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***Basic Study***

**CXCR7/CXCL12 axis is involved in lymph node and liver metastasis of gastric carcinoma**

Xin Q *et al.* CXCR7/CXCL12 axis and gastric carcinoma

Qi Xin, Na Zhang, Hai-Bo Yu, Qin Zhang, Yan-Fen Cui, Chuan-Shan Zhang, Zhe Ma, Yan Yang, Wei Liu

**Qi Xin, Qin Zhang, Chuan-Shan Zhang, Zhe Ma,** The Third Central Clinical College of Tianjin Medical University, Department of Pathology, Tianjin Third Central Hospital, Tianjin Key Laboratory of Artificial Cells, Tianjin 300211, China

**Na Zhang, Yan Yang, Wei Liu,** Department of Pathology, Dagang Hospital, Tianjin 300211, China

**Hai-Bo Yu,** The Second Hospital of Tianjin Medical University, Tianjin 300211, China

**Yan-Fen Cui,** Tianjin Medical University Cancer Institute and Hospital, Tianjin 300070, China

**Author** **contributions**: Xin Q, Zhang N, Bo Hai Y contributed equally to this work; Xin Q, Zhang N, Yu Hai B designed research; Xin Q, Cui Yan F, Ma ZH, Yang Y, Liu W performed research; Yu Hai B, Zhang Chuan SH contributed new reagents or analytic tools; Xin Q, Zhang N, Yu Hai B analyzed data; Xin Q, Zhang Q wrote the paper.

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**Correspondence to: Dr. Na Zhang,** Department of Pathology, Dagang Hospital, Tianjin Binhai New Area, Tianjin 300211, China. [13802108605@163.com](mailto:13802108605@163.com)

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**Abstract**

***AIM***

To investigate the role of CXC chemokine receptor (CXCR)-7 and CXCL12 in lymph node and liver metastasis of gastric carcinoma.

***METHODS***

In 160 cases of gastric cancer, the expression of CXCR7 and CXCL12 in the tumors and corresponding adjacent non-cancer tissues, in the lymph nodes around the stomach and in the liver was detected using immunohistochemistry to analyze the relationship between CXCR7/CXCL12 and clinical pathological features and to determine whether CXCR7 and CXCL12 constitute a biological axis to promote lymph node and liver metastasis of gastric cancer. Furthermore, the CXCR7 gene was silenced and overexpressed by a small hairpin RNA-mediated lentiviral vector, by transfection into human gastric cancer cell line SGC-7901 cells. Cell proliferation, migration and invasiveness were measured by the MTT, wound-healing and Transwell assays, respectively.

***RESULTS***

The results demonstrated that CXCR7 expression was upregulated in gastric cancer tissues (*P =* 0.011). CXCR7 /CXCL12 axis expression was significantly related to poorly differentiated tumors, high tumor stage and lymph node (r = 0.338, *P =* 0.000) and liver metastasis (r = 0.629, *P =* 0.000). The expression of CXCL12 in lymph node and liver metastasis was higher than that in primary gastric cancer tissues (*χ2* = 6.669, *P* = 0.010; *χ2 = 25379, P* = 0.000), and the expression of CXCL12 in lymph node and liver metastasis of gastric cancer was consistent with the positive expression of CXCR7 in gastric cancer (*r* = 0.338, *P =* 0.000; *r =* 0.629, *P =* 0.000). Overexpression of the CXCR7 gene up-regulated cell proliferation, migration and invasion. Silencing of the CXCR7 gene suppressed SGC-7901 cell proliferation, migration and invasion. Human gastric cancer cell lines expressed CXCR7 and showed vigorous proliferation and migratory responses to CXCL12.

***CONCLUSION***

The CXCR7/CXCL12 axis was involved in lymph node and liver metastasis of gastric cancer. CXCR7 was considered a potential therapeutic target in the treatment of gastric cancer.

**Key words**: Gastric cancer; Stromal cell derived factor-1; CXC chemokine receptor-7; Lymph node metastasis; Liver metastasis

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**C****ore tip:** The CXC chemokine receptor (CXCR)-7/CXCL12 axis could play an important role in cell metastasis in certain cancers. However, little is known about the effect of CXCR7/CXCL12 on the process of gastric cancer. This study investigated the role of CXCL12 and CXCR7 in lymph node and liver metastasis of gastric carcinoma. We found that the CXCR7/CXCL12 axis was involved in the lymph node and liver metastasis of gastric cancer. Overexpression of the CXCR7 gene up-regulated cell proliferation, migration and invasion. Silencing of the CXCR7 gene suppressed these processes. CXCR7 was considered a potential therapeutic target in the treatment of gastric cancer.

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**Introduction**

Gastric carcinoma is a disease with a high death rate, making it the second most common cause of cancer death worldwide, following lung cancer. The high mortality of gastric cancer is due to metastasis, and the most common metastatic site is the lymph nodes, followed by the liver, indicating an urgent need for new diagnostic markers and treatment approaches[1,2].

In recent years, chemokines and their receptors have been found to be expressed on cancer cells and may mediate cancer progression and metastasis. Malignant cells can express chemokine receptors and respond to chemokine gradients, which may be related to the growth and spread of cancer. Stromal cell-derived factor 1 (SDF-1) is a very important chemotactic factor that stimulates proliferation, dissociation, migration, and invasion in a wide variety of tumor cells, including gastric cancer[[3-5](#_ENREF_3)]. For many years, CXCR4 was believed to be the only receptor for CXCL12. However, several recent reports have provided evidence that CXCR7 (RDC-1) is an identified chemokine receptor that shares the same ligand (CXCL12) as CXCR4. CXCL12 binds to CXCR7 with greater affinity than CXCR4 (Kd = 0.4 nM *vs* 3.6 nM)[[2](#_ENREF_2)]. In humans, CXCR7 is expressed in embryonic neuronal and heart tissue, some hematopoietic cells, and activated endothelium[[6](#_ENREF_6),7], but on few other normal cell types. Moreover, CXCR7 is expressed by various cancers, including breast cancer[[8](#_ENREF_8)], lung cancer[[9](#_ENREF_9)], and glioma[[10](#_ENREF_10)], and was shown to promote the growth and metastasis of various tumor models[[9](#_ENREF_9),10]. The main ligand for CXCR7 is CXCL12, which binds to CXCR7 with high affinity, but CXCR7 may also bind the alternative ligand CXCL11 with low affinity.

