Expressions of CCR7 and CXCR4 Are Associated with Differentiation in Gastrointestinal Cancer

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ABSTRACT

Purpose: The chemokine receptors CCR7 and CXCR4 have been shown to play an important role in cancer invasion and metastasis. This study was aimed to investigate CCR7 and CXCR4 expressions and evaluate the association between their expressions and the clinicopathological features in gastrointestinal cancer.

Method: 27 paired tissue samples from patients who had curative surgery for gastrointestinal cancer were obtained. Quantitative real-time PCR, immunohistochemistry assay and western blot analysis were carried out to investigate the expressions of CCR7, CXCR4 expressions in gastrointestinal cancer.

Results: The cancer tissues expressed significant higher level of CCR7 (P = 0.000) and CXCR4 (P = 0.000) protein than the adjacent normal mucosa. Expressions of CCR7 (P = 0.002) and CXCR4 (P = 0.003) protein in cancer tissues exhibited significant correlation with differentiation in gastrointestinal cancer.

Conclusion: Expressions of CCR7 and CXCR4 protein were associated with differentiation in gastrointestinal cancer. CCR7 and CXCR4 may be predictive factors for poor prognosis in patients with gastrointestinal cancer.

Keywords: Chemokine Receptor; CCR7; CXCR4; Gastrointestinal Cancer

1. Introduction

Chemokines belong to small-molecule chemoattractive cytokine family and are categorized into four groups (CXC, CC, CX3C, and C) [1-3]. Usually, chemokines are molecules that are structurally and functionally similar to growth factors. They bind to G-protein-coupled receptors on leukocytes and stem cells, and work through guanine-nucleotide-binding (G) proteins to initiate intracellular signaling cascades that promote migration towards the chemokine source [1-3].

Chemokine receptors are seven-transmembrane receptors coupled to G-proteins, all with their N-terminus outside the cell surface, three extracellular and three intracellular loops as well as a C-terminus in the cytoplasm. One of the intracellular loops of the chemokine receptors couples with heterotrimeric G-proteins, and that mediate ligand binding to the receptor which initiates signal transduction cascade [4].

To date, at least 20 chemokine receptors (CCR1-11, CXCR1-7, XCR1, and CX3CR1) have been identified. Chemokines and their receptors have been known to play important roles in inflammation, infection, tissue injury, allergy, cardiovascular diseases, and malignant tumors [5].

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investigated CCR7 and CXCR4 expressions in gastro-
ocolorectal tumor specimens to evaluate the association
between their expressions and the clinicopathological
features of gastrointestinal cancer.

2. Patients and Methods

2.1. Patients Enrollment and Tissue Samples

This study was approved by the Research Ethics Com-
mittee of Gongli Hospital, Shanghai Pudong New Area,
China. Written informed consent was obtained from all
of the patients enrolled in this study. All specimens were
handled and made anonymous according to the ethical
and legal standards.

In this study, paired tumour specimens were obtained
from 27 patients who underwent radical surgery for gas-
tro-colorectal cancer in our hospital from July to De-
cember 2010. The baseline characteristics of enrolled
patients were as showed in Table 1. Tumor specimens
were obtained at the time of surgery and reserved in
pathological laboratory in a −80°C refrigerator. None of
the patients had received radiotherapy or chemotherapy
prior to surgery.

2.2. Immunohistochemistry Assay

Sections of 4-um thickness were obtained from repre-
sentative central and para-tumor areas of each tumor
specimen and were mounted on to glass slides for im-
munostaining. Briefly, after being sealed in goat serum
specimen and were mounted on to glass slides for im-

Table 1. Baseline characteristics of enrolled patients.

<table>
<thead>
<tr>
<th>Clinical grade</th>
<th>II (B-C1)</th>
<th>III (C2)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>59.47 ± 9.52</td>
<td>55.63 ± 14.12</td>
<td>0.414</td>
</tr>
<tr>
<td>Gender</td>
<td>Male</td>
<td>9</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>10</td>
<td>4</td>
</tr>
<tr>
<td>Differentiation level</td>
<td>Middle</td>
<td>7</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Well</td>
<td>5</td>
<td>1</td>
</tr>
</tbody>
</table>

2.3. Western Blot Analysis

Western blotting was also used to detect CCR7 and
CXCR4 protein. The whole specimens were separated
into central and para-tumor areas and sonicated re-
spectively. The cells were collected by centrifugation,
washed in phosphate-buffered saline (PBS), and lysed by
the addition of SDS sample buffer [62.5 mM Tris-HCl
(pH 6.8), 6% (w/v) SDS, 30% glycerol, 125 mM DTT,
and 0.03% (w/v) bromophenol blue]. Equal amounts of
protein from each sample were electrophoresed on 10%
SDS-polyacrylamide gels and transferred to nitrocellu-
lose membranes. The membranes were blocked for 1
hour with Tris-buffered saline (TBS) containing 5% (w/v)
milk and 0.1% Tween, and then incubated with the pri-
mary antibody CCR7 (abcam®, ab32527) and CXCR4
(abcam®, ab2074) overnight at 4°C. The blots were
washed with TBS containing Tween, incubated with hor-
seradish peroxidase-labeled antimouse immunoglobulin
(Sigma®, A6154) for 1 hour at 37°C, then add ECL
solution to record the image.

