Original Article

Expression of the CXCL12/CXCR4 and CXCL16/CXCR6 axes in cervical intraepithelial neoplasia and cervical cancer

Yu Huang[1], Jia Zhang[1,2], Zhu-Mei Cui[1], Jing Zhao[1] and Ye Zheng[1,3]

Abstract

The chemokine CXCL12 is highly expressed in gynecologic tumors and is widely known to play a biologically relevant role in tumor growth and spread. Recent evidence suggests that CXCL16, a novel chemokine, is overexpressed in inflammation-associated tumors and mediates pro-tumorigenic effects of inflammation in prostate cancer. We therefore analyzed the expression of CXCL12 and CXCL16 and their respective receptors CXCR4 and CXCR6 in cervical intraepithelial neoplasia (CIN) and cervical cancer and further assessed their association with clinicopathologic features and outcomes. Tissue chip technology and immunohistochemistry were used to analyze the expression of CXCL12, CXCR4, CXCL16, and CXCR6 in healthy cervical tissue (21 cases), CIN (65 cases), and cervical carcinoma (60 cases). The association of protein expression with clinicopathologic features and overall survival was analyzed. These four proteins were clearly detected in membrane and cytoplasm of neoplastic epithelial cells, and their distribution and intensity of expression increased as neoplastic lesions progressed through CIN1, CIN2, and CIN3 to invasive cancer. Furthermore, the expression of CXCR4 was associated significantly with the histologic grade of cervical carcinoma, whereas the expression of CXCR6 was associated significantly with lymph node metastasis. In Kaplan-Meier analysis, patients with high CXCR6 expression had significantly shorter overall survival than did those with low CXCR6 expression. The elevated co-expression levels of CXCL12/CXCR4 and CXCL16/CXCR6 in CIN and cervical carcinoma suggest a durative process in cervical carcinoma development. Moreover, CXCR6 may be useful as a biomarker and a valuable prognostic factor for cervical cancer.

Key words Cervical cancer, cervical intraepithelial neoplasia, chemokine, CXCL12, CXCL16

Cervical cancer is the third most common cancer in women and the seventh most common cancer worldwide[1]. Infection with high-risk human papillomavirus (HPV) is central to the pathogenesis of invasive cervical cancer[2]. Many other cancers also arise from sites of infection, chronic irritation, and inflammation[3]. Indeed, more than 15% of malignancies worldwide can be attributed to infection[4]. Recent studies have expanded the concept that inflammation is a crucial component of tumor progression[5,6], though the mechanism triggering the transformation from inflammation to malignancy is still unknown. Many women, for example, are infected with high-risk HPV, but only a subset of infected women will ever develop cervical cancer, suggesting that other cofactors must be present for the development of a malignancy[7].

Chemokines were initially defined as soluble factors that regulate the directional migration of leukocytes during states of inflammation. The chemokine receptor system extends to most human neoplastic cells and was found to be altered dramatically in neoplastic tissue, particular at the leading edge of invasion[8]. Their diverse roles in tumor biology include direct effects, such as transformation, survival, proliferation, metastasis, and indirect effects, such as angiogenesis and leukocyte

Authors' Affiliations: [1]Department of Gynecology, [3]Department of Reproductive Medicine, The Affiliated Hospital of Medical College, Qingdao University, Qingdao, Shandong 266003, P. R. China; [2]Department of Gynecology, The People's Hospital of Jimo, Jimo, Shandong 266200, P. R. China.

Corresponding Author: Yu Huang, Department of Gynecology, The Affiliated Hospital of Medical College, Qingdao University, No. 16 Road Jiangsu, Qingdao, Shandong 266003, P. R. China. Tel: +86-532-82913051; Fax: +86-532-80775298; Email: romanhuangyu@yahoo.com.cn.

doi: 10.5732/cjc.012.10063

recruitment to tumor sites[3]. Among more than 50 members of the chemokine family, CXCL12 (α-chemokine stromal cell-derived factor-1, SDF-1α) and its receptor CXCR4 have attracted much interest in the field of oncology. CXCR4 is the most consistently expressed receptor in human cancers, including tumors of epithelial, mesenchymal, and hematopoietic origin. Furthermore, CXCR4 level in tumor tissue has been associated with clinical outcomes[9-11]. Recently, CXCL12 expression in cervical cancer was reported to be directly associated with known determinants of immune dysregulation, such as HPV infection and FoxP3+ T cell infiltration in cervical cancer[12]. CXCR4 was also found to participate in metastasis of cervical cancer cells to lymph nodes *in vivo* and in direct migration *in vitro*[13].