Although CXCR7 is expressed by many different tumors, studies of CXCR7 expression in gastric cancer are few in number. Zhi *et al*[[11](#_ENREF_11),12] have reported that CXCR7 tran­scripts have been detected in gastric cancer cells, including MGC‑803, SGC‑7901 and BGC‑823 cells, and Lee *et al*[[5](#_ENREF_5)] reported that CXCR7 was differentially expressed in gastric adenocarcinoma tissues. However, most of the studies concerning CXCL12 and CXCR7 have been conducted *in vitro*, and the definitive pathophysiological functions of the CXCR7/CXCL12 axis in human gastric cancer require further research.

In this study, we investigated the expression of CXCR7 and CXCL12 in gastric tissues, normal mucous membranes, lymph nodes and liver. Using a combination of overexpression and RNA interference in a stable receptor expression study, we precisely interrogated the role of CXCR7 in gastric cancer cell growth, migration and invasion *in vitro*.

**MATERIALS AND METHODS**

***Materials***

The study included surgically resected specimens from 160 patients (72 men and 88 women, aged 65.7 ± 11.4 years) with gastric cancer. All of the patients underwent gastrectomy at the Tianjin Medical University Cancer Institute and Hospital. Non-tumoral gastric tissues were obtained at least 5 cm from the tumor at the same time. The cases were almost evenly divided between the two major types of gastric cancer: intestinal (120 patients) and diffuse (40 patients). These two types are defined by the histological classification of Lauren[[13](#_ENREF_13)]. According to the Union for International Cancer Control tumor‑node‑metas­tasis (TNM) classification[[14](#_ENREF_14)], cancers were classified as pT1 +T2 (*n =* 66), pT3 +pT4 (*n =* 94), with positive nodal involvement in 96 cases (all confirmed by histopathological examination) and 30 cases having liver metastasis at the time of gastrectomy (confirmed by either histopathological examination or computed tomography). The lymph nodes around the stomach did not have metastasis in 64 cases. The 29 cases of livers with no metastasis came from resected specimens with non-neoplastic diseases. The 29 cases with liver metastasis were of the intestinal type of gastric cancer (after the imaging diagnosis of liver metastasis of gastric cancer, one of the 30 patients refused to undergo fine-needle aspiration). Patients enrolled in the study had not received any chemo- or radiotherapy before diagnosis. Routine chemotherapy had been given to the patients with advanced-stage disease after operation, but no radiation treatment was performed in any of patients included in our study. Patients were excluded if they had previously been exposed to any targeted therapy, chemotherapy, radiotherapy, or intervention therapy for gastric cancer.

***Reagents***

The human recombinant CXCL12 and the mouse anti-human CXCR7 monoclonal antibody are were obtained from Dako Company. CXCR7-specific siRNA and overexpressed RNA were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). The CCK-8 reagent kit was purchased from Sigma (USA). Total RNA extraction kits (RNAfast200) were purchased from Fastagen Biotechnology (Shanghai); reverse transcription kits were purchased from TaKaRa (Japan). PCR primers were synthesized by Shanghai Bioengineering & Technology Services. Millicell small chambers were purchased from Millipore (USA); Matrigel and MTS kits were purchased from BD Biosciences (USA). The PCR amplification apparatus was produced by Gene Company; all of the primers used in RT-PCR were designed and synthesized by Beijing Aoke.

***Cell Lines and Cell Culture***

Human gastric cancer SGC-7901 cells were maintained under standard conditions (37 °C and 5% CO2) in tissue culture flasks and were grown in minimum essential medium supplemented with 10% fetal bovine serum (FBS). Cells in the logarithmic growth phase were used in all experiments.

***Immunohistochemistry***

Immunohistochemistry (IHC) staining was performed using the Ultra Tek HRP and anti-CXCR7 antibody according to the manufacturer’s instructions. In brief, sections were prepared from gastric cancer blocks, mounted on charged slides with APES (Sigma), and fixed for 1-2 h at 60 °C before staining. Next, the sections were deparaffinized in xylene and rehydrated in graded alcohol solutions. After antigen retrieval by heating (95 °C) in citrate buffer (pH 6) for 15 min, endogenous peroxidase was blocked by the treatment of sections with 3% hydrogen peroxidase for 10 min. After blocking with 2% BSA for 10 min, the slides were incubated with anti-CXCR7 antibody (1:200) diluted in antibody diluents (S3022; Dako) overnight in humid chamber at 4℃. The slides were washed and then were incubated with the anti-mouse biotinylated secondary antibody for 20 min, followed by incubation with HRP-conjugated streptavidin for 20 min. The slides were washed and treated with 3,3’-diaminobenzidine (DAB) chromogen for 5 min and were counterstained with Mayer’s hematoxylin, followed by mounting.

The cell membrane or cytoplasm shows light yellow or brown yellow staining in the cell, and 5 randomly selected high-magnification (× 400) images were assessed using the following scoring scales. Positive cells: 0, no positive cells; 1, 1%-29%; 2, 30%-59%; 3, ≥ 60% staining. Strength: 0, negative; 1, mild yellow staining; 2, moderate brown staining; 3, stained dark brown yellow. According to the range of positive cells ×staining intensity score, (-) negative referred to a score < 2 and (+) positive referred to a score ≥ 2.

***Establishment of stable overexpression and silenced cell lines***

Human gastric cancer SGC-7901 cells were cultured in RPMI supplemented with 10% fetal calf serum, under the conditions of 37 °C, 5% CO2, and saturated humidity. The human CXCR7 sequence was digested out from the pcDNA 3.1 + plasmid (kindly provided by ChemoCentryx, Mountain View, CA, USA). CXCR7 siRNA (sc-35421) was purchased from Santa Cruz Biotechnology. Transfections were performed using Lipofectamine 2000 according to the manufacturer’s instructions. The samples were treated with EndoFectinTM, and the CXCR7 overexpression cell line and CXCR7 silenced expression cell line were established. The following groups were part of the study: A group: the blank control group (Control), the blank vector group (Vector), the CXCR7 overexpression group; B group: the blank control group (Control), the blank vector group (Vector), the CXCR7 silenced expression group.

