Abstract

Purpose: The biological axes of chemokines and chemokine receptors, such as CXCR4/CXCL12, CCR7/CCL19 (CCL21), CCR9/CCL25, and CXCR5/CXCL13, are involved in cancer growth and metastasis. This study is aimed at the potential regulatory role of atypical chemokine binder CCX-CKR, as a scavenger of CCL19, CCL21, CCL25, and CXCL13, in human breast cancer.

Experimental Design: The role of CCX-CKR in human breast cancer was investigated in cell lines, animal models, and clinical samples.

Results: Overexpression of CCX-CKR inhibited cancer cell proliferation and invasion in vitro and attenuated xenograft tumor growth and lung metastasis in vivo. CCX-CKR can be regulated by cytokines such as interleukin-1β, tumor necrosis factor-α, and IFN-γ. Lack or low expression of CCX-CKR correlated with a poor survival rate in the breast cancer patients. A significant correlation between CCX-CKR and lymph node metastasis was observed in human breast cancer tissues. CCX-CKR status was an independent prognostic factor for disease-free survival in breast cancer patients.

Conclusion: We showed for the first time that CCX-CKR is a negative regulator of growth and metastasis in breast cancer mainly by sequestration of homeostatic chemokines and subsequent inhibition of intratumoral neovascularity. This finding may lead to a new therapeutic strategy against breast cancer.

Body of convincing evidence has shown that CCR7/CCL19 (CCL21), CCR9/CCL25, and CXCR5/CXCL13 interactions played a significant role in tumor survival, proliferation, and metastasis. CCR7 was found to be highly expressed in breast cancer cells (4, 6, 7). CCR7/CCL19 (CCL21) could promote the pathogenesis and progression of breast cancer, melanoma, nonsmall cell lung cancer, gastrointestinal cancer, head and neck cancer, hematologic cancer, etc. (8–11). CCR9/CCL25 axis role has been shown in breast carcinoma, prostate cancer, ovarian cancer, and cutaneous melanoma (12, 13). CXCR5/CXCL13 was also involved in various cancers (14–16). Fortunately, CCX-CKR, as a member of atypical chemokine binders, could bind and scavenge its cognate ligands CCL19 (ELC), CCL21 (SLC, 6Ckine), CCL25 (TECK), and CXCL13 (BCA-1, BLC; refs. 17–19). CCX-CKR (Chemocentryx chemokine receptor), as also known as CCR11, CCRL1, was a third atypical chemokine binder, which was found after Duffy antigen receptor for chemokine (DARC) and D6 (20–22).

Chemokine decoy receptors, also called as atypical chemokine binders, are a new subfamily of chemokine receptors that do not signal along classic G-protein-mediated pathways. Instead, they efficiently internalize their cognate chemokine ligands and act as scavengers. It contains at least three members: DARC, D6, and CCX-CKR. We have previously reported the antitumor effect of DARC and D6 in human breast cancer (23, 24). Similar to DARC, CCX-CKR binds chemokines of both CC and CXC subfamilies; unlike DARC and D6, only the “homeostatic” ones: CCL19, CCL21, CCL25, and CXCL13. These chemokines participate in the axis role of CCR7/CCL19 (CCL21), CCR9/CCL25, and CXCR5/CXCL13 in both the process of leukocytes and cancer cell migration. Therefore, we hypothesized that CCX-CKR might also correlate with human cancer.
Tumor. To further investigate the role and mechanism involved in CCX-CKR and human breast cancer, we constructed CCX-CKR overexpression breast cancer cell lines and do further experiments. Our finding indicated that the chemokine-binding protein CCX-CKR could inhibit the proliferation and migration of human breast cancer cells, particularly the potential of invasion and metastasis. A significant correlation between CCX-CKR and lymph node metastasis was observed. CCX-CKR status was an independent prognostic factor for disease-free survival in the breast cancer patients. We showed for the first time that CCX-CKR is a negative regulator of growth and metastasis in breast cancer mainly by sequestration of homeostatic chemokines and subsequent inhibition of intratumoral neovascularity. This finding may lead to a new therapeutic strategy against breast cancer.

