion ( $p<0.001$ ), infiltration type 'c' ( $p<0.001$ ), hepatic metastasis ( $p<0.001$ ), peritoneal metastasis ( $p<0.001$ ), other metastasis ( $p<0.001$ ), and age  $\geq 70$  years ( $p=0.002$ ). The multivariate analysis revealed that the factors that were significantly correlated with poor survival were CXCL1-positive expression ( $p=0.039$ ), macroscopic type 4 ( $p<0.001$ ), high T invasion ( $p=0.009$ ), lymph node metastasis ( $p<0.001$ ), hepatic metastasis ( $p=0.001$ ), peritoneal metastasis ( $p<0.001$ ), other metastasis ( $p<0.001$ ), and age  $\geq 70$  years ( $p=0.006$ ).

## Discussion

We investigated the expression of the CXCR2 ligands CXCL1, CXCL2, CXCL3, CXCL5/CXCL6, CXCL7, and CXCL8 in 590 gastric tumors. Expression on cancer cells was frequent for CXCL1 (46.2%), followed by CXCL7 (36.4%), CXCL2 (20.7%), and CXCL3 (17.1%). In contrast, CXCL5/CXCL6 (2.9%) and CXCL8 (1.7%) expression on GC cells was rare. CXCL1 has been reported to be expressed in 36%-56% of GC cases (8-10), and our present observation of a 46.2% CXCL1 expression rate is similar to these reports. CXCL1 has been described as a prognostic factor in

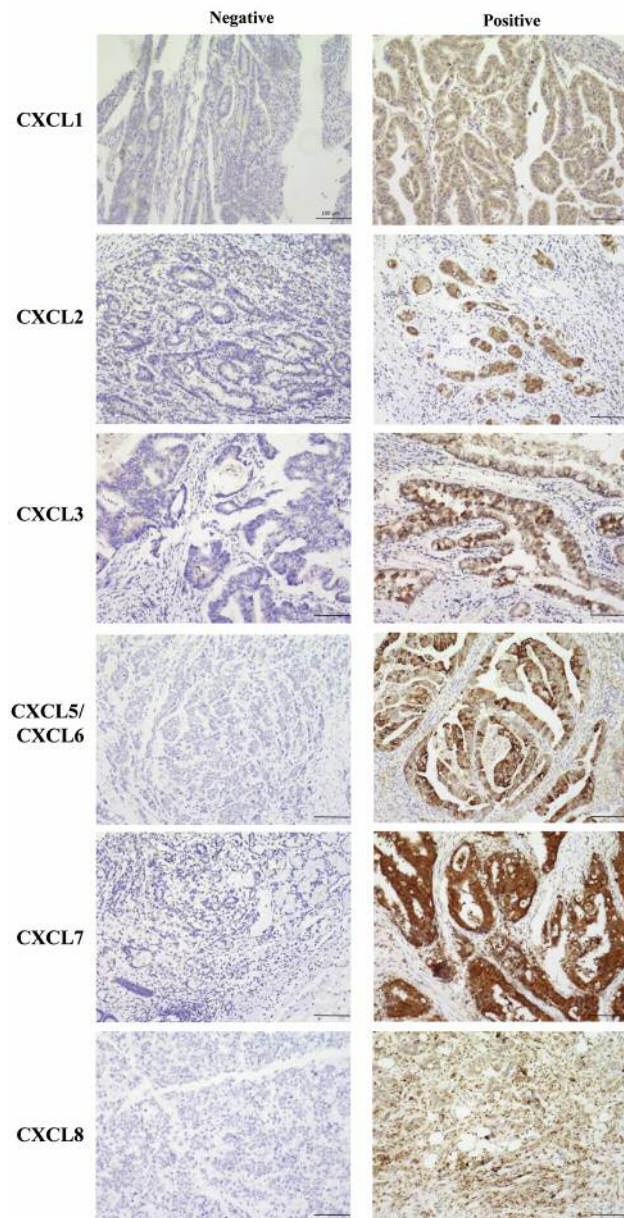


Figure 1. Representative images of CXCL1, CXCL2, CXCL3, CXCL5/6, CXCL7, and CXCL8 expression in gastric cancer. CXCL1, CXCL2, CXCL3, CXCL5/6, CXCL7 and CXCL8 were mainly expressed at the cytoplasm of cancer cells. ( $\times 200$ ). Bar: 100  $\mu$ m.

various cancers (11) including hepatocellular carcinoma (HCC) (12), laryngeal squamous cell carcinoma (13), and colorectal cancer (14). In our present series of GC patients, CXCL1 was correlated with poor prognosis, and CXCL1 expression was correlated with features of the malignant potential of cancer, *i.e.*, high T-stage, lymph node metastasis,

lymphatic invasion, and venous invasion. These findings indicated that CXCL1 might play an important role in the progression of GC.