***Real-time PCR assay***

Cells were cultured to reach 70%–80% confluency in six-well plates. The total mRNA was extracted from the cells using the Ultrapure RNA Kit (CWbio.Co.Ltd, *Cat. #CW0581*) according to the manufacturer’s instructions, and the gene expression was measured. The reaction temperature was 94 °C. After 5 min of denaturation, 40 cycles of amplification were performed; each cycle consisted of [predegeneration](http://dict.cn/predegeneration) to 95 °C for 10 min, degeneration at 95 °C for 15 s and distention at 60 °C for 60 s. The reaction was activated at 72 °C for 10 min, and was then terminated at 4 °C by the ABI 7500 real time PCR system. GAPDH was used as an internal control. Chemi Image 5500 automated electrophoresis gel image analyzer was used to determine the relative mean gray values (A) of the target product and β-actin internal control; the expression index (I) of the target product mRNA was calculated using the formula: I = Aproduct/A β-actin.

***Western blotting assay***

Cells were cultured to reach 70%–80% confluency in six-well plates, and then the cells were digested and collected. Whole protein was obtained by RIPA lysis buffer and centrifuged at 12,000 g for 10 min. The total protein concentration was measured by the bicinchoninic acid (BCA) method. Next, 100 mg of protein lysates were separated by 12% SDS-PAGE. The proteins were transferred to PVDF membranes and were blocked with 5% skimmed milk with PBST containing 0.05% Tween 20 at room temperature for 2 h. The primary antibody (CXCR7, 1:200) was added and incubated at room temperature for 2 h. The membrane was washed by PBST three times and was incubated with the secondary antibody for 1 h. The immunoreactive bands were washed and observed. α-Actin was used as an internal control.

***Cell proliferation assay***

Following intervention, the cells were digested with trypsin, prepared into 5 × 103 cells/well suspensions with serum-free medium, and then were inoculated into a 96-well culture plate, 200 μL of the cell suspension per well, followed by the addition of 20 μL of CCK-8 solution and 100 ng/ml CXCL12. The plate was returned to a 37 °C, 5% CO2 incubator with saturation humidity for 0 h, 48 h, or 96 h. Finally, the plate was placed in an Enzyme-Linked Immunoassay Analyzer (ELISA), and the absorbance value (OD) of each well was measured at 450 nm. Each group had 6 wells; the cell proliferation values of each group were calculated.

***Cell migration assay***

The cell migration assay was conducted as previously described. After incubation for 6 h, the growth medium was then changed to basal medium with CXCL12 (100 ng/mL). 24 h later, the wounds were observed using bright-field microscopy, and multiple images were taken at areas flanking the intersections of the wound and marker lines at the start and end of the experiment. The gap distance of the wound was measured at three different sites using Photoshop software, and the data were normalized to the average of the control. Graphs were plotted against the percentage of the migration distance the cells moved before and after treatment.

***Cell invasion assay***

Cell invasion in response to CXCL12 was assayed in the Biocoat Matrigel invasion chamber (Becton Dickinson, USA) using an 8-μm porosity polycarbonate filter membrane that was coated with Matrigel. The Transwell chamber was pre-cooled at 4 °C. Next, the upper chamber was evenly laid with 20 mL of Matrigel and was incubated at 37 °C for 3 h. Approximately 3 × 105 cells were added into the upper chamber, and 600 mL of medium with 0.1% BSA was added into the lower chamber with FN (50 mg/mL). Next, the cells were cultured at 37 °C for 24 h. The cells were fixed on the upper layer of the membrane by formalin. The number of invasive cells was determined by counting the hematoxylin-stained cells. For quantification, the cells were counted under a microscope in five fields (up, down, median, left, and right. ×200).

***Statistical analysis***

SPSS17.0 software was used for data processing. The measurement data were expressed as the mean ± standard deviation and were compared using ANOVA. Pair-wise comparisons between groups were performed using the SNK method. The above hypothesis test was two-sided; associations between these expression levels in gastric cancer and the clinicopathological features were deter­mined using a χ2-test. The vari­ables considered for the univariate analysis consisted of patient-related and tumor-related variables. Pearson correlation analysis was used for correlation analysis. *P <* 0.05 was considered to be statistically significant.

**RESULTS**

***Expression levels of CXCL12, CXCR7 protein in*** ***gastric carcinoma and adjacent normal gastric tissues***

To ascertain whether the CXCL12 and CXCR7 protein is elevated in gastric cancer tissues, we first evaluated CXCL12 and CXCR7 expression by immunohistochemically analyses in tumor tissues and normal gastric tissues. In cancer tissues, CXCR7 was highly expressed in gastric cancer (Figure 1A) and the positive expression rates of CXCR7 was 78.75% (126/160). CXCL12 was highly expressed in gastric cancer (Figure 1D ) and the expression rates of CXCL12 was 68.13% (109/160).but in normal gastric tissue, CXCL12 and CXCR7 were expressed at a very low level (Figure 1B, E).CXCL12 and CXCR7 in cancer tissues were significantly higher than normal tissues (*P =* 0.011, *P =* 0.011) (Figure 1C, F).and the expression of CXCL12 and CXCR7 were correlative in gastric cancer tissue (*P =* 0.000, Figure 1G). We also found CXCR7 staining was observed in inflammatory cells and some parts of [mesenchymal tissue](http://dict.cn/mesenchymal%20tissue). CXCR7 was detected on tumor-associated blood vessels in nearly all specimens of gastric cancer tissue, but not in blood vessels from nonmalignant tissue. In gastric carcinoma, CXCR7 in lymph and blood vessels within the lumen of the gastric cancer cells were strongly positive expression, and stronger than the expression intensity in gastric cancer tissues itself.