**Translational Relevance**

The biological axes of chemokines and chemokine receptors, such as CXCR4/CXCL12, CCR7/CCL19 (CCL21), CCR9/CCL25, and CXCR5/CXCL13, are involved in cancer growth and metastasis. This study is aimed at the potential regulatory role of atypical chemokine binder CCX-CKR, as a scavenger of CCL19, CCL21, CCL25, and CXCL13, in human breast cancer. The role of CCX-CKR in human breast cancer was investigated in cell lines, animal models, and clinical samples. Overexpression of CCX-CKR inhibited cancer cell proliferation and invasion in vitro and attenuated xenograft tumor growth and lung metastasis in vivo. A significant correlation between CCX-CKR in cancer tissues and lymph node metastasis was observed. CCX-CKR status was a significant independent prognostic factor for disease-free survival in the breast cancer patients. We showed for the first time that CCX-CKR is a negative regulator of growth and metastasis in breast cancer mainly by sequestration of homeostatic chemokines and subsequent inhibition of intratumoral neovascularity. This finding may lead to a new therapeutic strategy against breast cancer.

**Materials and Methods**

**Cells, regents, and cDNA construction.** Human breast cancer cell lines MDA-MB-435, MDA-MB-231, MCF-7, ZR-75-1, and T47D were obtained from the American Type Culture Center. HBL-100, a breast epithelial cell line from healthy woman, was purchased from the Cell Bank, Chinese Academy of Sciences. MDA-MB-435HM and MDA-MB-231HM with high pulmonary metastasis potential were established in our laboratory.

The goat anti-human CCR11 (CCX-CKR) polyclonal antibody was purchased from Abcam. The rat anti-mouse CD34 monoclonal antibody was from BD Biosciences. ELISA kits for CCL19/CCL21/ CCL25 and hCXCL13 were from R&D Systems. The plasmid pcDNA3.1 containing human CCX-CKR cDNA was kindly provided by Dr. Thomas J. Schall (Chemocentryx).

**Stable transfections.** The expression plasmid pcDNA3.1 with or without CCX-CKR were transfected using the Lipofectamine reagent (Life Technologies) and G418 was added to select stable transfectants. The CCX-CKR-positive colonies were identified by real-time PCR (Life Technologies) and G418 was added to select stable transfectants.

**RNA extraction and RT-PCR.** The specific primers of CCX-CKR and other relevant molecules used in the experiment were the following: CCX-CKR up GGGAATCCATGGTGTGCGC (132 bp) and down TGGATACAGCCCCCAGGG (55°C) and GAPDH up GGGAGCTCATCATCCTC (353 bp) and down CCATGCCAGTCGCCGGTT (60°C).

**Western blotting.** Western blot using goat anti-human CCX-CKR antibody was done according to standard protocols. Blot quantification was done with a Molecular Dynamics Laser Densitometer (model PSD) and the Image Quant Version 1 software.

**Proliferation and invasion assay.** Cell proliferation was done by using Cell Counting Kit-8 (Dojindo). Invasion experiments were conducted with a Matrigel invasion chamber (BD Labware).

**Experimental metastasis assay and immunohistochemical staining.** The tumorigenicity and spontaneous metastatic capability of the cell lines were determined by injection into the mammary fat pad of mouse. Animals were killed and autopsied at 8 and 6 weeks post-inoculation. Metastasis formation was assessed by macroscopic observation of all major organs for secondary tumors and confirmed by histologic examination of organs.

Tumor sections were subjected to immunohistochemical staining for CCX-CKR (for cancer cells) and CD34 (for microvessels).

**ELISA analysis.** The protein level of mouse CCL19, CCL21, CCL25, and CXCL13 present in conditioned cell supernatants and xenografts was determined using a sandwich ELISA kit (DuoSet; R&D Systems).

**In vivo selection and metastasis assay.** MDA-MB-231HM, generated as described in Materials and Methods, metastasized consistently to the lungs in 4 weeks in 100% of athymic nude mice compared with <30% lung metastasis in wild-type MDA-MB-231.

**Statistical analysis.** ANOVA and Student’s t test were used to determine the statistical significance of differences between experimental groups. The Kaplan-Meier method was used to analyze breast cancer patient cumulative survival rate. Cox risk proportion model was used to analyze the independent prognostic factor for breast cancer. Statistical significance was defined as P < 0.05.

