World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/1007-9327office
wjg@wjgnet.com
doi:10.3748/wjg.v17.i19.2389

World J Gastroenterol 2011 May 21; 17(19): 2389-2396
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

ORIGINAL ARTICLE

CXCR4/SDF-1 axis is involved in lymph node metastasis of gastric carcinoma

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Supported by The National Natural Science Foundation of China, No. 30772542
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Received: August 19, 2010 Revised: November 13, 2010
Accepted: November 20, 2010
Published online: May 21, 2011

Abstract

AIM: To investigate the role of CXC chemokine receptor-4 (CXCR4) and stromal cell-derived factor-1 (SDF-1) in lymph node metastasis of gastric carcinoma.

METHODS: In 40 cases of gastric cancer, expression of CXCR4 mRNA in cancer and normal mucous membrane and SDF-1 mRNA in lymph nodes around the stomach was detected using quantitative polymerase chain reaction (PCR) (TaqMan) and immunohistochemistric assay. SGC-7901 and MGC80-3 cancer cells were used to investigate the effect of SDF-1 on cell proliferation and migration.

RESULTS: Quantitative reverse transcription PCR and immunohistochemistry revealed that the expression level of CXCR4 in gastric cancer was significantly higher than that in normal mucous membrane (1.6244 ± 1.3801 *vs* 1.0715 ± 0.5243, $P < 0.05$). The expression level of CXCR4 mRNA in gastric cancer with lymph node metastasis was also significantly higher than that without lymph node metastasis (0.823 ± 0.551 *vs* 0.392 ± 0.338, $P < 0.05$). CXCR4 expression was significantly related to poorly differentiated, high tumor stage and lymph node metastasis. Significant differences in the expression level of SDF-1 mRNA were found between lymph nodes in metastatic gastric cancer and normal nodes (0.5432 ± 0.4907 *vs* 0.2640 ± 0.2601, $P < 0.05$). The positive expression of SDF-1 mRNA in lymph nodes of metastatic gastric cancer was consistent with the positive expression of CXCR4 mRNA in gastric cancer ($r = 0.776$, $P < 0.01$). Additionally, human gastric cancer cell lines expressed CXCR4 and showed vigorous proliferation and migratory responses to SDF-1. AMD3100 (a specific CXCR4 antagonist) was also found to effectively reduce the migration of gastric cancer cells.

CONCLUSION: The CXCR4/SDF-1 axis is involved in the lymph node metastasis of gastric cancer. CXCR4 is considered as a potential therapeutic target in the treatment of gastric cancer.

Key words: Gastric carcinoma; Chemokines; Stromal cell-derived factor-1; CXC chemokine receptor-4; Lymph node metastasis

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Zhao BC, Wang ZJ, Mao WZ, Ma HC, Han JG, Zhao B, Xu HM. CXCR4/SDF-1 axis is involved in lymph node metastasis of gastric carcinoma. *World J Gastroenterol* 2011; 17(19): 2389-2396 Available from: URL: http://www.wjgnet.com/1007-9327/full/v17/i19/2389.htm DOI: http://dx.doi.org/10.3748/wjg.v17.i19.2389

INTRODUCTION

Gastric cancer is one of the most commonly diagnosed malignancies and the main cause of cancer-related deaths in Asian populations. Most deaths from gastric cancer are caused by metastasis, of which lymph node metastasis is the most common cause, which leads to the failure of surgery, chemotherapy or radiotherapy. Therefore, inhibition of metastatic gastric cancer is an important therapeutic strategy. However, the molecular mechanisms involved in this process have not been fully elucidated.

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). Stromal cell-derived factor (SDF)-1 is a member of the CXC subfamily, which was first cloned from murine bone marrow[1]. SDF-1 exerts an effect through interaction with its specific receptor CXC chemokine receptor-4 (CXCR4). Many studies have proven that CXCR4 is the major chemokine receptor expressed in many types of cancer cells[2,3]. and demonstrated that the CXCR4/SDF-1 axis plays a major role in cell survival, proliferation, migration and adhesion of several tumor cells, including those from colon cancer[4], breast cancer[5,6], non-small cell lung cancer[7], prostate cancer[8], melanoma[9,10], cholangiocarcinoma[11], and oral squamous cell carcinoma[12], However, most of the studies about SDF-1 and CXCR4 have been conducted *in vitro*, and the definitive pathophysiological functions of the CXCR4/SDF-1 axis in human diseases, especially cancer, require further research.