***Association between CXCL12 and CXCR7 expression and clinical characteristics in patients with gastric cancer***

In gastric carcinoma, the expression of CXCL12 and CXCR7 related to tumor size, depth of invasion, Lauren’s classification of the tumor, lymph node metastasis, and clinical stage. We did not observe any other association between pattern of CXCR7 expression and other clinical findings such as age, gender, differentiation. (Table 1) Analysis of CXCL12+ CXCR7+, CXCL12+ CXCR7-/ CXCL12-CXCR7+, CXCL12-CXCR7- in gastric carcinoma and the clinical pathological characteristics and its relationship with lymph node and liver, CXCRL12+CXCR7+ gastric cancer cells more prone to lymph node and liver metastasis, and it was positively correlated with tumor size, depth of invasion and clinical stage. It suggests that CXCL12+CXCR7+ is more likely to grow and metastasis in gastric cancer (Table 2).

***Expression levels of CXCL12 in*** ***lymph nodes with or without cancer cell*** ***metastasis***

Among the 160 lymph nodes that we collected, 96 had cancer metastasis and the remaining nodes were normal. The expression of CXCL12 was also significantly higher in the lymph nodes with metastasis than in the lymph nodes without metastasis (*χ2* = 49.313, *P* = 0.000). And the expression of CXCL12 in lymph node metastasis was higher than in primary gastric cancer tissues (*χ2* = 6.669, *P =* 0.010 )(Figure 2A，B, C). in metastatic lymph nodes, the expression of CXCL12 is also expressed in the peripheral inflammatory cells.

***Expression levels of CXCL12 in livers with or without cancer cell metastasis***

Among the 58 livers that we collected, 29 livers had cancer metastasis and the remaining livers were normal. The expression of CXCL12 was also significantly higher in the livers with metastasis than in their normal counterparts (*χ2* = 5.317, *P* = 0.021). And the expression of CXCL12 in liver metastasis of gastric cancer cells were higher than primary type of gastric cancer (*χ2* = 25.379,*P* = 0.000) (Figure 2D, E, F). The expression of CXCL12 also was found in the normal around liver tissues of the liver metastasis.

***Correlation analysis of CXCL12 expression in lymph nodes/livers and CXCR7 expression in gastric cancer***

Pearson correlation analysis showed that the positive expression of CXCL12 in lymph node and liver metastasis of gastric cancer was consistent with the positive expression of CXCR7 in gastric cancer (r = 0.338, *P =* 0.000; r = 0.629, *P =* 0.000) (Figure 3A, B).

***Expression levels of CXCL12/CXCR7 in lymph nodes with intestinal gastric cancer and diffuse gastric cancer metastasis***

Based on the above experimental results, it is shown that CXCL12/CXCR7 can promote lymph node and liver metastasis of gastric cancer. Our previous work found that CXCL12/CXCR7 biological axis can promote lymph node and liver metastasis of intestinal type gastric cancer by immunohistochemistry, so we studied the difference of CXCL12/CXCR7 expression between lymph node metastasis with intestinal gastric cancer and lymph node with diffuse gastric cancer metastasis. Statistical analysis showed that there was no statistical difference in the expression of CXCL12/CXCR7 in these two groups (*χ2* = 0.042*, P =* 0.837*; χ2* = 0.265, *P =* 0.606*)* (Figure 4A, B).

***The vector stably expressing over-expressing CXCR7/CXCR7siRNA causes effective and specific up/down -regulation of CXCR7 expression***

In order to study the potential role of CXCR7 in SGC-7901 cell, we established the CXCR7 over-expression and CXCR7siRNA vector and then scrambled the overexpression and siRNA vector used to transfect SGC-7901 cells. Two groups were built: A group: the control group (Control), the blank vector group (Vector), the CXCR7 overexpression group; B group: the control group (Control), the blank vector group (Vector), the CXCR7 silencing expression group. The result was tested using RT-PCR and Western blot. As shown in Figure 5A, CXCR7mRNA levels were increased in CXCR7 overexpressing transfected cells, compared with the control cells (*P =* 0.000) and the blank vector group (*P =* 0.000). At the same time, CXCR7mRNA levels were reduced in CXCR7 siRNA transfected cells (*P =* 0.000, *P =* 0.000) (Figure.5C). Like RT-PCR results, in CXCR7 over-expressing transfected cells the expression level of CXCR7 protein were increased, and the expression level of CXCR7 protein were reduced in CXCR7 siRNA transfected cells (Figure 5B, D). These results demonstrated that the expression of CXCR7 was specifically increased/silenced in SGC-7901 cells.

***The effect of CXCR7 overexpression and silencing on the proliferation ability of human gastric cancer SGC-7901 cells in vitro***

CCK-8 experiments showed that cells overexpressing CXCR7 proliferated more rapidly than the control cells, whereas cells with depleted CXCR7 grew more slowly than control (Figure 6). Moreover, the CXCR7-transfected SGC-7901 cells showed a substantial increase in cell numbers in the presence of CXCL12 (100 ng/mL), compared with nontreatment ones; but proliferation was not increased in the transfected blank vector cells by CXCL12 stimulation. The results indicated that CXCL12 engagement to CXCR7 can induce proliferation.

***The effect of CXCR7 overexpression and silencing on the migration ability of SGC-7901 cells in vitro***

Besides enhanced growth advantage of tumor cell, increased cell invasion is a determinant hallmark of metastatic tumor cell. Thus, we sought to explore the influence of CXCR7 on cell migration. Ectopic overexpression of CXCR7 in SGC7901 cells significantly enhanced cell migration induced by CXCL12 *via* wound-healing experiment compared with the control cells and Vector cells. (Figure 7A, *P =* 0.011); in contrast, the reversed effects were observed when CXCR7 was silenced in SGC7901 cells (Figure 7B, *P =* 0.004).

***The effect of CXCR7 overexpression and silencing on the invasion ability of SGC-7901 cells in vitro***

Invasion is also one of the important steps in tumor metastasis. The CXCR7/CXCL12 interaction was reported to regulate invasive and metastatic behavior of several tumors. We measured the effect of CXCR7 overexpression and silencing on the invasion ability of SGC-7901 cells *in vitro* by Transwell assay. As shown in Figure 8A and 8B, SGC-7901 cells spontaneously invaded through artificial basement membrane in the absence of CXCL12. In addition, we found that CXCL12 induced a significant increase of cancer cell invasion through Matrigel, overexpressing CXCR7 cells had displayed increased invasive ability compared with the control cells and Vector cells. We next evaluated the effect of silencing, the CXCR7 siRNA cells displayed decreased invasive ability compared with the control cells and Vector cells (Figure 8). Taken together, these findings indicate that CXCR7overexpressing could potently enhances the invasive ability of SGC-7901 cells induced by CXCL12 and that silencing of CXCR7 inhibits the invasive behavior of the cells.