As a newly identified chemokine/receptor pair, CXCL16/CXCR6 has drawn less attention than did CXCL12/CXCR4 in cancer research. CXCR6, an exclusive receptor of CXCL16, is preferentially expressed on Th1 and Tc1 polarized memory CD4+ and CD8+ lymphocytes and has been detected in large proportions of tissue-infiltrating lymphocytes from patients with inflammatory disorders[14]. CXCL16 is one of the only two known plasma membrane chemokines. Its soluble form induces chemotaxis of activated T cells and bone marrow plasma cells via CXCR6[15]. CXCL16 expression has been reported to be associated with a number of human inflammatory diseases including rheumatoid arthritis, interstitial lung diseases, atherosclerosis, coronary artery disease, and liver injury. Several recent reports focused on the consistently high expression of CXCR6 in prostate cancer and suggested that CXCL16 and CXCR6 may be markers of cancer arising in inflammatory milieu. In addition, they may mediate pro-tumorigenic effects of inflammation through a direct effect on cancer cell growth and by inducing the migration and proliferation of tumor-associated leukocytes[16]. However, to the best of our knowledge, there are no reports on the CXCL16/CXCR6 axis in cervical cancer to date.

Materials and Methods

Patients and tissue samples

Formalin-fixed, paraffin-embedded tissue samples were prepared from 60 patients with cervical cancer, 65 with cervical intraepithelial neoplasia (CIN), and 21 with normal cervix. The cervical cancer patients ranged in age from 26 to 68 years, with a mean age of 42 years. Among the 65 patients with CIN, 6 had CIN1, 16 had CIN2, and 43 had CIN3, with a mean age of 36 years. The normal control cervical tissue specimens were obtained from patients without cancer (e.g., patients with uterine functional bleeding, uterine myoma, or uterine prolapse), whose mean age was 44 years.

The specimens, which were collected between January 2004 and June 2007, were obtained from the archives of the Department of Pathology at the Affiliated Hospital of Qingdao University Medical College. Patient records were retrieved and clinical data, histopathologic reports, and treatment information were all reviewed. No patients underwent chemotherapy or other adjuvant treatments before the tissues were obtained at surgery. The study was approved by the Ethics Commission of the Affiliated Hospital of Qingdao University Medical College. Informed consent was obtained from each patient. All samples were reviewed by the same pathologist to define histologic types and grades. We obtained follow-up information in July 2011 through visits or telephone interview with either patients or their relatives. The follow-up time ranged from 11 to 90 months, and the mean time was 63 months.

Construction of tissue microarray

Tissue microarray blocks were constructed with formalin-fixed, paraffin-embedded samples of normal cervical tissue, CIN tissue, and cervical cancer tissue stored in the Department of Pathology of the Affiliated Hospital of Qingdao University Medical College. The tissue microarray slides were stained with hematoxylin and eosin to confirm the diagnosis of invasive cervical cancer and CIN. Afterwards, morphologic characteristics were documented, and two typical tumor regions were marked on the donor blocks.

Tissue microarray blocks were created by punching a cylinder using a 2-mm hollow needle into the two selected areas of each donor block. The tissues were then inserted into an empty recipient paraffin block. Subsequently, these blocks were sectioned into 4-μm slides and prepared for immunohistochemical (IHC) analysis.

IHC analysis

Protein expression in human cervical cancer and CIN was analyzed by using IHC staining. In this process, sections were deparaffinized in xylene prior to rehydration using gradient alcohol. Endogenous peroxidase activity was then blocked with methanol containing 3% H_2O_2 for 20 min. For antigen retrieval, sections were treated with citrate buffer saline (pH 6.0) for 15 min at 95°C in a microwave oven. After blocking with 7% horse normal serum for 30 min at room temperature, sections were incubated with mouse monoclonal anti-CXCL12 (R&D, dilution 1:25), mouse monoclonal anti-CXCR4 (R&D, dilution 1:80), goat

polyclonal anti-CXCL16 (R&D, dilution 1:10), or mouse monoclonal anti-CXCR6 (R&D, dilution 1:40) overnight at 4℃.

Following incubation, sections were washed with phosphate buffered solution (PBS) and incubated with horseradish peroxidase (HRP)−conjugated goat anti-mouse IgG or rabbit anti-goat IgG for 40 min at room temperature. Staining was performed using 3,3'-diamino-benzidine (DAB). Sections were counterstained with hematoxylin followed by dehydration and mounting. Negative controls were prepared using matched immunoglobulin in lieu of the primary antibody. IHC results were evaluated by a pathologist.

Scoring of immunostaining

Sections were read by two pathologists blinded to patient clinicopathologic parameters. Both intensity and percentage of positive cells were considered. The intensity was determined by comparing the staining of tumor cells and mesenchymal cells. The positive cell percentage was determined by calculating the percentage of positive tumor cells among the total number of observed cells. When staining for CXCR4, CXCL12, CXCR6, and CXCL16, tumor cells with brown cytoplasm or membrane were considered positive and then scored based on four classes: none (0); weak brown (1+); moderate brown (2+); and strong brown (3+). The percentage of stained tumor cells was categorized into four classes: 0 for <10%; 1 for 10%–49%; 2 for 50%–74%; and 3 for ⩾75%. Staining index (SI), obtained by multiplying the intensity and percentage scores, was used for analysis[17]. An SI score of 0–3 indicated low expression; a score of 4–6 indicated high expression.