Kaplan–Meier survival curves indicated that the 5-year overall survival rate of the GC patients in the CXCL1-positive group was significantly worse than that of the CXCL1-negative group. In addition, the multivariate analysis revealed that CXCL1 expression was an independent prognostic factor. These findings suggest that CXCL1 could be a useful prognostic factor for patients with GC.

The reported expression of CXCL7 in Wilms tumor ( $n=40$ ) was 87.5% (15). CXCL7 expression was detected in 36.4% of the GC cases in the present investigation, and was correlated with the diffuse type, lymph node metastasis, lymphatic invasion, and venous invasion, although no significant difference in the 5-year survival rate was found between the CXCL7-positive and CXCL7-negative groups. CXCL7 might be partially associated with the malignant potential of GC cells.

CXCL2 expression in tumors has been reported only in HCC (16); there are no reports of CXCL2 expression in other cancers, including GC. The rate of CXCL2 expression in our series of 590 gastric cancer cases was 20.7%. No significant difference in the 5-year survival rate was shown between the CXCL2-positive and CXCL2-negative groups, but the expression of CXCL2 was negatively correlated with T-stage, lymph node metastasis, and cytology. These findings indicate that CXCL2 might not play an important role in the progression of GC.

The rate of CXCL3 expression in cases of prostate cancer has been reported to be 21.7% (17). In the present study, the rate of CXCL3 expression in gastric cancer was 17.1%. We observed that CXCL3 expression was associated with the infiltration pattern but with no other clinicopathological factors, including prognosis. Together these findings suggest that CXCL3 does not play an important role in the progression of GC.

Our results suggest that CXCL1, CXCL7, CXCL2, and CXCL3 produced from GC cells are involved in CXCR2 signaling. CXCL1 and CXCL7 and especially CXCL1 may have important functions in the progression of gastric cancer, but CXCL2 and CXCL3 expression may not.

CXCL5/CXCL6 expression was rare (2.9%,  $n=589$ ) in this study. The CXCL5 expression in 155 cases of gastric cancer was reported to be 45.2% (8). CXCL6 expression has not been studied in any type of cancer. CXCL8 expression was also rare among our present patients (1.7%;  $n=589$ ), but a study of 22 patients with GC reported that CXCL8 expression was 100% (10). One of the possible reasons for these differences in the rates of CXCL5/CXCL6 and CXCL8 expression is based on the difference in the antibodies used in each study. The expression of CXCL5/6 and CXCL8 was not correlated with any of the clinicopathological prognostic factors in the present

Table I. Correlation between clinicopathological factors and CXCR2 ligands in 589 gastric cancer.