Recently, it has been suggested that the interaction between CXCR4 and SDF-1 plays an important role in the development of peritoneal carcinomatosis from gastric cancer[13]. We hypothesize that the CXCR4/SDF-1 axis also participates in lymph node metastasis of gastric cancer. To verify the hypothesis, we examined the expression of CXCR4 and SDF-1 in gastric cancers, normal mucous membranes, and their related lymph nodes. We also investigated the relationship between CXCR4 expression and clinicopathological features, and determined whether CXCR4 expression influenced the proliferation and migration of gastric cancer cells *in vitro*.

MATERIALS AND METHODS

Patients and tissue samples

A total of 40 patients with gastric cancer who underwent surgery at the Department of General Surgery, Beijing Chaoyang Hospital, between 2008 and 2009 were enrolled. The patient population consisted of 31 men and nine women, with a mean age of 55 years (range, 31-76 years). Patients who were receiving preoperative chemotherapy and/or radiotherapy were not included. The specimens included the tumor tissue, normal mucous membranes (5 cm away from the tumor), and the lymph nodes around the stomach. All the specimens were collected within 30 min after resection and each specimen was divided into two parts: one was fixed in 4% formalin and embedded in paraffin; and the other was snap-frozen in liquid nitrogen and kept at -80℃. Tumor stage was determined according to the TNM classification system of the International Union against Cancer. Histological diagnosis was confirmed for each specimen. Informed consent was obtained from all patients.

Cell culture

The SGC-7901 and MGC-803 gastric cancer cell lines were grown in RPMI 1640 medium (Sigma, USA) that contained 10% fetal bovine serum (FBS; Sijiqing, Hangzhou, China), 100 U/mL penicillin and 100 μg/mL streptomycin (Sigma). The suspension was placed into T25 flasks and allowed to incubate at 37℃ in a humidified chamber that contained 5% CO_2. The adherent cells were then cultured with medium changed at a 3-d interval. Cells at passage 1-6 were used for all experiments.

Cell proliferation assay

Gastric cancer cells (SGC-7901 and MGC-803) were seeded into 96-well plates at a density of 5×10^3 cells per well without FBS. After 24 h, the cultures were washed and re-fed with medium alone (control) or with medium that contained SDF-1 at various concentrations. After 3 d, the number of viable cells was counted using an MTT assay (Beyotime, China) according to the manufacturer's instructions. The quantity of formazan product measured at 490 nm was proportional to the number of live cells in the culture. The experiments were repeated in triplicate.

Cell migration assays

The invasion potential of cancer cells was assayed using 24-well chemotaxis chambers (Corning, Corning, NY, USA). The upper and lower cultures were separated by 8-μm-pore-size polyvinylpyrrolidone-coated polycarbonate filters. Gastric cancer cells were suspended at 1×10^5 cells/mL in serum-free medium, and 0.2 mL cell suspension was added to the upper chamber. Then 0.5 mL serum-free medium with various concentrations of SDF-1 was added to the lower chamber. In another set of experiments, 0.5 mL serum-free medium with 10 nmol/L SDF-1 (fixed concentration) plus various concentrations of AMD3100 (Sigma) was added to the lower chamber. The chambers were incubated for 12 h at 37℃ in a humid atmosphere of 5% CO_2. After incubation, non-migrated cells were removed from the upper surface of the filters, and the migrated cells adherent to the filters were fixed with ethanol and stained with Giemsa solution. Each experiment was done in triplicate, and cells migrated to the underside of the filter were counted in five fields (10 × magnification) in each well under light microscope.