**RESULTS**

***Expression levels of CXCL12 and CXCR7 proteins in gastric carcinoma and adjacent normal gastric tissues***

To ascertain whether the CXCL12 and CXCR7 proteins are elevated in gastric cancer tissues, we first evaluated CXCL12 and CXCR7 expression by immunohistochemistry analyses in tumor tissues and normal gastric tissues. In cancer tissues, CXCR7 was highly expressed in gastric cancer (Figure 1A), and the positive expression rate of CXCR7 was 78.75% (126/160). CXCL12 was highly expressed in gastric cancer (Figure 1D), and the expression rate of CXCL12 was 68.13% (109/160). However, in normal gastric tissue, CXCL12 and CXCR7 were expressed at very low levels (Figure 1B, E). CXCL12 and CXCR7 expressions in cancer tissues were significantly higher than those in normal tissues (*P =* 0.011, *P =* 0.011) (Figure 1C, F). Additionally, the expressions of CXCL12 and CXCR7 were correlated in gastric cancer tissue (*P =* 0.000, Figure 1G). We also observed CXCR7 staining in inflammatory cells and some parts of [mesenchymal tissue](http://dict.cn/mesenchymal%20tissue). CXCR7 was detected in tumor-associated blood vessels in nearly all specimens of gastric cancer tissue but not in blood vessels from nonmalignant tissue. In gastric carcinoma, CXCR7 in lymph and blood vessels within the lumen of the gastric cancer cells showed strongly positive expression, stronger than the expression intensity in gastric cancer tissue.

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In gastric carcinoma, the expression of CXCL12 and CXCR7 was related to tumor size, depth of invasion, Lauren’s classification of the tumor, lymph node metastasis, and clinical stage. We did not observe any other association between the pattern of CXCR7 expression and other clinical findings such as age, gender, and differentiation (Table 1). Analysis of CXCL12+ CXCR7+, CXCL12+ CXCR7-/CXCL12-CXCR7+, CXCL12-CXCR7- in gastric carcinoma and clinical pathological characteristics and its relationship with the lymph nodes and liver revealed that CXCRL12+CXCR7+ gastric cancer cells were more prone to lymph node and liver metastasis, and were positively correlated with tumor size, depth of invasion and clinical stage. Thus, CXCL12+CXCR7+ is more likely to grow and metastasize in gastric cancer (Table 2).

***Expression levels of CXCL12 in lymph nodes with or without cancer cell metastasis***

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Besides the enhanced growth advantage of tumor cells, increased cell invasion is a determinant hallmark of metastatic tumor cells. Thus, we sought to explore the influence of CXCR7 on cell migration. The ectopic overexpression of CXCR7 in SGC7901 cells significantly enhanced cell migration induced by CXCL12 *via* wound-healing experiments compared with the control cells and Vector cells (Figure 7A, *P =* 0.011); by contrast, the reversed effects were observed when CXCR7 was silenced in SGC7901 cells (Figure 7B, *P =* 0.004).

***Effect of CXCR7 overexpression and silencing on the invasion ability of SGC-7901 cells in vitro***

Invasion is also one of the important steps in tumor metastasis. The CXCR7/CXCL12 interaction was reported to regulate the invasive and metastatic behavior of several tumors. We measured the effect of CXCR7 overexpression and silencing on the invasion ability of SGC-7901 cells *in vitro* by the Transwell assay. As shown in Figure 8A and 8B, SGC-7901 cells spontaneously invaded through the artificial basement membrane in the absence of CXCL12. In addition, we found that CXCL12 induced a significant increase in cancer cell invasion through Matrigel; overexpressing-CXCR7 cells displayed increased invasive ability compared with that in control cells and Vector cells. We next evaluated the effect of silencing, and the CXCR7 siRNA cells displayed decreased invasive ability compared with that of the control cells and Vector cells (Figure 8). Taken together, these findings indicate that CXCR7 overexpression could potently enhance the invasive ability of SGC-7901 cells induced by CXCL12 and that silencing of CXCR7 inhibits the invasive behavior of the cells.

**DISCUSSION**

Chemokine and chemokine receptor pairs have been identified to play pivotal roles in cancer initiation and progression. CXCL12 and its receptors (CXCR4 and CXCR7) are important members. They are implicated in several aspects, including the migration, adhesion, proliferation and survival of tumor cells, and the formation of tumor-associated vessels and invasion[[15](#_ENREF_15)] . CXCR7, initially named receptor Dog cDNA1/RDC1, is a second receptor for CXCL12, and it can bind chemokines CXCL11/ITAC and macrophage migratory inhibitory factor (MIF)[[16](#_ENREF_16)],[[17](#_ENREF_17)]. Due to the mutation at the “DRAILIV” motif[[18](#_ENREF_18)], CXCR7 cannot activate G proteins that are associated with typical chemokine receptors. CXCR7 has been proposed as a “decoy” receptor, mainly functioning to shape the CXCL12 gradient for the guidance of cell migration in different models[[19](#_ENREF_19),20]. By contrast, other studies provided evidence that CXCR7 can activate downstream signal transduction molecules and cytokine production, promote the survival of tumor cells by preventing apoptosis, and impact cell adhesion and invasion through complex signaling processes[[9](#_ENREF_9),[10](#_ENREF_10),21].