Clinicopathologic features		CXCL1		p-Value	CXCL2		p-Value	CXCL3		p-Value
		Positive (n=258)	Negative (n=300)		Positive (n=122)	Negative (n=468)		Positive (n=101)	Negative (n=488)	
Age	<70	139 (40.4%)	205 (59.6%)	<0.001	75 (20.5%)	291 (79.5%)	0.887	61 (16.7%)	305 (83.3%)	0.692
	≥70	119 (55.6%)	95 (44.4%)		47 (21.0%)	177 (79.0%)		40 (17.9%)	183 (82.1%)	
Gender	female	110 (46.2%)	128 (53.8%)	0.994	54 (20.7%)	207 (79.3%)	0.995	38 (14.6%)	222 (85.4%)	0.147
	male	148 (46.3%)	172 (53.8%)		68 (20.7%)	261 (79.3%)		63 (19.1%)	266 (80.9%)	
Macroscopic type	Other type	232 (46.1%)	271 (53.9%)	0.871	112 (21.1%)	418 (78.9%)	0.418	92 (17.4%)	437 (82.6%)	0.641
	Type-4	26 (47.3%)	29 (52.7%)		10 (16.7%)	50 (83.3%)		9 (15.0%)	51 (85.0%)	
Histological type	Intestinal-type	121 (43.2%)	159 (56.8%)	0.151	69 (22.9%)	232 (77.1%)	0.169	44 (14.6%)	257 (85.4%)	0.096
	Diffuse-type	137 (49.3%)	141 (50.7%)		53 (18.3%)	236 (81.7%)		57 (19.8%)	231 (80.2%)	
T invasion	T1	70 (26.9%)	190 (73.1%)	<0.001	69 (25.1%)	206 (74.9%)	0.013	49 (17.9%)	225 (82.1%)	0.659
	T2, T3, T4	188 (63.1%)	110 (36.9%)		53 (16.8%)	262 (83.2%)		52 (16.5%)	263 (83.5%)	
Lymph node metastasis	Negative	98 (31.3%)	215 (68.7%)	<0.001	79 (23.9%)	251 (76.1%)	0.033	62 (18.8%)	267 (81.2%)	0.195
	Positive	159 (65.7%)	83 (34.3%)		43 (16.7%)	214 (83.3%)		38 (14.8%)	219 (85.2%)	
Lymphatic invasion	Negative	66 (26.7%)	181 (73.3%)	<0.001	60 (23.0%)	201 (77.0%)	0.224	45 (17.3%)	215 (82.7%)	0.94
	Positive	192 (61.9%)	118 (38.1%)		62 (18.9%)	266 (81.1%)		56 (17.1%)	272 (82.9%)	
Venous invasion	Negative	180 (39.7%)	273 (60.3%)	<0.001	103 (21.2%)	383 (78.8%)	0.504	84 (17.3%)	401 (82.7%)	0.811
	Positive	78 (74.3%)	27 (25.7%)		19 (18.3%)	85 (81.7%)		17 (16.3%)	87 (83.7%)	
Infiltration	a, b	185 (51.5%)	174 (48.5%)	0.24	85 (22.7%)	290 (77.3%)	0.111	79 (21.1%)	295 (78.9%)	0.002
	c	63 (45.7%)	75 (54.3%)		25 (16.4%)	127 (83.6%)		15 (9.9%)	137 (90.1%)	
Hepatic metastasis	Negative	248 (45.6%)	296 (54.4%)	0.56	119 (20.7%)	457 (79.3%)	0.944	98 (17.0%)	477 (83.0%)	0.667
	Positive	10 (71.4%)	4 (28.6%)		3 (21.4%)	11 (78.6%)		3 (21.4%)	11 (78.6%)	
Peritoneal cytology	Negative	210 (46.8%)	239 (53.2%)	0.113	103 (21.9%)	367 (78.1%)	0.01	82 (17.5%)	387 (82.5%)	0.054
	Positive	33 (57.9%)	24 (42.1%)		5 (7.9%)	58 (92.1%)		5 (7.9%)	58 (92.1%)	
Peritoneal metastasis	Negative	240 (45.6%)	286 (54.4%)	0.242	117 (21.1%)	438 (78.9%)	0.336	95 (17.1%)	459 (82.9%)	0.999
	Positive	18 (56.3%)	14 (43.8%)		5 (14.3%)	30 (85.7%)		6 (17.1%)	29 (82.9%)	
Metastasis	Negative	250 (46.0%)	294 (54.0%)	0.407	121 (21.1%)	452 (78.9%)	0.126	98 (17.1%)	474 (82.9%)	0.956
	Positive	8 (57.1%)	6 (42.9%)		1 (5.9%)	16 (94.1%)		3 (17.6%)	14 (82.4%)	