Immunohistochemistry

Immunohistochemistry was performed using the Histostain-SP kits (Boster, Wuhan, China) according to the manufacturer's recommendations. Sections (4 μm thick) were de-

paraffinized, placed in 0.01 mol/L citrate buffer (pH 6.0), and treated by microwave heating for 15 min. The sections were then placed in a solution of 97% methanol and 3% hydrogen peroxide for 10 min at room temperature, to quench endogenous peroxidase activity. Subsequently, the slides were pretreated with 1% bovine serum albumin in phosphate-buffered saline (PBS) and incubated with anti-SDF-1 antibody (Boster; dilution 1:100) and anti-CXCR4 antibody (Boster; dilution 1:50) for 1 h at room temperature. The primary antibody was washed away with PBS, and the biotinylated secondary antibody was used. After 20 min, the sections were washed with PBS, and treated with peroxidase-conjugated streptavidin for 20 min. Finally, the slides were incubated in 3,3'-diaminobenzidine tetrahydrochloride with 0.05% H_2O_2 for 3 min and counterstained with Carazzi's hematoxylin, dehydrated and mounted.

Evaluation of immunostaining

The slides were examined blindly by three pathologists who had no clinicopathological knowledge of the patients. The intensity of staining and percentage of positive cells were determined by the three observers. The intensity, staining percentage, and pattern of staining (nuclear and cytoplasmic) were assessed for CXCR4 and SDF-1. The intensity of staining (brown color) was scored semi-quantitatively as follows: +, weak; ++, medium; +++, strong; and ++++, very strong. The immunostained sections were scanned under light microscope. Samples with a score of ++ or greater were considered CXCR4 or SDF-1-positive.

Determination of CXCR4 and SDF-1 mRNA expression

Total RNA (500 ng) was isolated from frozen tissues and cell pellets using RNArose reagent (Fulin, Qingdao, China) according to the manufacturer's instructions. Reverse transcription was performed in a final volume of 10 μL that contained 5 × PrimeScript™ Buffer (2 μL), PrimeScript™ RT Enzyme Mix (2 μL), Oligo dT Primer (50 μmol/L) (0.5 μL), Random 6 mers (100 μmol/L) (0.5 μL), RNase Free dH_20 (4.5 μL) using a Reverse Transcription System kit (Takara, Japan). The reverse transcription reaction was performed at 37℃ for 15 min, and 85℃ for 5 s. Gene expression of CXCR4 and SDF-1 was detected by quantitative real-time polymerase chain reaction (PCR) (TaqMan) using the 7500 sequence detector (AB Applied Biosystems, USA) and SDS analysis software. The primers and fluorescent probe for human CXCR4, SDF-1 and GAPDH are shown in Table 1. GAPDH served as a control for efficiency of the amplification in the reactions. Thermal cycle conditions were 95℃ for 10 s for one cycle, followed by 40 cycles of 95℃ for 5 s, and 60℃ for 45 s. The expression level of CXCR4 mRNA and SDF-1 mRNA was obtained by $2^{-\Delta\Delta CT}$ calculation. All PCR products were analyzed on a 2% agarose gel with ethidium bromide staining.

Statistical analysis

The SPSS version 12.0 software was used for statistical analysis. The Pearson χ^2 test or Fisher exact test was used to compare qualitative variables. Quantitative variables were analyzed using Student's *t* test. Results were presented as mean ± SE. Pearson correlation analysis was used for correlation analysis. Probability values < 0.05 were considered significant. All experiments were repeated two or three times with triplicate samples, and similar results were obtained.

Table 1 Primers and fluorescent probe for human CXC chemokine receptor-4, stromal cell-derived factor-1 and GAPDH

Primers	5'-3'	Product (bp)
CXCR4	Forward: TGGCCTTATCCTGCCTGGTAT	173
	Reverse: GGAGTCGATGCTGATCCCAAT	
	Taqman: AGAAGCGCAAGGCCCTCAAGACCA	
SDF-1	Forward: GAGCCAACGTCAAGCATCTCA	103
	Reverse: TTCGGGTCAATGCACACTTGT	
	Taqman: CTGTGCCCTTCAGATTGTAGCCCGG	
GAPDH	Forward: TCATGGGTGTGAACCATGAGAA	146
	Reverse: GGCATGGACTGTGGTCATGAG	
	Taqman: TCATCAGCAATGCCTCCTGCACCA	

CXCR4: CXC chemokine receptor-4; SDF-1: Stromal cell-derived factor-1; GAPDH: Glyceraldehyde-3-phosphate dehydrogenase.

