CXCR7 expression in nasopharyngeal carcinoma tissues correlates with disease severity

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Abstract: The chemokine CXCL12 and its receptors, CXCR4 and CXCR7, are important contributors to the pathogenesis of multiple types of tumors. CXCL12/CXCR4 was previously demonstrated to be upregulated in nasopharyngeal carcinoma (NPC) tissues, but the status of CXCR7 in NPC remains unknown. Here, 62 nasopharyngeal carcinoma specimens were obtained from patients who received rhinitis biopsy in our hospital in 2012 and 2013. Another 30 samples were collected from patients with nasopharyngitis who did not have NPC, to serve as a control group. Expression of CXCR7 protein and mRNA in NPC and normal tissues was detected by immunohistochemistry and quantitative real-time polymerase chain reaction, respectively. CXCR7 protein was detected in just 7.1% (2/30) of normal nasopharyngeal samples, but 61.3% (38/62) of tumor tissues (P<0.05). The staining patterns (proportion of stained cells/sample as well as staining intensity) were correlated with lymph node metastasis, TNM staging, and disease severity (P<0.01). Thus, CXCR7 may promote disease progression in nasopharyngeal carcinoma, and may be useful as a predictor of metastasis and prognosis.

Keywords: Nasopharyngeal carcinoma, chemokine receptor 7 (CXCR7), metastasis, recurrence

Introduction

Nasopharyngeal carcinoma (NPC) is a multiple malignancy commonly found in China. NPC is poorly differentiated and has rich lymphatic tissues at the site of rhinitis. Indeed, lymph node metastases have occurred in 70% of NPC patients at the time of diagnosis, and NPC invasion and metastasis are significant contributors to its mortality [1].

The pathogenesis and metastasis of NPC are linked to the expression and activity of chemokines and their receptors. Chemokines regulate cell trafficking through interaction with G-protein coupled receptors. The first-described chemokine, stromal-derived factor 1 (SDF-1/CXCL12), activates leukocytes, typically in response to pro-inflammatory stimuli. CXCL12 binds with multiple receptors, including CXCR4 and CXCR7 [2]. The CXCL12/CXCR7 pathway plays an important role in tumor development. In particular, CXCR7 contributes to the vascularization and engineering of tumors [3, 4]. In fact, CXCR7 is highly expressed in breast, lung, prostate, and malignant neuroglioma tumors, and is believed to promote tumor invasion and metastasis [5, 6].

Some previous studies found that CXCR4 is detected at high levels in NPC tissues; further, it is correlated with poor prognosis [7, 8]. Therefore, we speculated that CXCR7, as another high-affinity receptor of CXCL12, may have an effect similar to that of CXCR4 in NPC. Currently, no study has described the expression of CXCR7 in this disease. Therefore, in the current study, immunohistochemical staining was used to detect CXCR7 in nasopharyngeal carcinoma tissues, and investigate its staining patterns in relation to clinicopathological characteristics and disease prognosis.

Materials and methods

Participants

The study used nasopharyngeal carcinoma specimens obtained from 62 patients who received rhinitis biopsy in The First Affiliated
Hospital, Chengdu Medical College hospital in Jan, 2012 and Dec, 2013. All cases were diagnosed and histopathologically confirmed as NPC. None of the patients received chemotherapy or radiotherapy, excluding patients with tumors in other regions. The cases included 34 males and 28 females, ages 42-66 years (mean age 51.2±7.6 years). A control group comprised 30 patients with nasopharyngeal inflammation who received surgical treatment during the same period in our hospital; these patients included 16 males and 14 females, ages 42-64 years (mean age 52.4±5.9 years). The age and gender were comparable between the two groups, with no statistically significant difference (P>0.05). The study was approved by the ethics committee of our hospital, and all patients provided written informed consent to participate in this research.

**Immunohistochemistry**

Standard immunohistochemical techniques were used to detect CXCR7 in nasopharyngeal tissue samples of all subjects. Nasopharyngeal tissue samples were fixed in 4% formalin and embedded in paraffin for serial sectioning. Tissue sections were stained then developed with DAB. CXCR7 staining was observed under the microscope (OLYMPUS BX53). Sections were evaluated for CXCR7 staining patterns by the proportion of positively stained tumor cells and the intensity of staining. For proportion of stained cells in one field, 0 indicated no positive cells; 2 indicated 10-50% of cells were positive; 3 indicated ≥50% of cells were positive. For intensity of staining, 0 indicated no coloring; 1 indicated slight coloring; 2 indicated medium coloring; and 3 indicated intense coloring. A total score <3 was deemed as negative (-) for CXCR7; a total ≥3 was considered as positive (+) for CXCR7. CXCR7 staining was assessed for correlation with age, pathological stage, myometrial invasion depth, and lymph node metastasis.