Statistical analyses

Data were analyzed using the SPSS17.0 software package. The expression of CXCL12, CXCR4, CXCL16, or CXCR6 among the groups was compared by using one-way ANOVA tests. Association of protein expression with clinicopathologic features of cervical cancer was analyzed with Fisher's exact test. The interrelation of chemokines and receptors was analyzed with Pearson correlation or Spearman's rank correlation test. Overall survival (OS) was calculated from the date of inclusion until death or the last follow-up examination. Kaplan-Meier analysis was performed to generate OS curves. For comparison of age between cervical cancer groups, we used unpaired two-tailed t test. P values < 0.05 were deemed significant. All statistical tests were two-sided.

Results

Expression of CXCL12, CXCR4, CXCL16, and CXCR6 in CIN and malignant cervical epithelial cells

Both CXCL12 and CXCR4 were clearly detected in the epithelia of malignant squamous and glandular lesions, which showed specific brown staining in the cytoplasm and on the cytomembrane. The epithelial staining was confined to the cytoplasm, with particularly strong staining of the membrane frequently observed. No nuclear staining was detected. An increase in intensity and distribution of CXCL12 or CXCR4 was noted as the lesions progressed. Both were present throughout the full thickness of the epithelia in cervical cancer and CIN3. Antigen expression of CIN1 and CIN2 was confined to the basal layers of the epithelia, whereas neither protein was detected in normal squamous epithelia of the ectocervix (Figure 1). The expression of CXCL12 in the cervical cancer group was significantly higher than that in the CIN1 and CIN2 groups (P < 0.05). In addition, the expression of CXCR4 in the cervical cancer group was significantly higher than that in the CIN1+CIN2 group and CIN3 group (both P < 0.001).

CXCL16 antigen was stained in both the epithelia and the stroma of neoplastic squamous as well as glandular lesions. The epithelial staining, which was confined to the cytoplasm and plasma membrane, was clearly intracellular and well defined, whereas stromal staining was generally diffuse and weak. Staining for CXCL16 was present diffusely and weakly in the epithelia and stroma of healthy ectocervix, but it became stronger in intensity and distribution in the epithelia as the lesions progressed through CIN1+CIN2 (P < 0.05), CIN3 (P < 0.001), and finally to cancer, where staining was the strongest (P < 0.001) (Figure 2). As observed for CXCL12 and CXCR4, staining for CXCR6 was essentially absent in the epithelia of the normal cervix but was present in the plasma membrane and cytoplasm of the epithelia of neoplastic lesions. Furthermore, this staining pattern increased in distribution and intensity as the cervical lesions progressed through CIN1, CIN2, and CIN3 to invasive cancer (Figure 2).