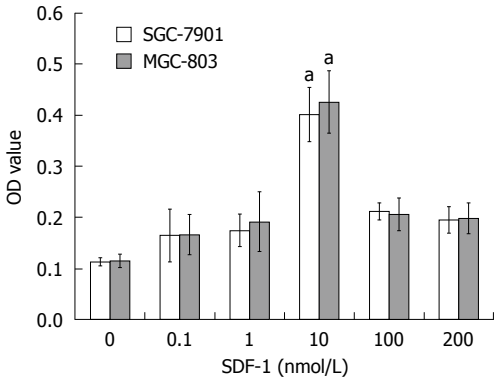

Figure 1 Effect of stromal cell-derived factor-1 on proliferation of gastric cancer cells. Gastric cancer cells were grown in serum-free medium with or without the indicated doses of stromal cell-derived factor-1 (SDF-1). SDF-1 significantly increased the number of SGC-7901 and MGC-803 cells. Maximum effect was observed with 10 nmol/L SDF-1 ($^aP < 0.05$).

RESULTS

Effect of SDF-1 on gastric cancer cell proliferation

The effect of SDF-1 on cell proliferation was examined in gastric cancer cell lines SGC-7901and MGC-803. After incubation for 72 h, cell proliferation was significantly and dose-dependently enhanced by SDF-1 at concentrations from 0.1 to 200 nmol/L (Figure 1).

Effect of AMD3100 on SDF-1-induced migration of gastric cancer cells

SDF-1 stimulated migration of gastric cancer cells (Figure 2). Maximal effect was observed at 10 nmol/L SDF-1 in all gastric cancer cell lines. The inhibitory effect of AMD3100 on SDF-1-induced migration was tested. The migration induced by SDF-1 at 10 nmol/L was inhibited by AMD3100 in SGC-7901 and MGC-803 cells (Figure 3).

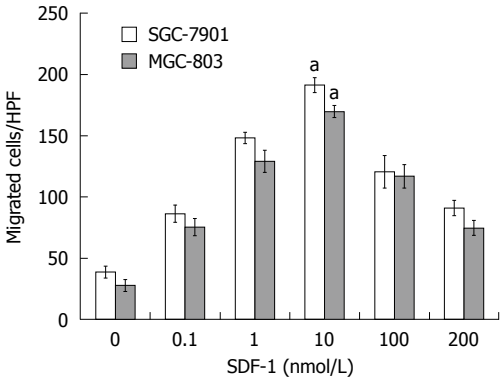

Figure 2 Effect of stromal cell-derived factor-1 on migration of gastric cancer cells. Stromal cell-derived factor-1 (SDF-1) stimulated migration of cancer cells. Maximum effect was observed at 10 nmol/L of SDF-1 ($^aP < 0.05$).

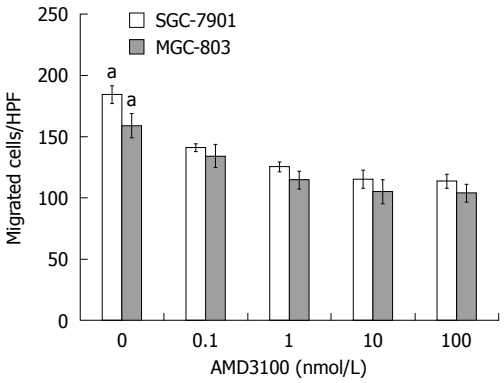

Figure 3 Effect of AMD3100 on stromal cell-derived factor-1-stimulated migration of gastric cancer cells. Gastric cancer cells were stimulated by stromal cell-derived factor-1 (SDF-1) at 10 nmol/L and various concentrations of AMD3100. Cell migration was decreased as the concentration of AMD3100 increased ($^aP < 0.05$).