2.4. Realtime-PCR

200 mg separated central or para-tumor sample from
each sonicated specimen was weighed to extracting total
RNA (Sangon total RNA extracting kit-SK1352, China)
for realtime-PCR on PRISM®7900HT. Every sample was
tested 3 times and the average value was calculated as
the results. The primers and fluorescent probes were de-
signed and synthesized by Sangon Biotech (Shanghai)
CO. Ltd. PCR was performed under the following con-
ditions: an initial cycle of denaturation at 94°C for 2
minutes, followed by 21 - 23 cycles of denaturation at
92°C for 45 seconds; annealing at 60°C for 45 seconds;
estinction at 72°C for 45 seconds; and a final extension at
72°C for 5 minutes. The sequences for qRT-PCR primers
were as follows:

- CCR7 forward, 5’-CTTCTTCAACGCCATGCTCTCT-
A-3’; reverse, 5’-GCTGAGACAGCCTGGACGAT-3’;
- CXCR4 forward, 5’-CAGTGCCGACCTCCTCTCTCTTT-
- reverse, 5’-CAGTTTGCCACGGCACTCA-3’; GAPDH for-
- 5’-CAGATCGGCCCAAGGAGAT-3’; reverse, 5’-TGAGGCTCCTCCTCCTCTTTGT-3’.

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2.5. Statistical Analysis

The quantitative data were measured as average and standard deviation. The association between the variables was tested using the chi-square test or T-test (quantitative data). Probability values of <0.05 were considered statistically significant. The statistic analysis was completed with PASW Statistics 18 Software.

3. Results

3.1. CCR7 and CXCR4 Protein Expression in Gastro-Colorectal Cancer

Of all 27 paired specimens, CCR7 and CXCR4 protein were both detected in central and para tumor tissue by either immunohistochemistry assay (Figure 1) or western blot analysis (Figure 2). The central-tumor tissue expressed significant higher level of CCR7 ($\chi^2 = 47.455$, $P = 0.000$) and CXCR4 ($\chi^2 = 47.600$, $P = 0.000$) protein than para-tumor tissue. However, CCR7 and CXCR4 protein expression between Stages II and III patients showed no variance (Table 2).

While comparing patients with various tumor differentiation levels, the CCR7 and CXCR4 protein expression of central tumor samples exhibited significant variance as demonstrated in Table 3.

3.2. CCR7 and CXCR4 mRNA Expression in Gastro-Colorectal Cancer

The realtime-PCR showed no mRNA expression difference between central and para tumor tissue for CCR7 (2.215 ± 0.462 vs. 1.962 ± 0.660, $P = 0.109$) and CXCR4 (1.543 ± 0.836 vs. 1.483 ± 1.197, $P = 0.832$). Based on clinical stage and tumor differentiation level, the statistic analysis showed none of them was a factor associated with CCR7 and CXCR4 mRNA expression (Table 4).

4. Discussion

This study analyzed the chemokine receptor CCR7, CXCR4 expressions in small series of human gastro-colorectal cancer specimens. Our results showed both CCR7 and CXCR4 protein expressions were significantly higher in cancer tissues than in adjacent normal tissues. Furthermore, CCR7 and CXCR4 protein expressions were significantly lower in better-differentiated tumor. This finding demonstrated that CCR7 and CXCR4 are involved in gastro-colorectal cancer progression just as in many other malignancies, meanwhile the fact that their expressions are associated with tumor differentiation also indicated CCR7 and CXCR4 may be predictive factors for faster progression and poorer prognosis of the diseases.

Our study also revealed that the protein and mRNA expressions of CCR7, CXCR4 showed no difference among patients with different stage. This finding does not mean the denial of commonly accepted opinion of the relevance of CCR7, CXCR4 expression with clinical stage in various types of cancer. For example, Schimanski reported that CXCR4 in colorectal cancer was significantly associated with the advanced UICC tumor stages [11]. The aim of our study was restrictively confined to candidates who were suitable for radical operation. That means all enrolled patients were stage II or III for gastric cancer and stage Duke’s B-C1 or C2 for colorectal cancer and with no distant metastasis. In other words, the tumor progression was at similar level. Theoretically, if surveying a wider stage of the cancer at the time of diagnosis, the results might be different.