Table I. Continued

Table I. *Continued*

Clinicopathologic features		CXCL5/6		p-Value	CXCL7		p-Value	CXCL8		p-Value
		Positive (n=17)	Negative (n=572)		Positive (n=215)	Negative (n=375)		Positive (n=10)	Negative (n=575)	
Age	<70	9 (2.5%)	356 (97.5%)	0.437	112 (30.6%)	254 (69.4%)	<0.001	9 (2.5%)	354 (97.5%)	0.066
	>70	8 (3.6%)	216 (96.4%)		103 (46.0%)	121 (54.0%)		1 (0.5%)	221 (99.5%)	
Gender	female	9 (3.5%)	251 (96.5%)	0.458	98 (37.5%)	163 (62.5%)	0.619	5 (1.9%)	253 (98.1%)	0.705
	male	8 (2.4%)	321 (97.6%)		117 (35.6%)	212 (64.4%)		5 (1.5%)	322 (98.5%)	
Macroscopic type	Other type	15 (2.8%)	514 (97.2%)	0.827	199 (37.5%)	331 (62.5%)	0.097	10 (1.9%)	516 (98.1%)	0.285
	Type-4	2 (3.3%)	58 (96.7%)		16 (26.7%)	44 (73.3%)		0 (0.0%)	59 (100.0%)	
Histological type	Intestinal-type	11 (3.7%)	289 (96.3%)	0.249	91 (30.2%)	210 (69.8%)	0.001	6 (2.0%)	290 (98.0%)	0.549
	Diffuse-type	6 (2.1%)	283 (97.9%)		124 (42.9%)	165 (57.1%)		4 (1.4%)	285 (98.6%)	
T invasion	T1	6 (2.2%)	269 (97.8%)	0.339	89 (32.4%)	186 (67.6%)	0.055	2 (0.7%)	272 (99.3%)	0.086
	T2, T3, T4	11 (3.5%)	303 (96.5%)		126 (40.0%)	189 (60.0%)		8 (2.6%)	303 (97.4%)	
Lymph node metastasis	Negative	6 (1.8%)	324 (98.2%)	0.076	108 (32.7%)	222 (67.3%)	0.026	3 (0.9%)	325 (99.1%)	0.09
	Positive	11 (4.3%)	245 (95.7%)		107 (41.6%)	150 (58.4%)		7 (2.8%)	247 (97.2%)	
Lymphatic invasion	Negative	5 (1.9%)	256 (98.1%)	0.207	79 (30.3%)	182 (69.7%)	0.005	2 (0.8%)	259 (99.2%)	0.113
	Positive	12 (3.7%)	315 (96.3%)		136 (41.5%)	192 (58.5%)		8 (2.5%)	315 (97.5%)	
Venous invasion	Negative	13 (2.7%)	472 (97.3%)	0.519	167 (34.4%)	319 (65.6%)	0.023	6 (1.2%)	476 (98.8%)	0.061
	Positive	4 (3.8%)	100 (96.2%)		48 (46.2%)	56 (53.8%)		4 (3.9%)	99 (96.1%)	
Infiltration	a, b	11 (2.9%)	363 (97.1%)	0.833	167 (44.5%)	208 (55.5%)	<0.001	7 (1.9%)	365 (98.1%)	0.929
	c	5 (3.3%)	147 (96.7%)		32 (21.1%)	120 (78.9%)		3 (2.0%)	147 (98.0%)	
Hepatic metastasis	Negative	17 (3.0%)	558 (97.0%)	0.514	209 (36.3%)	367 (63.7%)	0.614	10 (1.8%)	561 (98.2%)	0.617
	Positive	0 (0.0%)	14 (100.0%)		6 (42.9%)	8 (57.1%)		0 (0.0%)	14 (100.0%)	
Peritoneal cytology	Negative	13 (2.8%)	456 (97.2%)	0.385	184 (39.1%)	286 (60.9%)	0.034	8 (1.7%)	459 (98.3%)	0.411
	Positive	3 (4.8%)	60 (95.2%)		16 (25.4%)	47 (74.6%)		2 (3.2%)	60 (96.8%)	
Peritoneal metastasis	Negative	17 (3.0%)	537 (96.9%)	0.293	204 (36.8%)	351 (63.2%)	0.525	9 (1.6%)	541 (98.4%)	0.589
	Positive	0 (0.0%)	35 (100.0%)		11 (31.4%)	24 (68.6%)		1 (2.9%)	34 (97.1%)	
Metastasis	Negative	17 (3.0%)	555 (97.0%)	0.471	209 (36.5%)	364 (63.5%)	0.921	10 (1.8%)	558 (98.2%)	0.581
	Positive	0 (2.9%)	17 (100.0%)		6 (35.3%)	11 (64.7%)		0 (0.0%)	17 (100.0%)	