Expression of CXCR4 in gastric cancer tissues and paired normal samples

In the normal gastric epithelium adjacent to the tumor, weak immunoreactivity for CXCR4 was detected in the non-neoplastic epithelial cells. In gastric cancer tissues, CXCR4 immunoreactivity was strong in cancer cells. Staining was observed predominantly in the cytoplasm and plasma membrane of tumor cells (Figure 4A). Twenty (50%) of the 40 gastric cancers were positive for CXCR4 expression at the invasive front, whereas only three (7.5%) of 40 normal mucous membranes were positive for CXCR4. The levels of CXCR4 mRNA were significantly higher in gastric cancers (1.624 ± 1.380) than in its normal counterpart (1.072 ± 0.524, $P = 0.015$) (Figure 5A and Table 2). The levels of CXCR4 mRNA were significantly higher in gastric cancers with lymph node metastasis (32/40) (0.823 ± 0.551) than in those without (8/40) (0.392 ± 0.338, $P = 0.042$) (Table 3).

Localization of CXCR4 proteins in gastric cancer cell lines

Total RNA from the gastric cancer cell lines SGC-7901 and MGC-803 was isolated using RNArose reagent (Fulin, Qingdao, China) according to the manufacturer's instructions. Reverse transcription PCR was carried out as described above. The PCR products were analyzed on a 2% agarose gel with ethidium bromide staining. We found that both SGC-7901 and MGC-803 cells expressed CXCR4 protein (Figure 5B).

Table 2 Expression of CXC chemokine receptor-4 in primary gastric carcinoma and normal mucous membrane (mean ± SE)

Tissues	No.	CXCR4-mRNA	*t*	*P*
Gastric cancer	40	1.6244 ± 1.3801	2.554	0.015
Normal mucous membrane	40	1.0715 ± 0.5243		

CXCR4: CXC chemokine receptor-4.

Table 3 Expression of CXC chemokine receptor-4 in primary gastric carcinoma with or without lymph nodes metastasis (mean ± SE)

Lymph node metastasis	No.	CXCR4-mRNA	*t*	*P*
Present	32	0.823 ± 0.551	2.101	0.042
Absent	8	0.392 ± 0.338		

CXCR4: CXC chemokine receptor-4.

Relationship between CXCR4 expression and clinicopathological features in gastric cancer

CXCR4 expression was significantly positive in gastric cancer with poor differentiation, high tumor stage and lymph node metastasis. However, other parameters, age, sex, tumor location and tumor size, had no significant relationship with CXCR4 expression. Clinical and pathological characteristics of patients are listed in Table 4.

Expression of SDF-1 in lymph nodes with or without cancer cell metastasis

Among the 40 lymph nodes that we collected, 24 (60%) had cancer cell metastasis and the remaining nodes were normal. Sixteen (66.7%) of 24 lymph nodes with cancer cell metastasis were positive for SDF-1 expression, whereas only 5/16 (31.3%) were positive in the lymph nodes without metastasis (Figure 4C and D). The levels of SDF-1 mRNA were also significantly higher in the lymph nodes with metastasis (0.5432 ± 0.4907) than in their normal counterparts (0.2640 ± 0.2601, $P = 0.025$) (Figure 5C and Table 5).

Correlation analysis of SDF-1 expression in lymph nodes and CXCR4 expression in gastric cancer

Pearson correlation analysis showed that the positive expression of SDF-1 mRNA in lymph node metastasis of gastric cancer was consistent with the positive expression of CXCR4 mRNA in gastric cancer ($r = 0.776$, $P < 0.01$).