**Quantitative real-time polymerase chain reaction (qRT-PCR)**

Tissue specimens that had been stored in liquid nitrogen were used for RNA extraction. To each 50 mg tissue sample, 1 mL Trizol reagent (Invitrogen) homogenate was added to extract total RNA according to manufacturer’s instructions. Total RNA was assessed for purity and quantity with an ultraviolet spectrophotometer and 1% agarose gel electrophoresis. Reverse transcription was performed in accordance with instructions in the Takara PrimeScript™ RT-PCR Kit (Code No. DR014A, TaKaRa). The system consisted of 4 μL MgCl₂ (25 mmoL/L), 2 μL reaction buffer (10× RT Butter), 2 μL dNTP compound (10 mmoL/L), 0.5 μL RNA enzyme inhibitor, 15 U AMV reverse transcriptase, 0.5 μg random primer, and 1 μg total RNA (pre-incubation at 70°C for 10 minutes), and nucleic acid-free water to a final volume of 20 μL.
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Reaction conditions were as follows: room temperature for 10 minutes; 42°C for 15 minutes; 95°C for 5 minutes; and at 4°C for 5 minutes. The synthesized cDNA was stored at -20°C for amplification. The qRT-PCR using cDNA as a template was performed in a 20-μL reaction system on an ABI PRISM 7500 instrument. Primer 5 was used to design primers, as follows: CXCR7 upstream, 5'-AAACAGGGCTCACCAAGCTCA-3'; CXCR7 downstream, 5'-ATAGGCGCCATACACCTGTTC-3'; internal reference (U6) upstream, 5'-TGGCACCCAGCACATTGAA-3'; internal reference (U6) downstream, 5'-CTAAGTCATAGTCGGCTGTAAGCA-3'. The anticipated fragment length for CXCR7 was 154 bp; for the reference gene was 186 bp. PCR amplification was performed under the following conditions: pre-degeneration at 95°C for 5 minutes; 40 cycles of 95°C for 15 seconds and 60°C for 60 seconds. A dissolution profile was prepared.

The corrected Ct value \(2^{-\Delta\Delta CT}\) method was used to compare the difference in CXCR7 mRNA expression between the experimental group and the control group.

**Statistical methods**

SPSS17.0 statistical software was used for analysis. \(\chi^2\) test was to compare numerical data. \(P<0.05\) was considered statistically significant.

**Results**

**CXCR7 expression in cancerous and non-cancerous nasopharyngeal tissues**

Immunohistochemical staining of nasopharyngeal tissues indicated that CXCR7 was detect-
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ed in fewer cells and with lower intensity in normal nasopharyngeal tissues compared to cancerous tissues (Figure 1). CXCR7 staining in nasopharyngeal carcinoma tissues was detected mainly in tumor cells, but was also noted in vascular endothelial cells. Thirty-eight of 62 (61.3%) nasopharyngeal carcinoma patients were positive for CXCR7 in tumor tissue cases had positive CXCR7 expression; in contrast, CXCR7 was detected in only 2/30 normal nasopharyngeal tissue samples (7.1%). These differences were statistically significant ($\chi^2=8.72$, $P<0.01$; Table 1).

Similarly, qRT-PCR using RNA extracted from nasopharyngeal tissues samples detected significantly higher relative expression of CXCR7 mRNA ($2.14$) in nasopharyngeal carcinoma compared to the adjacent normal tissues ($0.487$; $P=0.02$). The CXCR7 gene dissolution curve is shown in Figure 2.

Correlation between CXCR7 expression and pathological characteristics of nasopharyngeal carcinoma

CXCR7 staining pattern scores were used to determine whether positive or negative staining was associated with clinical and pathological characteristics of NPC. Of the 62 NPC cases, 28 who were positive for CXCR7 staining were in stages III+IV, while 10 cases positive for CXCR7 were in stages I+II; the difference in these distributions was statistically significant ($P<0.05$). Similarly, there were statistically significant differences in distribution of cases positive for CXCR7 and the M stage as well as presence of lymph node metastasis: 10 cases with CXCR7 had metastasis, 28 cases did not ($P<0.05$, Table 2).

Discussion

Prior work indicates that CXCR7 can promote tumor angiogenesis and tumor cell proliferation, and lead to tumor invasion and directional metastasis [5, 9-12]. Thus, its role in cancer progression appears important. Indeed, CXCR7 is expressed at different levels in gastric cancer and breast cancer, and its expression is related to metastasis to lymph nodes. Further, it is widely distributed, existing in most types of tumor cells [13, 14]. In this study, immunohistochemical staining was able to detect CXCR7 protein nasopharyngeal carcinoma tissues, indicating that its expression occurs in yet another type of tumor. Further, detection of CXCR7 was common in NPC, with more cells affected and a stronger intensity than in normal nasopharyngeal tissue. Not surprisingly, qRT-PCR detection of CXCR7 mRNA levels revealed higher levels in NPC than normal tissues, suggesting that CXCR7 is upregulated in NPC. This increased expression of CXCR7 was also correlated with the degree of malignancy, consistent with the previous study results that indicated CXCR7 expression in a variety of tumor cell surfaces [14-17].

That CXCR7 expression was correlated with NPC clinical staging, M staging, and lymph node metastasis suggests that CXCR7 promotes disease progression and metastasis. CXCR7, in binding with CCL21 in surrounding tissues of NPC, may induce a local Ca$^{2+}$ influx, thereby causing morphological changes and rearrangement of the actin cytoskeleton in tumor cells. These changes can enhance the invasiveness of the tumor cells, thereby enabling metastasis and local infiltration of the tumors. Further, CXCR7 can promote maturation of the lymphatic system and hematopoietic function in the body, and since tumor cell metastasis must be supported with nutrients through new vessels, CXCR7 may promote angiogenesis to support metastasis [18].

In summary, CXCR7 appears to be upregulated in nasopharyngeal carcinoma and correlated with clinical staging and lymph node metastasis. Thus, CXCR7 may be useful in the diagnosis/prognosis of NPC during clinical practice, as well as offering a potential new treatment target for NPC. Further in vivo and in vitro studies uncovering the specific mechanisms and signaling pathways through which CXCR7 promotes metastasis of nasopharyngeal carcinoma are required.

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Disclosure of conflict of interest

None.
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