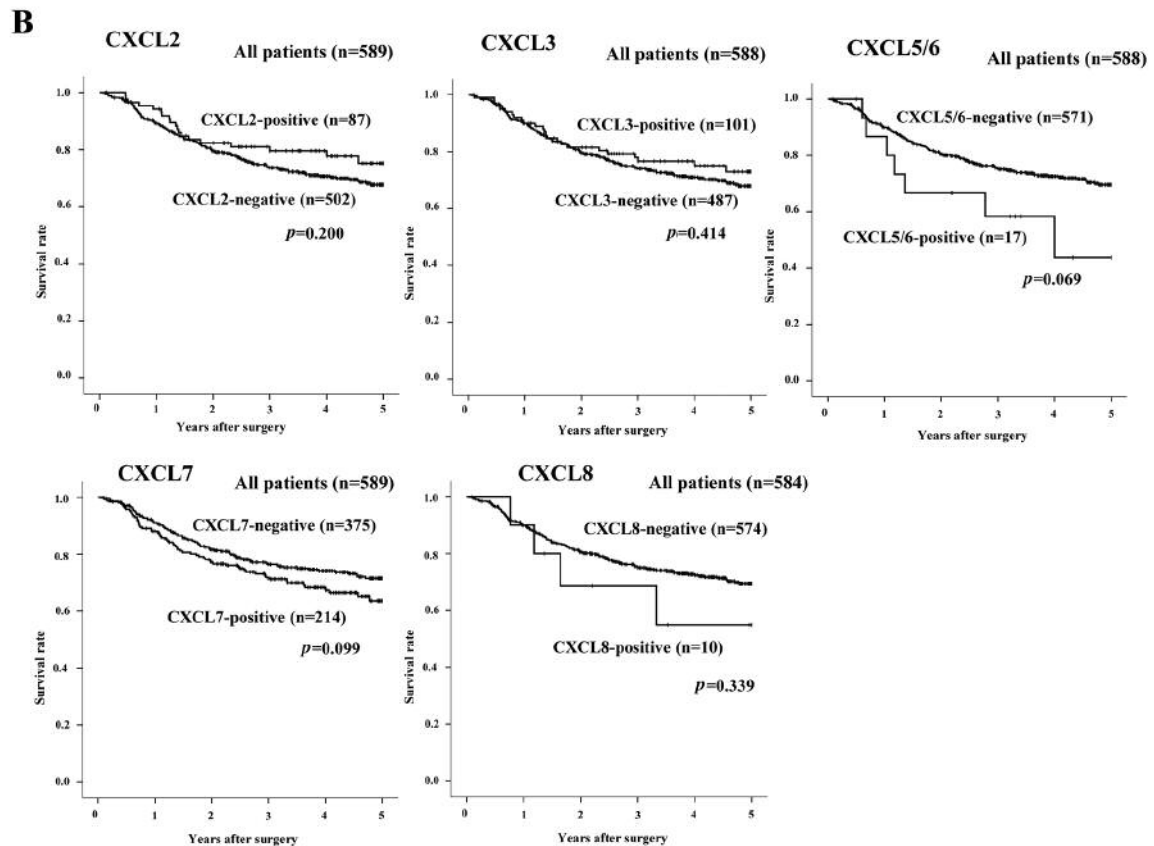
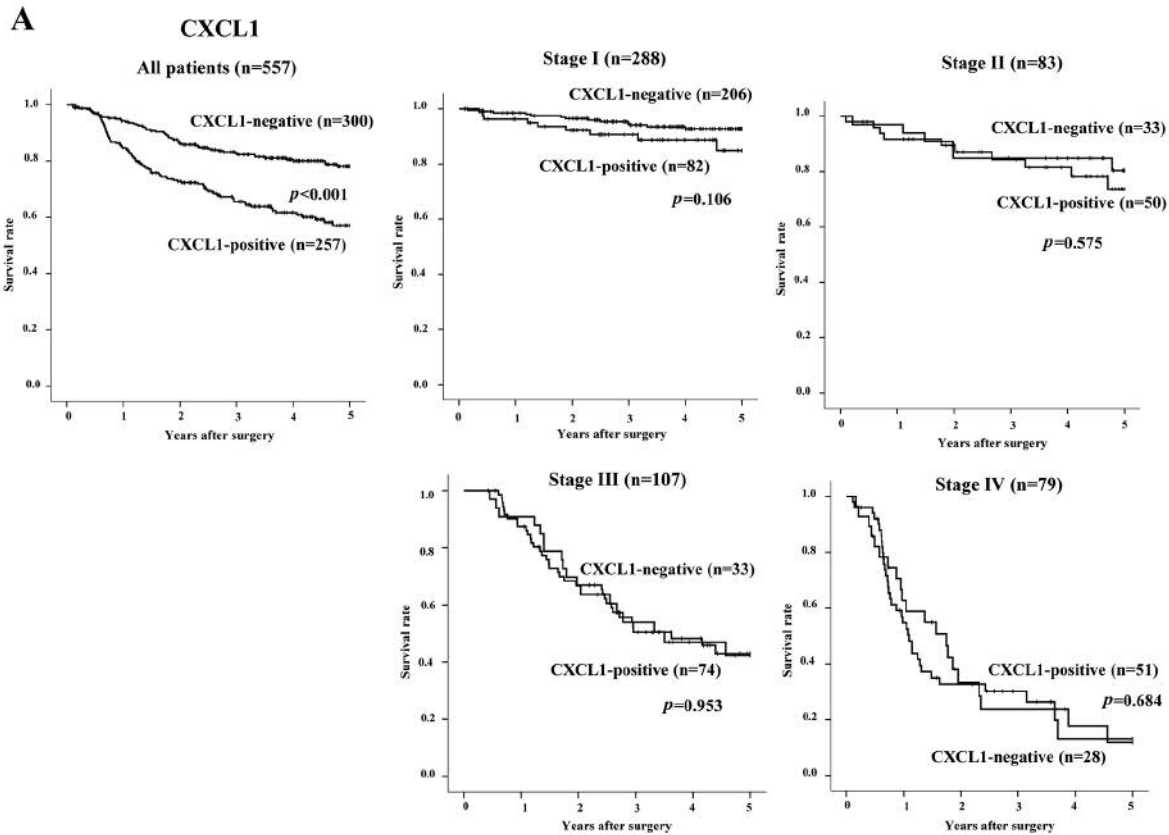