DISCUSSION

The mechanisms of lymph node metastasis in gastric

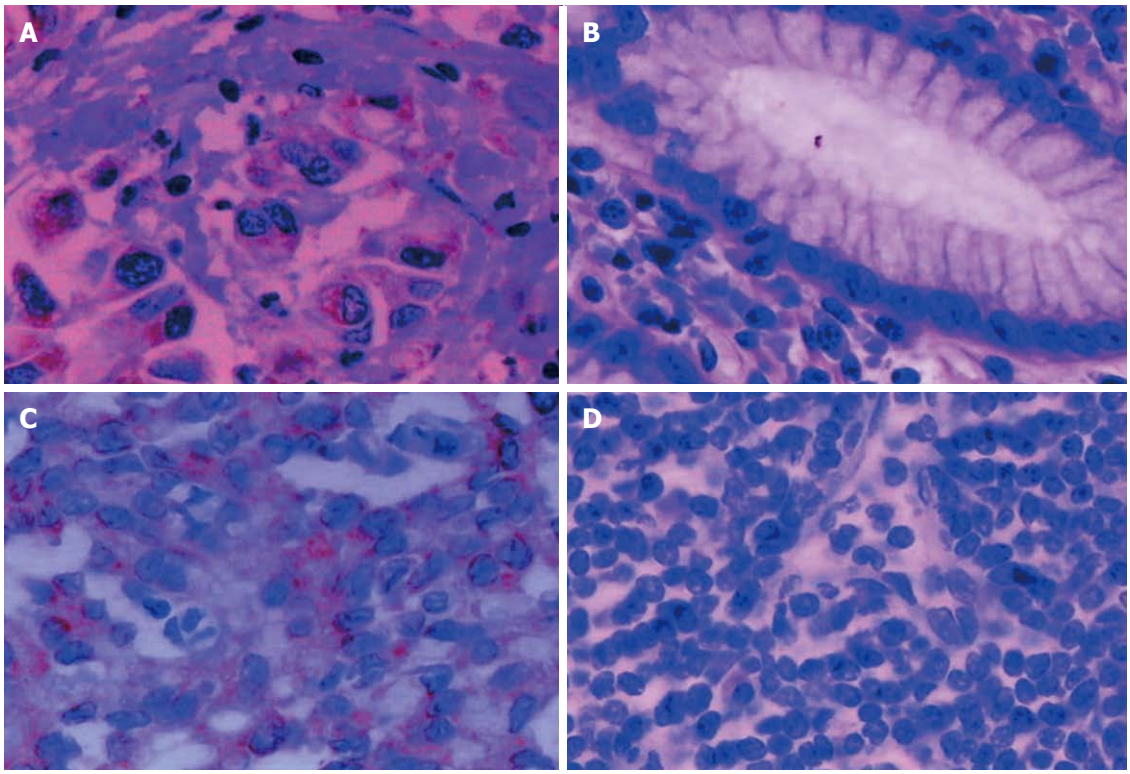

Figure 4 Expression of CXC chemokine receptor-4 in gastric carcinoma tissues and stromal cell-derived factor-1 in lymph nodes. A: CXC chemokine receptor-4 (CXCR4) protein was detected by immunohistochemistry in primary gastric carcinoma tissues; B: CXCR4 protein was not detected in normal mucous membrane; C: Stromal cell-derived factor-1 (SDF-1) protein was detected by immunohistochemistry in lymph nodes with gastric cancer cell metastasis; D: SDF-1 protein was not detected in normal lymph nodes (400 ×).

Table 4 Relationship between CXC chemokine receptor-4 expression and clinicopathological features in gastric cancer

Clinicopathologic parameters	No.	CXC chemokine receptor-4			
		Positive	Negative	χ^2	*P* value
Sex					
Male	31	18	13	0.006	0.938[2]
Female	9	6	3		
Age (yr)					
> 56	19	12	7	0.007	0.935[1]
≤ 56	21	13	8		
Tumor size (cm)					
≥ 5	25	16	9	0.064	0.800[1]
< 5	15	9	6		
Tumor location					
Cardia of stomach	2	2	0	-	0.811[3]
Fundus of stomach	1	1	0		
Body of stomach	6	4	2		
Antrum of stomach	31	18	13		
Differentiation					
Moderate/well	6	1	5	-	0.021[3]
Poor	34	24	10		
Lymph node metastasis					
Present	32	26	6	4.146	0.042[2]
Absent	8	3	5		
Stage					
Ⅱ and Ⅲa	23	8	15	5.013	0.025[1]
Ⅲb and Ⅳ	17	12	5		

[1]χ^2 test; [2]Modified χ^2 test; [3]Fisher exact test.

