Expression of chemokine receptor CXCR5 in gastric cancer and its clinical significance

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Received January 8, 2016; Accepted March 22, 2016; Epub July 1, 2016; Published July 15, 2016

Abstract: The increased expression of chemokine receptor CXCR5 in cancers has been demonstrated. In order to characterize the expression pattern of CXCR5 in cell lines and tissues of gastric cancer and to assess clinical implications, the expression of CXCR5 mRNA in gastric cancer tissues and adjacent tissues was evaluated by real-time RT-PCR. Meanwhile, the expression of CXCR5 in cell lines of human gastric cancer was also analyzed by flow cytometry. Tissue microarray and immunohistochemistry were used to detect the protein expression of CXCR5 in human gastric cancer tissues and adjacent normal tissues. Flow cytometry results revealed the positive expression of CXCR5 in human gastric cancer cell lines such as BGC-823, SGC-7901 and HGC-27 cells. The immunohistochemistry results showed higher expression of CXCR5 in 52.87% of gastric cancer tissues. The expression of CXCR5 in patients with tumor size less than 2.8 cm subgroup was significantly lower than that in patients with tumor size larger than 2.8 cm subgroup (P = 0.0456). There was no significant correlation between the expression of CXCR5 and other clinical parameters in gastric cancer. Moreover, the survival analysis showed that the overall survival rate of the patients with higher CXCR5 expression was lower than that of the patients with lower CXCR5 expression (P = 0.0579, HR = 1.810, 95% CI: 0.9803-3.341). The results suggest that CXCR5 is involved in the oncogenesis and progression of gastric cancer.

Keywords: Gastric cancer, CXCR5, immunohistochemistry, microarray, flow cytometry

Introduction

Gastric cancer is the most common malignant tumor of upper digestive tract. Lymph node metastasis and abdominal implantation are the major causes of metastasis, which finally leads to cancer recurrence or cancer-related death [1]. Despite extensive studies on the identification of novel diagnostic and therapeutic agents, patients with advanced gastric cancer often suffer from a poor prognosis, and the treatment is still mainly dependent on conventional cytotoxic chemotherapy [2]. Thus, the advances in understanding novel factors involved in the progression of gastric cancer, and the development of prognostic and predictive markers, can potentially improve the therapeutic outcomes of this malignancy.

As a group of small molecule proteins, chemokines can bind target cells by chemotaxis through their receptors, which plays an essential role in the recruitment of leukocytes from the circulation system to local inflammatory sites [3]. Chemokines are able to regulate the infiltration of leukocytes in tumor, activate specific immune response, and stimulate the proliferation and migration of tumor cells by autocrine or paracrine models. The overwhelming evidences have supported that chemokine-chemokine receptor systems can regulate transformation, growth, neovascularization and metastasis of tumor cells [4].

The chemokine receptor CXCR5 expressed by B cells and certain T cells can control the migration of these cells into and within lymph nodes [5]. The abnormal expression of CXCR5 could be observed in many tumors and was closely associated with tumor progression [6]. However, the role of CXCR5 and its ligand in gastric cancer has not been investigated. The aim of the
present study was to detect the expression of CXCR5 in gastric cancer tissues and to analyze its clinical significance.

Materials and methods

Patients and tissue microarray

Tissue microarray for human gastric cancer (HStm-Ade180Sur-05) was purchased from Shanghai Outdo Biotech Co., LTD (Shanghai, China). The tissue microarray included 90 cases of gastric cancer and adjacent normal tissues. All patients including 60 male and 30 female patients underwent surgical stomach resection between July 2006 and April 2007. The median age of these patients was 65 years and survival data were available, with follow-up to 2013 August. In addition, the gastric cancer tissues and adjacent normal tissues from 14 cases during the surgical resection were collected to determine the expression of CXCR5 mRNA. No patients received pre-operative chemotherapy or radiotherapy before surgical resection. The clinical parameters of these patients are shown in Table 1.