Concordance of CXCL12 and CXCR4, CXCL16 and CXCR6 in CIN and cervical cancer

We then investigated the correlation between the two chemokine axes with IHC staining for CXCL12, CXCR4, CXCL16, and CXCR6. CXCL12 expression was positively correlated with CXCR4 expression in the CIN1+CIN2, CIN3, and cervical cancer groups. Likewise, a positive correlation of CXCL16 expression with CXCR6 expression was observed in the CIN3 and cervical
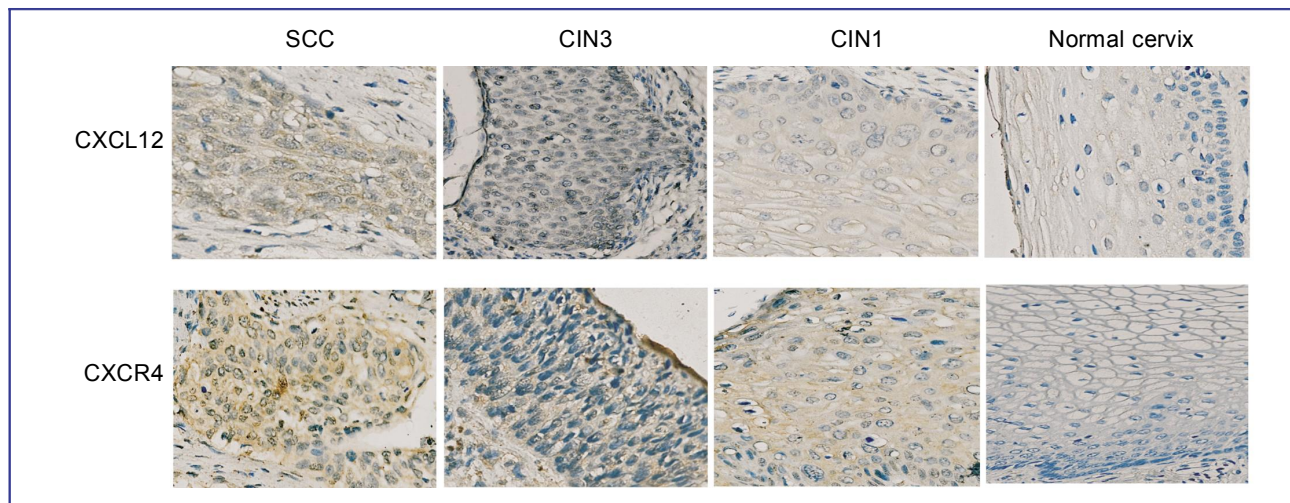
Figure 1. CXCL12 and CXCR4 are expressed in the epithelia of malignant neoplastic tissue. Immunohistochemistry (IHC) was used to detect the expression of CXCL12 and CXCR4 in tissue sections. Sections of invasive squamous cell carcinoma (SCC), cervical intraepithelial neoplasia 3 (CIN3), CIN1, and normal cervix are stained for CXCL12 and CXCR4 (×200). Positive staining for CXCL12 or CXCR4 is detected in the cytoplasm and is frequently and strongly observed on the membrane. Neither protein is detected in the nuclei. In squamous neoplastic lesions, the intensity of CXCL12 in cervical cancer is the highest and that in CIN3 is the second highest; CXCL12 is present throughout the full thickness of the epithelium in the two types of tissues. In CIN1, the area positive for either CXCL12 or CXCR4 is confined to the basal layers of the epithelia, whereas normal ectocervical epithelia shows no staining.
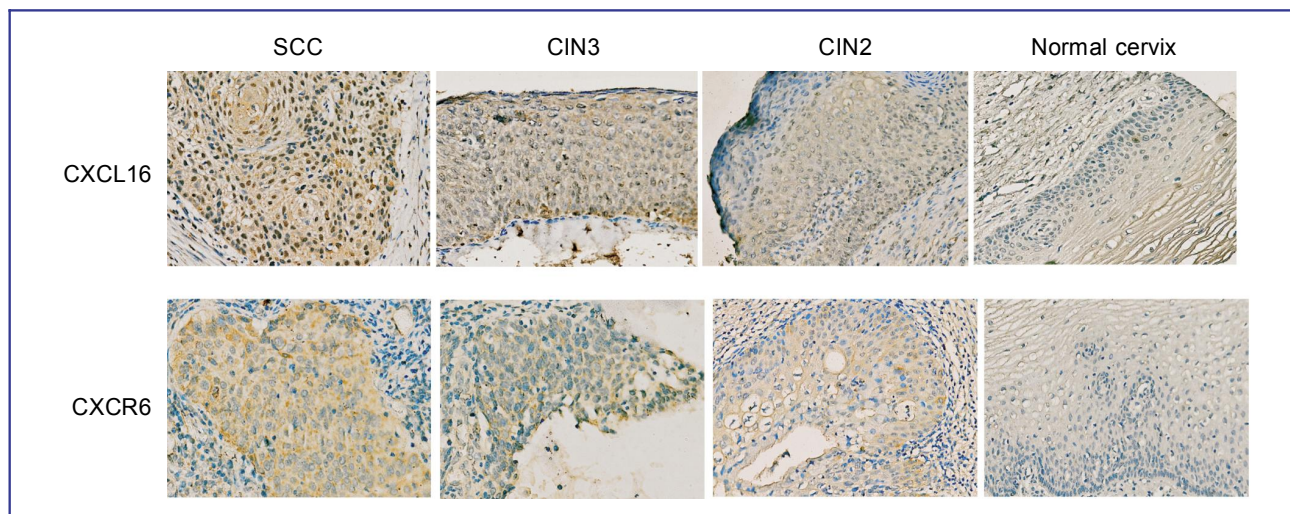
Figure 2. CXCL16 and CXCR6 are expressed strongly in the epithelia of malignant neoplastic tissue. Immunohistochemistry was used to detect the expression of CXCL16 and CXCR6 in tissue sections. Sections of invasive SCC, CIN3, CIN2, and normal cervix were stained for CXCL16 and CXCR6 (×200). Both CXCL16 and CXCR6 antigens are expressed in the epithelia and stroma of neoplastic squamous and glandular lesions. The epithelial staining is clearly intracellular and well defined, whereas stromal staining is generally diffuse and weak. CXCL16 and CXCR6 exhibit strong expression in the cytoplasm and membrane throughout the full thickness of the epithelium in SCC and CIN3. In contrast, CXCL16 or CXCR6 staining is decreased and confined to the basal layers of the epithelia in CIN2 lesions. The normal ectocervix shows weak staining for CXCL16 in epithelia and stroma but is consistently negative for CXCR6.

cancer groups (Table 1). These results indicate a tendency towards coexpression of chemokine ligands and their receptors in tumors. Moreover, positive correlations between chemokines CXCL12 and CXCL16 as well as between their respective receptors CXCR4 and CXCR6 were detected during neoplastic progression (Table 1). Hence, the expression of these two chemokine axes is likely to be tightly linked in the

evolution of cervical cancer.