**Results**

**Expression of CCX-CKR in breast cancer cells and tissues that can be down-regulated by interleukin-1β, tumor necrosis factor-α, and IFN-γ.** We examined the expression of CCX-CKR mRNA by RT-PCR in seven breast cancer cell lines (Fig. 1A) and six breast cancer samples (Fig. 1B) and found that CCX-CKR mRNA expressed differentially. To further confirm the difference of CCX-CKR mRNA expression in the cells, we detected the CCX-CKR mRNA expression in six breast cancer cells and a normal mammary epithelial cell line HBL-100 by quantitative RT-PCR (data not shown). It was clear that MDA-MB-435 cells had higher expression of CCX-CKR than MDA-MB-435HM cells with high metastatic potential, and HBL-100 cells have higher expression than the cancer cell lines. These results indicated that CCX-CKR might play an anticancer role in tumor development.

It is known that cytokines and chemokines have complicated interactions with each other. Therefore, we selected three cytokines interleukin-1β, tumor necrosis factor-α, and IFN-γ to investigate the regulation of cytokines in CCX-CKR. After treatment of cytokines in different concentration for 24 h, all the expression levels of CCX-CKR mRNA decreased: CCX-CKR expression decreased 84.67%, 85.33%, and 83.45% by interleukin-1β at concentration of 0.5, 1.0, and 2.0 ng/mL compared with control and 74.98%, 84.02%, and 84.98% by tumor necrosis factor-α at the concentration of 10, 20, and 40 ng/mL and 84.6%, 92.35%, and 90.26% by IFN-γ at the concentration of 10, 20, and 40 ng/mL.
Stable transfection of CCX-CKR cDNA in MDA-MB-435 and MDA-MB-231HM cells. CCX-CKR may play an anticancer role in breast cancer development. To test this hypothesis, we transfected CCX-CKR expression vector into MDA-MB-231HM and MDA-MB-435HM cells and generated stable transfectants. CCX-CKR expression was detected by RT-PCR and Western blot (Fig. 1C-E). Transfectant clones from MDA-MB-435 and MDA-MB-231 HM cells with high expression levels of CCX-CKR protein were named 435-CCX-CKR1, 435-CCX-CKR2, 231HM-CCX-CKR1, and 231HM-CCX-CKR2. These CCX-CKR overexpression cell lines were used in further experiments.

CCX-CKR inhibits the proliferation and invasion of cells in vitro, but the mRNA expression of metastatic relative genes does not change. Proliferation assays were done to determine the variation of proliferation potential of breast cancer cells after CCX-CKR transfection (Fig. 2). The proliferation potential was significantly attenuated in CCX-CKR-transfected clones in contrast to mock-transfected clones. Compared with mock-transfected cells, MDA-MB-231HM-CCX-CKR cells were significantly growth slower ($P < 0.05$; Fig. 2B). The similar result was observed in MDA-MB-435 group ($P < 0.05$; Fig. 2A).

We examined the invasion activities of CCX-CKR stable transfectants and their parental cells using the Boyden chamber coated with Matrigel. As a result, invasion was significantly inhibited in CCX-CKR stable transfectants in 12, 24, and 48 h ($P < 0.01$; Fig. 2C and D), which was almost completely blocked in MDA-MB-435-CCX-CKR cells in 12 h. Compared with the attenuation of growth potential by CCX-CKR in transfectants, the inhibition of invasion activity was more noticeable.

Because CCX-CKR could bind and scavenge its cognate chemokines, we detected the amount of its ligands in cells by RT-PCR and in the conditioned cell supernatant by ELISA. The results showed that the significant ligand decrease was seen in supernatant of CCX-CKR transfectants, whereas no mRNA expression change could be detected (data not shown). In addition, no significant changes could be detected on matrix metalloproteinase (MMP)-1, MMP-2, MMP-7, MMP-9, vascular endothelial growth factor, and basic fibroblast growth factor mRNA expression (data not shown).

CCX-CKR slows the tumor growth and attenuates lung metastasis in vivo. To further support for the idea that
CCX-CKR could attenuate the proliferation and invasion in vitro and to determine whether CCX-CKR could inhibit the growth and metastasis in vivo, we used orthotropic xenograft tumor models in the nude mice.