To the best of our knowledge, this study is the first demonstrating that CXCR7 was widely expressed in human gastric cancer tissue by immunohistochemistry and that this expression of CXCR7 was increased compared with that in normal gastric tissues. CXCR7 was present on tumor-associated blood vessels but not on the normal vasculature. Immunohistochemistry of human breast and lung cancer tissue also revealed extensive CXCR7 expression on tumor-associated blood vessels and cancer cells[[9](#_ENREF_9)]. Additionally, in hepatocellular carcinoma, the suppression of CXCR7 expression by RNA interference impairs *in vitro* cellular VEGF secretion and angiogenesis[[22](#_ENREF_22)]. Thus, CXCR7 may contribute to gastric cancer development by regulating angiogenesis; in human hepatocellular carcinoma cells, the overexpression of CXCR7 induces the angiogenic capacity *via* the AKT signaling pathway[[23](#_ENREF_23)]. In gastric cancer cells, CXCR7 is highly expressed, but CXCR7 is poorly expressed in normal gastric cells. This result suggests that CXCR7 might play a role in gastric tumorigenesis. In our *in vitro* studies, we found that the proliferation of gastric cancer cells was significantly increased in the presence of overexpressing CXCR7 and showed significant profile responses to CXCL12. Importantly, silencing CXCR7 can effectively suppress this proliferative capacity of gastric cancer cells. This suggested that the CXCR7/CXCL12 ligand can promote the proliferation of gastric cancer cells, and the anti-proliferative effect of the down-regulation of CXCR7 may be an important factor in the growth mechanism. Combined with our experiments, we believe that CXCR7/ CXCL12 can promote the growth of gastric cancer. These findings are similar to observations in hepatocellular and breast carcinoma models[[24](#_ENREF_24)],[[8](#_ENREF_8)]. However, in thyroid cancer K1 cells and TFF3-dependent activation of cells, the overexpression of CXCR7 had no effect on cell proliferation[[25](#_ENREF_25),26]. Different results have described that in different tumor cell types, depending on the differentiation status and environment, CXCR7 may play a different role. In prostate cancer, IL-8-regulated CXCR7 stimulates EGFR signaling to promote prostate cancer growth, and the growth-promoting activity does not require its ligands[[27](#_ENREF_27)]. However, in hepatocellular carcinoma, CXCR7 activates the ERK pathway to mediate cell proliferation. The mechanisms by which CXCR7 participates in the growth of gastric cancer warrant further investigation, and the specific mechanism will be further studied in our next work. Additionally, we found that the expression of CXCR7 in the cancer cells of metastatic vascular lumen was higher than that in gastric carcinoma tissues, suggesting that CXCR7 may have a function in the metastasis of gastric cancer.

Our clinicopathological study revealed that CXCR7 expression was significantly positive in gastric cancers with a high tumor stage and lymph node and liver metastasis. The larger the tumor diameter and infiltration depth were, the stronger the expression of CXCR7 was. Regarding the different clinical stages, the expression level of CXCR7 in stage III + IV was significantly higher than that in stage I + II gastric cancer. These results suggested that the expression of CXCR7 might be involved in the invasion and metastasis of gastric cancer cells, and CXCR7-positive gastric cancer may have a strong migratory potential. CXCL12+CXCR7+ gastric cancer tissues are more prone to lymph node and liver metastasis compared to CXCL12+CXCR7-/CXCL12-CXCR7+ and CXCL12-CXCR7- gastric cancer tissues. A tumor diameter greater than 3.5 cm, depth T3 + T4, and stage III + IV showed stronger expression of CXCL12+CXCR7+ in gastric cancer. Our results from the experiments *in vitro* appeared to support this hypothesis. In this study, we also found that CXCR7, by combining with CXCL12, can promote invasion in SGC7901 cells, and CXCR7/ CXCL12 can promote gastric cancer cell invasion, confirming the results of a previous study in other parts of tissues and cells. Additionally, in prostate cancer, CXCR7 potentially promoted invasion through its downstream targets of CD44 and cadherin-11[[28](#_ENREF_28)].

It has been shown that higher levels of CXCL12 in target organs such as the liver or lymph nodes attract and recruit cancer cells, which subsequently form liver or lymph node metastases[[29](#_ENREF_29)]. In gastric cancer, the CXCR4/CXCL12 signaling axis played an important role in the process of lymph node metastasis and liver metastasis[[15](#_ENREF_15),30]. Additionally, CXCR4-expressing gastric carcinoma cells are preferentially attracted to the peritoneal cavity, where its ligand, SDF-1, is produced abundantly by peritoneal mesothelial cells[[31](#_ENREF_31)]. Unlike CXCR4, however, CXCR7 fails to signal through Gαi proteins and does not facilitate cell migration in response to CXCL12; it appears to act as a CXCL12 scavenger controlling the chemokine levels in favor of CXCR4-mediated chemotaxis[[32](#_ENREF_32)]. Additionally, some reports have found that CXCR7 could not mediate cancer cell migration[[33](#_ENREF_33),34]. However, a significant increase in CXCL12 was found in lymph nodes and livers with cancer cell metastasis compared with that in normal lymph nodes and livers, and the positive expression of CXCL12 in lymph node and liver metastasis of gastric cancer was consistent with the positive expression of CXCR7 in gastric cancer. This confirmed that cancer cells with highly expressed CXCR7 can migrate towards a CXCL12 gradient established in specific target organs. Additionally, *in vitro*, the overexpression of CXCR7 in SGC7901 cells significantly enhanced cell migration by combining with CXCL12. By contrast, the reversed effects were observed when CXCR7 was silenced in SGC7901 cells. Our studies strongly supported that the CXCR7/CXCL12 signaling axis could promote migration in gastric cancer. CXCR7, which cannot signal directly through G protein-linked pathways, can nevertheless affect cellular signaling networks by forming a heteromeric complex with CXCR4. The CXCR4·CXCR7 heterodimer complex recruits â-arrestin, resulting in the preferential activation of â-arrestin-linked signaling pathways[[35](#_ENREF_35)]. In late neural progenitor cells (NPCs), CXCR7 mediates migration to CXCL12 in the absence of CXCR4 through extracellular signal-regulated kinases (ERK) 1/2[[36](#_ENREF_36)] but TFF3 induced cell migration independently from the ERK1/2 signaling pathway[[26](#_ENREF_26)].

In conclusion, the results in this study indicate that CXCR7 was highly expressed in gastric cancer tissue and was increased with tumor clinical stage. The CXCR7/CXCL12 signaling axis appears to be involved in the lymph node and liver metastasis of gastric cancer. Based on these results, specific therapies with chemokine receptor antagonists could be helpful in the treatment of patients with gastric cancer metastasis.