Association of CXCL12, CXCR4, CXCL16, and CXCR6 expression with clinicopathologic features

In 60 cervical cancer specimens, CXCL12 and CXCR4 expression ranged from high levels (SI 4–6) in 21 (35%) and 16 (27%) specimens, respectively, to an absence of staining in 12 (20%) and 12 (20%) specimens, respectively. CXCL16 and CXCR6 expression ranged from high levels (SI 4–6) in 14 (23%) and 11 (18%) specimens, respectively, to an absence of staining in 10 (17%) and 9 (15%) specimens, respectively. Most cases expressed CXCL16 and CXCR6 or CXCL12 and CXCR4 at a moderate level. The classic clinicopathologic parameters, such as histological type, FIGO stage, and lymph node metastasis after first laparotomy, were analyzed by using Fisher's exact test. The four molecules tested were associated significantly with FIGO

stage (0–II) in cervical cancer, with higher level of staining for CXCL12, CXCR4, CXCL16, and CXCR6 corresponding to higher stage (Table 2, Figure 3). Moreover, the expression of CXCR4 was associated with histologic grade of cancer cells, and CXCR6 status was associated with lymph node metastasis of cervical cancer (Table 2). Thus, the expression of both chemokine axes, CXCL12/CXCR4 and CXCL16/CXCR6, reflect the clinical status of cervical cancer.

Association of CXCL12, CXCR4, CXCL16, and CXCR6 expression with patient outcome

We then investigated whether CXCL12, CXCR4, CXCL16, and CXCR6 status affected OS in cervical cancer. For this analysis, we applied a single cut-off at an SI score of 3. Of the 60 specimens of cervical cancer, 14 had low CXCL12 expression and 46 had high CXCL12 expression; 12 had low CXCR4 expression and 48 had high CXCR4 expression; 11 had low CXCL16

Table 1. Correlation among CXCL12, CXCR4, CXCL16, and CXCR6

Variable	CIN1+CIN2		CIN3		Cervical cancer	
	Rho	P	Rho	P	Rho	P
CXCL12 and CXCR4	0.519	0.007	0.306	0.023	0.389	0.001
CXCL16 and CXCR6	0.158	0.241	0.445	0.010	0.548	<0.001
CXCL12 and CXCL16	0.431	0.045	0.419	0.005	0.534	<0.001
CXCR4 and CXCR6	0.369	0.091	0.571	<0.001	0.600	<0.001

Table 2. Association of CXCL12, CXCR4, CXCL16, or CXCR6 with clinicopathologic features

Clinical feature	Number of patients	CXCL12 expression			CXCR4 expression			CXCL16 expression			CXCR6 expression		
		+	–	P	+	–	P	+	–	P	+	–	P
FIGO stage													
CIN3	43	31	12	0.008	24	21	0.001	30	13	0.001	26	17	0.001
Ia-Ib	49	37	12		37	12		39	10		41	8	
IIa	11	11	0		11	0		11	0		10	1	
Histological grade													
G1	21	14	7	1.011	14	8	0.023	14	7	0.068	15	6	0.185
G2	15	14	1		13	2		14	1		14	1	
G3	24	20	4		22	2		22	2		22	2	
Histological type													
SCC	55	44	11	0.975	44	11	0.829	47	8	0.733	47	8	0.562
Others	5	4	1		4	1		3	2		4	1	
LN metastasis													
Yes	11	9	2	0.513	8	3	0.107	9	2	0.501	10	1	0.005
No	49	39	10		40	9		41	8		41	8	

All values are presented as number of patients. SCC, squamous cell cancer; LN, lymph node.
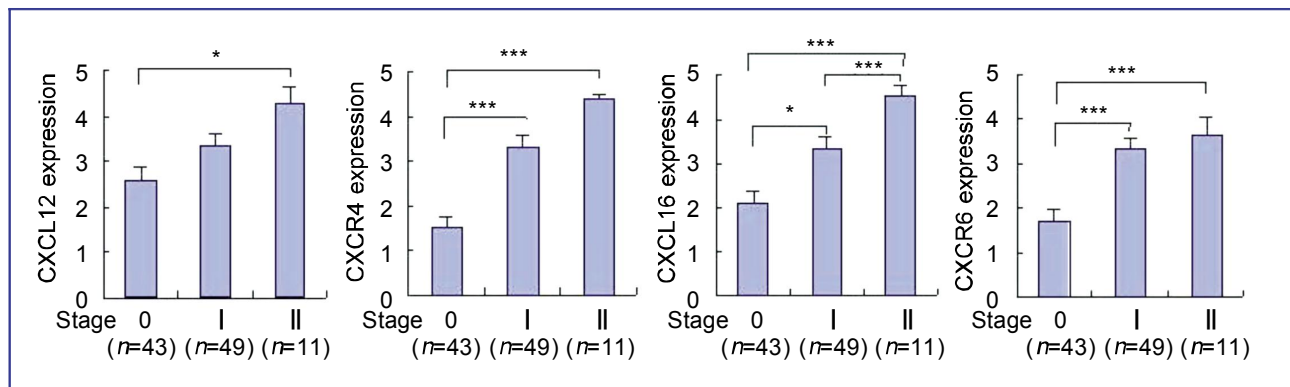
Figure 3. The expression of CXCL12, CXCR4, CXCL16, and CXCR6 are associated significantly with FIGO stage (0–II) in cervical cancer. Arrays containing cervical cancer tissue from 103 patients were stained for CXCL12, CXCR4, CXCL16, and CXCR6, and samples were scored for their expression as described in the Materials and Methods section. Samples were grouped according to stage as determined by the supplier, and mean scores for protein expression were calculated. The number of samples (n) in each stage group is shown. Stage 0 includes 43 cases of CIN3. Error bars show standard errors of measurement. Cross bars indicate comparisons that are significant. $*P < 0.05$; $***P < 0.001$.