Total RNA extraction, reverse transcription and real-time PCR

In brief, total RNA was extracted from tissues using a total RNA purification kit (BiocolorBioScience and Technology Company, Shanghai, China). Complementary DNA was synthesized with cDNA synthesis kit (Fermentas, Vilnius, Lithuania), according to the manufacturer’s instructions. The primers of CXCR5 and GAPDH were designed according to the National Center for Biotechnology Information (NCBI) database, and then synthesized by Sangon Biotech (Shanghai, China). The sequences of all primers used in this study are as follows: CXCR5-F: GGTCAACCTACC-ACATCGTC, CXCR5-R: GCCATTCAGC- TTGCGAGTTTG, GAPDH-F: ACAACTTGTATCGTGGAAGG, GAPDH-R: GC CATCACGCCACAGTTTC. The mRNA level of CXCR5 was measured by real-time PCR using SYBR Green method on the Applied Biosystems 7500 real-time PCR system (Applied Biosystem, USA). All experiments were performed in triplicate. Data were normalized to the housekeeping gene GAPDH, and the relative abundance of transcripts was calculated by the comparative ΔΔCT method.

Immunohistochemistry

Immunohistochemical staining was performed using the EnVision™ method according to the manufacturer’s instructions. The tissue microarray section was dewaxed in xylene, rehydrated and graded ethanol solutions. Antigen retrieval was conducted by heating tissue sections at 100°C for 30 min in EDTA (pH 9.0) solution. Then, the sections were immersed in a 3% hydrogen peroxide solution for 30 min to block endogenous peroxidase activity, rinsed in phosphate buffered saline (PBS) for 5 min, blocked with 3% BSA at room temperature for 30 min, and incubated with purified rabbit anti-human CXCR5 antibody (Millipore, USA) at 4°C over-
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Expression of CXCR5 mRNA in gastric tissues

As shown in Figure 1, the mRNA of CXCR5 could be observed in gastric tissues, and the mRNA expression level of CXCR5 in gastric cancer tissues was significantly higher than that in adjacent normal gastric tissues ($P = 0.0047$).

Expression of CXCR5 in gastric cancer cell lines, tumor and adjacent normal gastric tissues

As shown in Figure 2, high expression of CXCR5 was detected in BGC-823, SGC-7901 and HGC-27 gastric cancer cells. Immunostaining results

Intracellular staining and flow cytometry analysis

The expression of CXCR5 in cells was examined by flow cytometry using IntraPrepPermeabilization Reagent. Human gastric cancer cells such as BGC-823, SGC-7901 and HGC-27 cells were cultured in a standard condition (5% CO$_2$, 37°C). Harvested cells and dispersed cells (5 x 10$^6$-1 x 10$^6$) were washed with PBS and aliquoted into 100 mL of IntraPreppermeabilization reagent 1 (Immunotech, Marseille, France) for 15 min at room temperature. After washing once with 1 mL of Hanks’ buffered saline solution (HBSS) with 2% FBS, the cells were permeabilized for 5 min with IntraPrepempermeabilization reagent 2. The cytoplasmic expression of CXCR5 was detected by flow cytometry (FACSCanto II, BD, USA), and the data were analyzed by using FlowJo10.0.6.

Statistical analysis

All statistical analyses were performed using GraphPad Prism 5.0. The T-test was used to analyze the difference in the expression of CXCR5 protein between cancer tissues and adjacent normal tissues. The $\chi^2$ tests were used to analyze the relationship between CXCR5 expression and pathological parameters. Overall survival rate of patients with different clinic-pathological parameters was compared by log-rank survival analysis. A statistically significant difference was considered at $P < 0.05$.
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showed that CXCR5 was mainly localized on the membrane of tumor cells, and adjacent normal gastric tissues. In addition, there was no significant difference in CXCR5 expression between 87 cases of gastric cancer patients and adjacent normal mucosa tissues (Figure 3).

Relationship of CXCR5 expression and clinical parameters

All patients were divided into two groups according to the staining intensity: HS-score < 5 (41 cases) and HS-score ≥ 5 (46 cases). The results of tissue microarray and immunohistochemistry showed that the ratio of higher CXCR5 expression in gastric cancer tissues was 52.87%. The expression of CXCR5 differed significantly according to tumor size in patients (P < 0.05). Nevertheless, no significant correlation was observed between CXCR5 and other parameters such as gender, age, pathological grade, tumor location and TNM staging (P > 0.05, Table 1).