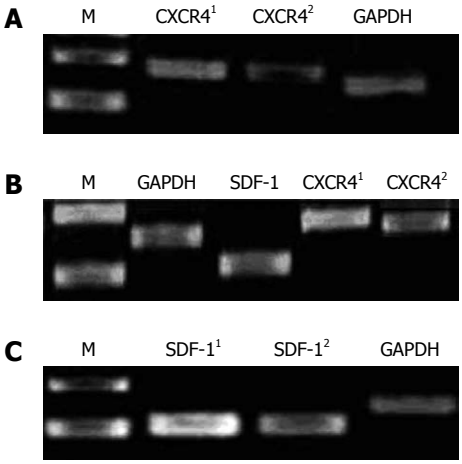

Figure 5 mRNA expression of CXC chemokine receptor-4 in gastric cancer cells, tumors and normal mucous membranes and of stromal cell-derived factor-1 in lymph nodes. A: CXCR[1]: The expression of CXC chemokine receptor-4 (CXCR4) in gastric carcinoma tissues; CXCR[2]: Expression of CXCR4 in normal mucous membrane; B: Stromal cell-derived factor-1 (SDF-1): Expression of SDF-1 in lymph nodes; CXCR[1]: Expression of CXCR4 in gastric cancer cell line SGC-7901; CXCR[2]: Expression of CXCR4 in gastric cancer cell line MGC-803; C: SDF-1[1]: Expression of SDF-1 in lymph nodes with gastric cancer cell metastasis; SDF-1[2]: Expression of SDF-1.

cancer are complex. The process involves the proteolysis of extracellular matrix, altered cancer cell adhesiveness, local invasion, angiogenesis, vascular dissemination, immune evasion and cancer cell survival in a new microenvironment. Some types of tumors show an organ-specific pattern of metastasis, and the "seed (cancer cells) and soil (factors in the organ environment)" hypothesis has been

Table 5 Expression of stromal cell-derived factor-1 in lymph nodes with or without gastric cancer cell metastasis (mean ± SE)

Lymph node metastasis	No.	SDF-1-mRNA	*t*	*P*
With	24	0.5432 ± 0.4907	2.338	0.025
Without	16	0.2640 ± 0.2601		

SDF-1: Stromal cell-derived factor-1.

introduced[14,15]. To date, the role of the CXCR4/SDF-1 signaling axis in the process of tumor metastasis has been extensively investigated. Most results have confirmed that increased expression of CXCR4 is mainly found in cancers, whereas SDF-1 tends to be overexpressed in normal tissues[16-18]. It has been reported that the signaling axis is involved in lymph node metastasis of breast cancer[13], colorectal cancer[19], nasopharyngeal cancer[20] and thyroid carcinoma[21], and also mediates melanoma metastasis to the lungs[22], prostate cancer metastasis to the bone[23], neuroblastoma metastasis to bone marrow[24], hepatocellular cancer metastasis to the bone[25], non-small cell lung cancer metastasis to the pleural space[26], and gastric cancer metastasis to the peritoneum[13]. Therefore, the CXCR4/SDF-1 signaling axis is essential for organ-specific metastasis, and has become a key determinant of tumor metastasis. The lymph nodes might also serve as the soil to promote the survival and proliferation of cancer cells that then cause lymph node metastasis.