expression and 49 had high CXCL16 expression; and 12 had low CXCR6 expression and 48 had high CXCR6 expression. There was no significant difference in patient age between the low and high level groups of any molecule. Follow-up investigation revealed that the median survival time was 66 months (ranging from 11 to 90 months). Twelve patients (20%) died of cervical cancer. Kaplan-Meier analysis revealed that patients with high CXCR6 expression had significantly lower OS than did those with low CXCR6 expression ($P = 0.049$), whereas the level of CXCL12, CXCR4, and CXCL16 expression was not associated with the OS of cervical cancer patients (Figure 4). Thus, CXCR6 expression by tumor epithelium may be a valuable prognostic factor of cervical cancer.

Discussion

This study has shown that the chemokine axes CXCL12/CXCR4 and CXCL16/CXCR6 have stage-dependent expression in neoplastic cervical epithelia. Although these findings must be further verified, the difference in CXCL12/CXCR4 expression and CXCL16/CXCR6 expression between normal cervical epithelium, precancerous lesions, and invasive cancer was significant.

The expression of CXCR4 was associated with histologic grade of cervical cancer, and up-regulation of CXCR4 expression was positively correlated with the expression of its ligand CXCL12 in both malignant and premalignant epithelia. This suggests a direct link between CXCL12 and CXCR4 in cervical tumorigenesis. As the expression of CXCR4 may render cervical

neoplastic cells a target for autocrine and paracrine regulation by locally produced CXCL12, the possible roles of the CXCL12/CXCR4 axis in cell proliferation and invasion should be explored. Recent reports provided evidence that CXCL12, through its interaction with CXCR4, could induce directed migration of HeLa cells and hepatocellular carcinoma cells and play a role in cell growth, survival, and scatter [13,18-20]. Furthermore, Jaafar *et al.* [12] suggested that high levels of CXCL12 led to retention or accumulation of FoxP3^{+} T cells, rather than infiltration of CD4^{+} and CD8^{+} T cells, during cervical cancer progression. Thus, the CXCL12/CXCR4 axis could be a main mechanism by which cervical cancer invades into adjacent tissue and subsequently into distant organs that produce CXCL12.

Notably, our study did not reveal an association between CXCL12 or CXCR4 expression and OS, which is consistent with findings in some studies of epithelial ovarian cancers [11,21]. This lack of prognostic value for CXCL12 and CXCR4 in cervical cancer is somewhat confusing, but there are two possible explanations. First, the cellular expression of CXCL12 may not provide a true reflection of its bioavailability, which depends principally on the presence of factors capable of disrupting CXCL12 from glycosaminoglycans in the tumor microenvironment [22]. Second, CXCL12 activity may be mediated by two receptors, CXCR4 and CXCR7. There is evidence showing crosstalk between CXCR7 and CXCR4 in CXCL12-mediated events, such as cell motility and chemotaxis [23,24]. Thus, the respective contributions of CXCR4 and CXCR7 to the pathologic activities of CXCL12 in cervical cancer should be further investigated.