Prognostic value of CXCR5 expression in gastric cancer tissues

As shown in Figure 4, 90 cases of gastric cancer patients with follow-up data were included for survival analysis. The log-rank survival analysis showed that patients with high CXCR5 expression had poorer overall survival rate than that of the patients with low CXCR5 expression (P = 0.0579, HR = 1.810, 95% CI: 0.9803-3.341).

Discussion

Chemokines are small molecular polypeptides with molecular weights of 8-10 kDa, and can lead to the infiltration of inflammatory cells in tumor tissues, and promote the angiogenesis, tumor cell proliferation, alive and dissemination [4]. Recent studies have demonstrated that chemokines and their receptors play an important role in organ-specific metastasis of malignant tumor [8, 9]. Chemokines and their receptors are highly expressed in certain tumor cells and tissues. The expression level is closely correlated with many clinical parameters. For example, higher expression of CXCR1, CXCR2 and CXCR4 with corresponding ligands of CXCL5, CXCL8 and CXCL12 is highly associated with tumor angiogenesis, metastasis, and poor survival of the patients with lung cancer [10, 11].

CXCR5, one of the members for CXC chemokine receptor family, is mainly distributed in mature B cells and a few T cell subsets. CXCR5 plays a significant role in B cell homing to secondary lymphoid tissue [12]. It is a transmembrane receptor containing 7 transmembrane domains coupled with guanosine triphosphate 2 (GTP2) [13]. CXCR5 contributes to the returning of B lymphocytes to lymph node follicles, and promotes lymph follicle assembly as one of the important lymphnode factors. CXCR5 is expressed primarily in mature, recirculating B cells and small subsets of CD4+ and CD8+ T cells. The migration of these leukocytes into
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Figure 3. Immunohistochemical staining of CXCR5 in gastric cancer and adjacent normal mucosa tissues. A, B. The high expression of CXCR5 in adjacent normal mucosa tissues; C, D. The low expression of CXCR5 in adjacent
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lymph nodes is controlled by CXCR5/CXCL13 interaction [14]. It has been demonstrated that the CXCR5/CXCL13 axis is involved in the development and the progression of solid tumors, such as breast and neuronal cancers [15, 16]. Moreover, the clinical and biological relevance of CXCL13/CXCR5 pathway has been demonstrated to have a critical role in invasion and migration of prostate cancer [17].

The increasing evidences suggest that multiple chemokines and their corresponding receptors are involved in the progression of gastric cancer and play significant roles in metastatic process. So far, there are few reports regarding the expression of CXCR5 in gastric cancer tissues. In the present study, the expression of CXCR5 in gastric cancer tissues was detected, and the role of CXCR5 in the development of gastric cancer, and its prognostic value in patients with gastric cancer were explored. We found that the expression level of CXCR5 mRNA in gastric cancer tissues was significantly higher than that in adjacent normal gastric tissues, indicating that over-expression of CXCR5 might be involved in the progression of gastric cancer. Meanwhile, CXCR5 expression was positively associated with tumor size. In contrast, no significant correlation of CXCR5 with gender, age, pathological grade, tumor location and TNM stage was observed. In addition, CXCR5 was highly expressed in gastric cancer cell lines. Given the expression of CXCR5 in a substantial proportion of pancreatic carcinomas, this option may be particularly interesting for this aggressive and incurable tumor type [18]. The exact mechanism for the regulation of abnormal expression of CXCR5 in human gastric cancer and the potential biological function need to be further explored in the future.

Acknowledgements

This study was supported by the grants from the National Science and Technology Support Projects (2015BAI12B12), The National Natural Science Foundation of China (No. 31570877, 31570908, 81201741, 81301960, 31428005 and 81171653), Natural Science Foundation of Jiangsu Province (BK2011246, BK2011247), the project of Six Batch of Major Talent Summit (BRA2010037), the Key R&D Project of Science and Technology Department of Jiangsu Province (BE2015633 and BE2015634), and the Innovative Talents Training Project of Changzhou Health Bureau.

Disclosure of conflict of interest

None.

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