Table II. Univariate and multivariate Cox multiple regression analysis with respect to overall survival after surgery in 589 patients with gastric carcinoma.

Parameter	Univariate			Multivariate		
	Hazard ratio	95%CI	p-Value	Hazard ratio	95%CI	p-Value
CXCL1 Positive	2.272	1.632-3.161	<0.001	1.469	1.109-2.117	0.039
CXCL2 Positive	0.732	0.453-1.182	0.202			
CXCL3 Positive	0.835	0.54-1.289	0.414			
CXCL5/6 Positive	1.992	0.933-4.252	0.075			
CXCL7 Positive	1.305	0.950-1.792	0.1			
CXCL8 Positive	1.615	0.598-4.360	0.344			
Macroscopic type: type-4	7.474	5.288-10.564	<0.001	2.619	1.669-4.109	<0.001
Histological type: diffuse-type	0.556	0.403-0.766	<0.001	0.843	0.556-1.254	0.399
T stage	7.98	4.992-12.756	<0.001	2.27	1.233-4.182	0.009
Lymph node metastasis: positive	8.337	5.556-12.610	<0.001	3.229	1.909-5.464	<0.001
Venous invasion: positive	3.291	2.379-4.554	<0.001	1.14	0.789-1.649	0.485
Infiltration type c	1.817	1.315-2.511	<0.001	1.008	0.668-1.520	0.971
Hepatic metastasis: positive	6.555	3.696-11.628	<0.001	3.088	1.599-5.964	0.001
Peritoneal metastasis: positive	9.11	6.016-13.796	<0.001	2.749	1.624-4.654	<0.001
Metastasis: Positive	6.386	3.741-10.900	<0.001	3.523	1.864-6.659	<0.001
Age ≥70	1.65	1.210-2.251	0.002	1.636	1.152-2.324	0.006

study. These findings may indicate that CXCL5, CXCL6, and CXCL8 do not play important role in the CXCR2 signaling in the gastric cancer tumor microenvironment.

In conclusion, among the CXCR2 ligands, CXCL7 and especially CXCL1 were frequently expressed on gastric cancer cells. CXCL1 was closely associated with the malignant potential of this cancer, and it was an independent prognostic factor. CXCL1 might play an important role in the malignant progression of gastric cancer *via* CXCR2 signaling.

## Conflicts of Interest

There are no financial or other interests with regard to the submitted manuscript that might be construed as a conflict of interest.

## Authors' Contributions

Y.Y. performed the experiments of this study, interpreted the data and wrote the manuscript; Y.M., designed the experiments of this study, interpreted the data and edited the manuscript; K.K., T.S.,

A.S., S.K., S.T., and T.O. contributed to the immunohistochemical analysis; M.Y., T.T., H.T., K.M. and S.N. collected the gastric cancer specimen; M.O. helped draft the manuscript.

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Figure 2. Overall survival of gastric cancer patients based on CXCLs expression in gastric cancer cells. A: The Kaplan-Meier survival curves indicate that the overall survival of patients, at all disease stages, with CXCL1-positive expression was significantly worse than that of patients with CXCL1-negative expression ( $p < 0.001$ ). B: In contrast, no significant difference in overall survival was found between patients with positive and negative expression of CXCL2, CXCL3, CXCL5/6, CXCL7, and CXCL8.

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