The CCR7 and CXCR4 have been considered as possible targets of anti-cancer drugs. Luo has reported that the blockage of CXCR4-SDF1 combination can inhibit
tumor cell growth and metastasis [12]. Our study found the deviation in expression of CCR7 and CXCR4 at protein level did not exhibit at mRNA level as well among various differentiation cancers. This phenomenon indicated that the difference in translation of mRNA might be a key step for the variant expression of CCR7 and

Table 2. Grading of CCR7 and CXCR4 protein expression between different clinical stage.

<table>
<thead>
<tr>
<th>Clinical Stage</th>
<th>Central-tumor</th>
<th>Para-tumor</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>II (B-C1)</td>
<td>III (C2)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CCR7</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>17 (62.96%)</td>
<td>8 (29.63%)</td>
</tr>
<tr>
<td></td>
<td>2+</td>
<td>6 (22.22%)</td>
<td>3 (11.11%)</td>
<td>1.089</td>
<td>0.580</td>
</tr>
<tr>
<td></td>
<td>3+</td>
<td>7 (25.93%)</td>
<td>4 (14.81%)</td>
<td>0.909</td>
<td>0.340</td>
</tr>
<tr>
<td></td>
<td>4+</td>
<td>6 (22.22%)</td>
<td>1 (3.70%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>17 (62.96%)</td>
<td>8 (29.63%)</td>
</tr>
<tr>
<td>CXCR4</td>
<td>2+</td>
<td>6 (22.22%)</td>
<td>2 (7.41%)</td>
<td>1.052</td>
<td>0.591</td>
</tr>
<tr>
<td></td>
<td>3+</td>
<td>8 (29.63%)</td>
<td>5 (18.52%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>4+</td>
<td>5 (18.52%)</td>
<td>1 (3.70%)</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 3. Grading of CCR7 and CXCR4 protein expression among various tumor cell differentiation.

<table>
<thead>
<tr>
<th>Differentiation</th>
<th>Central-tumor</th>
<th>Para-tumor</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Poor</td>
<td>Middle</td>
<td>Well</td>
<td>Poor</td>
<td>Middle</td>
</tr>
<tr>
<td>CCR7</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>2+</td>
<td>0</td>
<td>5</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>3+</td>
<td>4</td>
<td>5</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>4+</td>
<td>7</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>10</td>
<td>9</td>
</tr>
<tr>
<td>CXCR4</td>
<td>2+</td>
<td>0</td>
<td>4</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>3+</td>
<td>5</td>
<td>6</td>
<td>2</td>
<td>0</td>
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<tr>
<td></td>
<td>4+</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 4. CCR7, CXCR4 mRNA expression based on clinical stage and differentiation level.

<table>
<thead>
<tr>
<th>Clinical stage</th>
<th>II (B-C1)</th>
<th>III (C2)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>CCR7</td>
<td>central</td>
<td>2.171 ± 0.484</td>
<td>2.319 ± 0.417</td>
<td>0.228, 0.633</td>
<td></td>
</tr>
<tr>
<td></td>
<td>para</td>
<td>1.939 ± 0.748</td>
<td>2.017 ± 0.417</td>
<td>0.477, 0.490</td>
<td></td>
</tr>
<tr>
<td>CXCR4</td>
<td>central</td>
<td>1.537 ± 0.920</td>
<td>1.558 ± 0.648</td>
<td>0.282, 0.595</td>
<td></td>
</tr>
<tr>
<td></td>
<td>para</td>
<td>1.669 ± 1.377</td>
<td>1.042 ± 0.363</td>
<td>3.454, 0.063</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Differentiation level</th>
<th>Poor</th>
<th>middle</th>
<th>well</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>CCR7</td>
<td>central</td>
<td>2.158 ± 0.593</td>
<td>2.155 ± 0.273</td>
<td>2.419 ± 0.458</td>
<td>1.137, 0.566</td>
<td></td>
</tr>
<tr>
<td></td>
<td>para</td>
<td>1.806 ± 0.442</td>
<td>1.990 ± 0.821</td>
<td>2.200 ± 0.733</td>
<td>1.673, 0.433</td>
<td></td>
</tr>
<tr>
<td>CXCR4</td>
<td>central</td>
<td>1.769 ± 1.127</td>
<td>1.324 ± 0.551</td>
<td>1.494 ± 0.579</td>
<td>0.623, 0.732</td>
<td></td>
</tr>
<tr>
<td></td>
<td>para</td>
<td>1.585 ± 1.711</td>
<td>1.207 ± 0.544</td>
<td>1.757 ± 0.891</td>
<td>2.019, 0.364</td>
<td></td>
</tr>
</tbody>
</table>

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CXCR4. For anti-cancer therapy, inhibiting the translation of CCR7, CXCR4 mRNA may be another effective approach.

REFERENCES