**COMMENTS**

***Background***

Gastric cancer is one of the most commonly malignant tumors. Most deaths from gastric cancer are caused by metastasis, of which lymph node and liver metastasis are the most common cause, which leads to treatment failure. Therefore, inhibition of gastric cancer metastasis is thought to be an important therapeutic strategy. However, the molecular mechanisms involved in this process have not been fully elucidated.

***Research frontiers***

Many researchers have shown that CXC chemokine receptor-7 (CXCR7) was expressed in many types of cancer cells. The CXCR7/ and stromal cell-derived factor-12 (CXCL12) axis plays a major role in survival, proliferation, migration and adhesion of many kinds of tumor cells. However, little is known about the effect of CXCL12/CXCR7 on the process of gastric cancer. and the definitive pathophysiological function of the CXCR7/CXCL12 axis in lymph node and liver metastasis of gastric cancer needs further research.

***Innovations and breakthrough***

A little is known about the effect of CXCL12/CXCR7 on the process of gastric cancer. This study has found that the CXCR7 can express in gastric cancer and CXCR7/CXCL12 axis is also involved in lymph node and liver metastasis of gastric cancer. Furthermore, this *in vitro* study has suggested that silencing of CXCR7 gene suppressed proliferation, migration and invasion of gastric cancer cells.

***Applications***

By understanding how the CXCR7/CXCL12 axis is involved in lymph node and liver metastasis of gastric cancer, this study could represent a future strategy for therapeutic intervention in patients with lymph node and liver metastasis from gastric cancer.

***Terminology***

Chemokines are a family of small heparin-binding and secretory proteins, and through interactions with their corresponding receptors, they can control and activate many types of cells. According to the position of the four conserved cysteine residues in the amino acid sequence, they are classified into four groups: CXC, CX3C, CC and C. CXCL12 is a member of the CXC subfamily. For a long time, CXCR4 was thought to be the only receptor for CXCL12. However, several recent reports have provided evidence that CXCR7 is another receptor of CXCL12. CXCL12 binds to CXCR7 with greater affinity than to CXCR4.

***Peer-review***

The authors reported that CXCR7/CXCL12 axis could play an important role in cell metastasis in gastric carcinoma. They concluded that the CXCR7/CXCL12 axis was involved in the lymph node metastasis and liver of gastric cancer. This study was interesting and excellent, and this article was well written.

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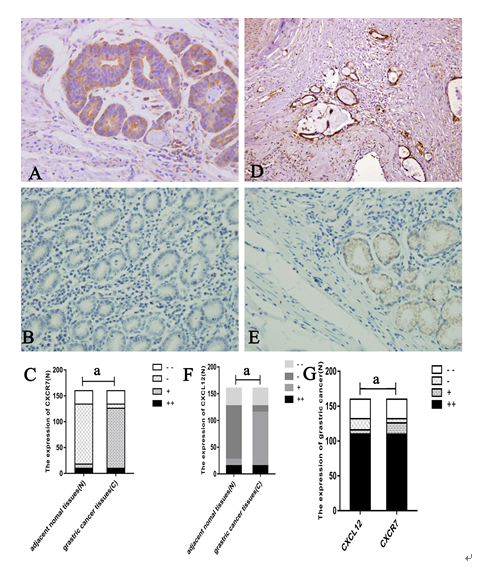
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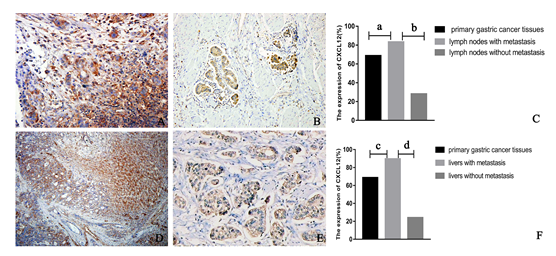
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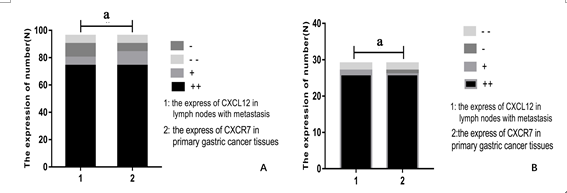
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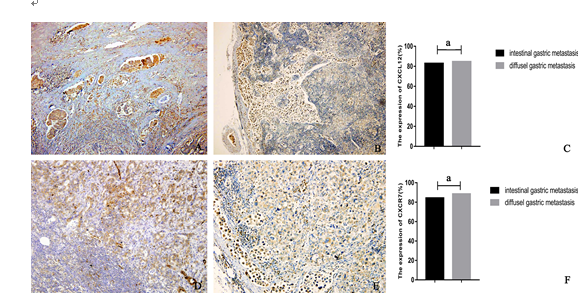
**Figure 1 CXCR7 and CXCL12 expression in non-tumoral tissues and gastric cancer.** A: gastric cancer tissue shows strong expression of CXCR7; B: Non-tumoral gastric tissue shows negative expression of CXCR7; C: CXCR7 expression in cancer tissues was significantly higher than in normal tissues; D: gastric cancer tissue shows strong expression of CXCL12; E: Non-tumoral gastric tissue shows weak expression of CXCL12; F: CXCR7 expression in cancer tissues was significantly higher than in normal tissues; G: The expression of CXCR7 and CXCL12 was correlative in gastric cancer tissues. aDenotes significant difference from controls and vector group,*P <* 0.05.