Taking all of these results together, we hypothesize that CXCR4/SDF-1 interaction is generally important for lymph node metastasis of gastric cancer. In the present study, we found that CXCR4 was expressed in 50% of gastric cancers and CXCR4 was upregulated more in gastric cancer than in normal gastric tissues, which confirmed the previous data[13]. We also found a significant increase in SDF-1 mRNA in lymph nodes with cancer cell metastasis in comparison with normal lymph nodes, which confirmed that cancer cells can migrate towards an SDF-1 gradient established in specific target organs. It has been shown that higher levels of SDF-1 in target organs such as liver or lymph nodes attract and recruit cancer cells, which subsequently form lymph node metastases[16]. Therefore, these studies strongly support our hypothesis that the CXCR4/SDF-1 signaling axis plays an important role in the process of lymph nodes metastasis of gastric cancer. In supporting this idea, our clinicopathological study revealed that CXCR4 expression was significantly positive in gastric cancers with a high tumor stage and lymph nodes metastasis. No significant correlation between CXCR4 expression and other clinicopathological factors was found. Our study involved a limited group of patients, and more studies with a larger number of cases are necessary to determine the exact role of the CXCR4/SDF-1 axis in the development of lymph node metastasis of gastric cancer.

In our *in vitro* studies, expression of CXCR4 was also found in the gastric cancer cell lines SGC-7901 and MGC-803. The two cell lines showed significant chemotactic responses to SDF-1 in a dose-dependent manner and the chemotactic responses were significantly blocked by neutralizing anti-CXCR4 antibody. SDF-1 also significantly and dose-dependently enhanced cancer cell proliferation. A similar result has been found in several other tumor cell lines, including small cell lung cancer[27], prostate cancer[28], and squamous cell carcinoma of the neck[29]. In contrast, some studies have demonstrated that SDF-1 has no proliferative effects on pancreatic cancer cell lines[30], rhabdomyosarcoma[31], and lymphohematopoietic cells[32]. These differences may be due to the different culture system or the different target cells used.

It has been shown that chemokine receptor CCR7-positive carcinoma cells were detected in 42 (66%) of 64 cases, and that there was a significant difference in lymph node metastasis and lymphatic invasion between CCR7-positive and CCR7-negative cases, which indicates that CCR7 and its ligands interaction are associated with preferential lymph node metastasis of gastric carcinoma[33]. Arigami *et al*[34] have found recently that levels of combined CCR7 and CXCR4 expression are significantly correlated with lymph node metastasis. Similar results have been also observed in esophageal squamous cell carcinoma[35] and oral[36] squamous cell carcinoma. Additionally, nuclear factor-κB[37], c-erbB-2[38], hypoxia-inducible factor 1[39] or nitric oxide[40] can induce CXCR4 expression, which then plays an important role in lymph node metastasis. Therefore, there are certainly many more factors and/or signaling pathways than we thought that are involved in the process of lymph node metastasis and the exact mechanisms need further studies.

In conclusion, the results in this study indicate that the CXCR4/SDF-1 signaling axis appears to be involved in lymph node metastasis of gastric cancer. CXCR4 overexpression in primary gastric cancers might be an independent risk factor for lymph node metastasis. CXCR4 receptor antagonists can inhibit chemotactic behavior of gastric cancer cells. 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 diagnosed malignant tumors. Most deaths from gastric cancer are caused by metastasis, of which lymph node metastasis is 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-4 (CXCR4) seems to be the major chemokine receptor that is expressed in many types of cancer cells. The CXCR4/ and stromal cell-derived factor-1 (SDF-1) axis plays a major role in survival, proliferation, migration and adhesion of many kinds of tumor cells. However, most of the studies about SDF-1 and CXCR4 have been conducted *in vitro*, and the definitive pathophysiological function of the CXCR4/SDF-1 axis in lymph node metastasis of gastric cancer needs further research.

Innovations and breakthroughs

Recent reports have highlighted the importance of the CXCR4/SDF-1 axis in

cancer metastasis. This study has found that the CXCR4/SDF-1 axis is also involved in lymph node metastasis of gastric cancer. Furthermore, this *in vitro* study has suggested that CXCR4 receptor antagonists could suppress the proliferation and migration of gastric cancer cells.

Applications

By understanding how the CXCR4/SDF-1 axis is involved in lymph node metastasis of gastric cancer, this study could represent a future strategy for therapeutic intervention in patients with lymph node 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. SDF-1 is a member of the CXC subfamily. CXCR4 is the only receptor of SDF-1 and is expressed in many kinds of tumor cells.

Peer review

This is a nice manuscript with good data. The conclusions fit the data.

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