The present study, to our knowledge, is the first to associate the CXCL16/CXCR6 chemokine axis with
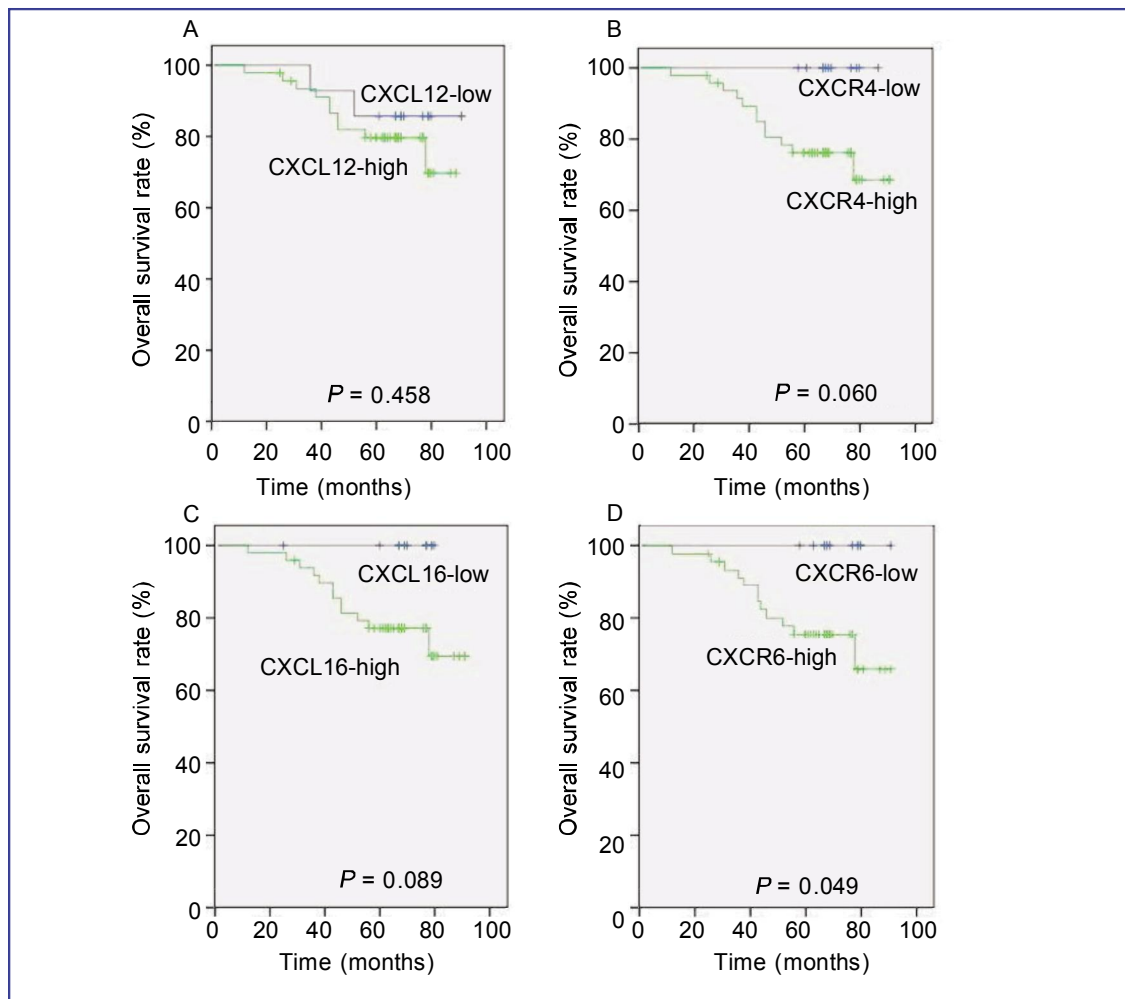
Figure 4. **CXCR6 is the only protein that is associated with overall survival among both chemokine axes.** The overall survival of cervical cancer patients was plotted by using Kaplan-Meier estimates according to low the expression level of CXCL12 (A), CXCR4 (B), CXCL16 (C), and CXCR6 (D). Low protein expression is identified with a staining index of 0–3; high protein expression is identified with a staining index of 4–6. Curves were compared using the log-rank test. Among all proteins, only CXCR6 expression had any impact on overall survival. In this group, overall survival rate was significantly lower in patients whose tumors expressed high levels of CXCR6 than in patients whose tumors expressed low levels of CXCR6.

cervical cancer, though a similar co-expression pattern has been verified in prostate cancer[16]. As a pro-inflammatory chemokine, CXCL16 and its exclusive receptor CXCR6 have been described in other inflammation-associated cancers. A recent study explored the role of this chemokine axis in pro-tumorigenic activity and tumor progression in prostate cancer [16]. In this study, the co-localization level of CXCL16 and CXCR6 in prostate cancer cells associated with poor prognostic features, including high stage and high grade. Furthermore, CXCL16 was up-regulated significantly in pre-neoplastic lesions associated with inflammation. Therefore, CXCL16 and CXCR6 may mark cancers arising in an inflammatory milieu and mediate pro-tumorigenic effects of inflammation through direct effects on cancer cell growth and induction of migration in tumor-associated leukocytes[16].

Interestingly, we observed that the expression of CXCR6 associated significantly with lymph node metastasis in 60 cases of invasive cervical cancer and that high expression of CXCR6 often indicated a worse prognosis. On the contrary, CXCL16 expression did not have the same prognostic value for lymph node metastasis or patient survival. Based on previous studies, we predict that CXCL16 and CXCR6 may be responsible for selective infiltration of tumor-associated leukocytes, as well as proliferation, survival, migration, and invasion of tumor cells at all stages of cervical cancer, including tumor-associated inflammation, cervical intraepithelial neoplasia, and invasive cervical cancer.

In summary, our findings suggest that the chemokine axes CXCL12/CXCR4 and CXCL16/CXCR6 are tightly linked and may form a network to mediate the evolution of cervical cancer. Our study also revealed that CXCR6 is the only one of these four molecules that has any prognostic impact on lymph node metastasis and OS of cervical cancer. Thus, we suggest CXCR6 may be the best candidate among the four molecules to serve as a biomarker in biopsies for cervical cancer and a valuable prognostic factor for invasive cervical cancer.