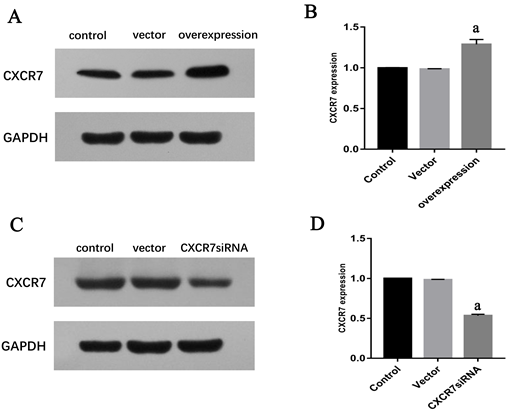
**Figure 2 CXCL12 expression in lymph node, liver and primary gastric cancer tissue.** A: lymph node with metastasis gastric cancer shows strong expression of CXCL12; B: primary gastric tissue shows strong expression of CXCL12; C: CXCL12 expression in lymph nodes with metastasis cancer tissues was significantly higher than in primary gastric tissue and lymph node with no metastasis; D: liver with metastasis gastric cancer tissue shows strong expression of CXCL12; E: primary gastric tissue shows strong expression of CXCL12; F: CXCl12 expression in liver with metastasis cancer tissues was significantly higher than in primary gastric tissue and liver with no metastasis. a-dDenotes significant difference from controls and vector group, *P <* 0.05.



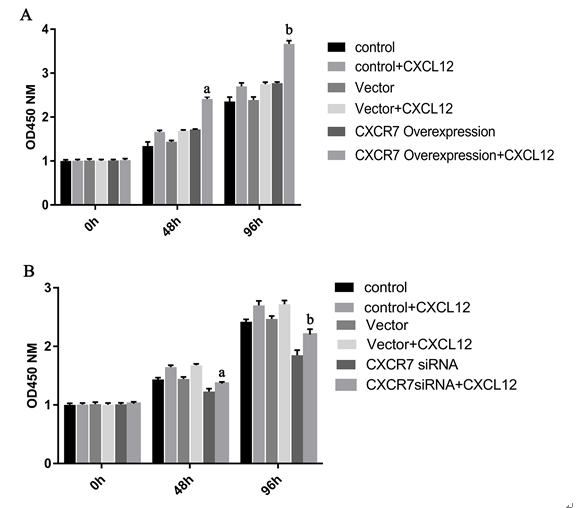
**Figure 3 The relation of CXCL12 and CXCR7.** A: Correlation analysis of CXCL12 expression in lymph nodes and CXCR7 expression in gastric cancer; B: Correlation analysis of CXCL12 expression in livers and CXCR7 expression in gastric cancer. aDenotes significant difference from controls and vector group, *P <* 0.05**.**



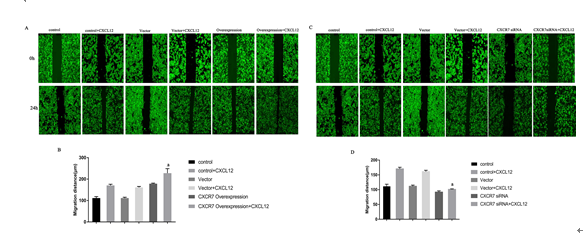
**Figure 4 CXCL12/CXCR7 expression in intestinal gastric cancer and diffuse gastric cancer.** A: CXCL12 expression in lymph nodes with intestinal metastasis cancer tissues; B: CXCL12 expression in lymph nodes with diffuse metastasis cancer tissues; C: CXCL12 expression in lymph nodes with intestinal metastasis cancer tissues and diffuse metastasis cancer tissues was no difference; D:CXCR7 expression in lymph nodes with intestinal metastasis cancer tissues; E: CXCR7 expression in lymph nodes with diffuse metastasis cancer tissues; F: CXCR7 expression in lymph nodes with intestinal metastasis cancer tissues and diffuse metastasis cancer tissues was no difference. aDenotes significant difference from controls and vector group, *P <* 0.05.



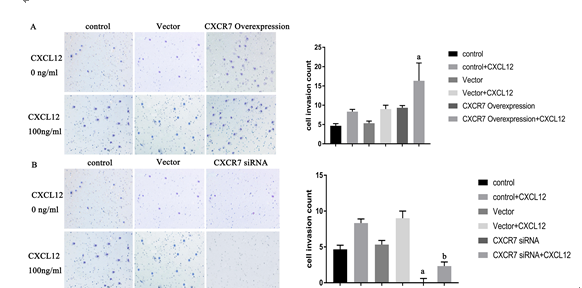
**Figure 5 Stable expression of CXCR7 in human SGC7901 cell lines.** A: Western blot assay showed increased CXCR7 expression in stable CXCR7-overexpressing human SGC7901 cell lines compared with the untreated control group and blank vector transfection control group; B: PCR assay showed the same results as the Western; C: Western blot assay showed decreased endogenous CXCR7 expression in stable CXCR7-silencing human SGC 7901 cell lines compared with the untreated control group and blank vector transfection control group; β-actin was used as an internal control; D: PCR assay showed the same results as the Western blot. aDenotes significant difference from controls and vector group. The data are presented as the mean ± SD; *n =* 4; bars indicate SD, *P <* 0.05.



**Figure 6 Effect of CXCR7 on proliferation in human gastric SGC7901 cell lines.** A: Overexpressing of CXCR7 promoted cell proliferation; B: Silencing of CXCR7 inhibited cell proliferation. Cell proliferation was measured by CCK-8 assay at 0, 48 and 96 h. a-bDenotes significant difference from control group and blank vector group. Data are presented as the mean ± SD; *n =* 5; bars indicate SD,*P <* 0.05.



**Figure 7 Effect of CXCR7 on migration in human gastric SGC7901 cell lines.** A: Overexpression of CXCR7 promoted CXCL12 induced enhancement on SGC-7901 cell migration *in vitro*; B: Mean wound width from three independent fields/well is indicated; C: Silencing of CXCR7 inhibits CXCL12 induced enhancement on SGC-7901 cell migration *in vitro*; D: Mean wound width from three independent fields/well is indicated. aDenotes significant difference from control group and blank vector group. The data are presented as the mean ± SD; *n =* 3; bars indicate SD, *P <* 0.05.



**Figure 8 Effect of CXCR7 on invasion in human gastric SGC7901 cell lines.** A: Overexpression of CXCR7 promoted CXCL12 induced enhancement on SGC-7901 cell invasion *in vitro*; B: silence of CXCR7 decreased CXCL12 induced enhancement on SGC-7901 cell invasion *in vitro*. a-bDenotes significant difference from control group and blank vector group. The data are presented as the mean ± SD; *n =* 3; bars indicate SD, *P <* 0.05.