References

[1] Ferlay J, Shin HR, Bray F, et al. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. Int J Cancer, 2010,127:2893–2917.

[2] Smith JS, Herrero R, Bosetti C, et al. Herpes simplex virus-2 as a human papillomavirus cofactor in the etiology of invasive cervical cancer. J Natl Cancer Inst, 2002,94:1604–1613.

[3] Coussens LM, Werb Z. Inflammation and cancer. Nature, 2002,420:860–867.

[4] Kuper H, Adami HO, Trichopoulos D. Infections as a major preventable cause of human cancer. J Intern Med, 2000,248:171–183.

[5] Nathan C. Points of control in inflammation. Nature, 2002,420:846–852.

[6] Hanahan D, Weinberg RA. The hallmarks of cancer. Cell, 2000,100:57–70.

[7] Goswami B, Rajappa M, Sharma M, et al. Inflammation: its role and interplay in the development of cancer, with special focus on gynecological malignancies. Int J Gynecol Cancer, 2008,18:591–599.

[8] Rossi D, Zlotnik A. The biology of chemokines and their receptors. Annu Rev Immunol, 2000,18:217–242.

[9] Balkwill F. The significance of cancer cell expression of the chemokine receptor CXCR4. Semin Cancer Biol, 2004,14:171–179.

[10] De Falco V, Guarino V, Avilla E, et al. Biological role and potential therapeutic targeting of the chemokine receptor CXCR4 in undifferentiated thyroid cancer. Cancer Res, 2007,67:11821–11829.

[11] Kajiyama H, Shibata K, Terauchi M, et al. Involvement of SDF-1α/CXCR4 axis in the enhanced peritoneal metastasis of epithelial ovarian carcinoma. Int J Cancer, 2008,122:91–99.

[12] Jaafar F, Righi E, Lindstrom V, et al. Correlation of CXCL12 expression and FoxP3+ cell infiltration with human papilloma-virus infection and clinicopathological progression of cervical cancer. Am J Pathol, 2009,175:1525–1535.

[13] Zhang JP, Lu WG, Ye F, et al. Study on CXCR4/SDF-1alpha axis in lymph node metastasis of cervical squamous cell carcinoma. Int J Gynecol Cancer, 2007,17:478–483.

[14] Kim CH, Kunkel EJ, Boisvert J, et al. Bonzo/CXCR6 expression defines type 1-polarized T-cell subsets with extralymphoid tissue homing potential. J Clin Invest, 2001,107:595–601.

[15] Abel S, Hundhausen C, Mentlein R, et al. The transmembrane CXC-chemokine ligand 16 is induced by IFN-γ and TNF-α and shed by the activity of the disintegrin-like metalloproteinase ADAM10. J Immunol, 2004,172:6362–6372.

[16] Darash-Yahana M, Gillespie JW, Hewitt SM, et al. The chemokine CXCL16 and its receptor, CXCR6, as markers and promoters of inflammation-associated cancers. PLoS One, 2009,4:e6695.

[17] Guo L, Cui ZM, Zhang J, et al. Chemokine axes CXCL12/CXCR4 and CXCL16/CXCR6 correlate with lymph node metastasis in epithelial ovarian carcinoma. Chin J Cancer, 2011,30:336–343.

[18] Majka M, Drukala J, Lesko E, et al. SDF-1 alone and in co-operation with HGF regulates biology of human cervical carcinoma cells. Folia Histochem Cytobiol, 2006,44:155–164.

[19] Balkwill F. The significance of cancer cell expression of the chemokine receptor CXCR4. Semin Cancer Biol, 2004,14:171–179.

[20] Uchida D, Begum NM, Almofti A, et al. Possible role of stromal-cell–derived factor-1/CXCR4 signaling on lymph node metastasis of oral squamous cell carcinoma. Exp Cell Res, 2003,290:289–302.

[21] Machelon V, Gaudin F, Camilleri-Broët S, et al. CXCL12 expression by healthy and malignant ovarian epithelial cells. BMC Cancer, 2011,11:97.

[22] Laguri C, Arenzana-Seisdedos F, Lortat-Jacob H. Relationships between glycosaminoglycan and receptor binding sites in chemokines—the CXCL12 example. Carbohydr Res, 2008,343:2018–2023.

[23] Burns JM, Summers BC, Wang Y, et al. A novel chemokine receptor for SDF-1 and I-TAC involved in cell survival, cell adhesion, and tumor development. J Exp Med, 2006,203:2201–2213.

[24] Hartmann TN, Grabovsky V, Pasvolsky R, et al. A crosstalk between intracellular CXCR7 and CXCR4 involved in rapid CXCL12-triggered integrin activation but not in chemokine-triggered motility of human T lymphocytes and CD34+ cells. J Leukoc Biol, 2008,84:1130–1140.