As expected, CCX-CKR transfectants grew much slower than mock transfectants and wild-type cells in nude mice. The reduction of tumor volume was observed at 73.4% and 69% of transfected MDA-MB-231HM-CCX-CKR1 (294.6 ± 90 mm³) tumors compared with the mock-transfected MDA-MB-231HM (1,108.8 ± 124 mm³) and wild-type MDA-MB-231HM (957.3 ± 115 mm³) tumors, respectively, in 39 days. Similar results were observed in another CCX-CKR transfectants of MDA-MB-231HM cell lines and CCX-CKR transfectant of MDA-MB-435 cell lines (Fig. 3A and B). The weight of xenograft tumors was also measured and the CCX-CKR-transfected tumors showed significant reduction of weight compared with the control tumors (P < 0.05; data not shown). The number of metastatic lung nodules in the mice injected with CCX-CKR transfectants was significantly lower than that in the control mice (P < 0.05; Fig. 3C and D). All the primary tumors and lung metastatic tumors were confirmed by pathologic examination. Results showed that CCX-CKR protein in CCX-CKR overexpression tumors is much higher than that in the control tumors (Fig. 3C), whereas microvessels were significantly less in mock-transfected tumors and wild-type tumors than CCX-CKR-transfected tumors (Fig. 3C and E). All the similar results could be seen in MDA-MB-435 xenografts (data not shown).

The influence of high expression of CCX-CKR on local concentration of target chemokines was also investigated. We observed a significant decrease in mouse CCL19, CCL21, CCL25, and CXCL13 protein levels in CCX-CKR-transfected xenografts compared with the mock-transfected and wild-type xenografts (P < 0.01; Fig. 3F and G).

Correlation between CCX-CKR and survival in human breast cancer patient. To determine whether CCX-CKR expression in primary tumor tissues was associated with clinical characteristics (Table 1), we performed immunohistochemical staining (Fig. 4A). We found a significantly negative correlation between CCX-CKR and lymph node metastasis. In human breast cancer tissues with lymph node metastasis, CCX-CKR was mostly undetectable, whereas, in the tissues without lymph node metastasis, CCX-CKR expression was mostly positive. Among the 98 breast cancer tissues that we examined, the correlation between CCX-CKR and lymph node metastasis was statistically significant (P < 0.05; Fig. 4B), supporting the conclusion that overexpression of CCX-CKR can inhibit tumor metastasis potential. However, CCX-CKR expression had no relationship with human epidermal growth factor receptor-2, estrogen receptor, and progesterone receptor status (data not shown). Importantly, CCX-CKR expression was found to be correlated with better survival in all the 98 patients and the lymph node metastasis-positive patients (P < 0.05; Fig. 4C and E) but no significant relationship in lymph node metastasis-negative patients (Fig. 4D).

To further investigate if this is an independent prognostic factor, we performed multivariate analysis by Cox risk proportion model. The result showed that CCX-CKR status was an independent prognostic factor for disease-free survival in patients (Table 2).

Discussion

CCX-CKR, acting as a scavenger for chemokines, binds and clears the constitutively expressed chemokines including CC chemokine ligand CCL19, CCL21, CCL25, and CXCL13, also known as homeostatic chemokines (20, 21, 25). CCX-CKR was

![Fig. 2. CCX-CKR inhibits the proliferation and invasion of breast cancer cells in vitro. A and B, growth curve of control cells and CCX-CKR-transfected MDA-MB-435 and MDA-mb-231 cells in proliferation assay. *, P < 0.05. C and D, quantification of invasion assays for the CCX-CKR-transfected cells and control cells. Invasive assays were done using the Boyden chamber assay coated with Matrigel, which mimics basement membrane composition. Cells were counted in triplicate wells and in three identical experiments. **, P < 0.01. Columns, mean of three independent experiments; bars, SD. HP, high-power objective.]
expressed in dendritic cells, T cells, spleen, lymph node, heart, kidney, placenta, trachea, brain (18), small intestine, lung (26, 27), astrocytes (28), and pulmonary sarcoidosis (29). Recently, observations have indicated that the silent chemokine receptor CCX-CKR, which is exclusively expressed by stroma cells, but not hematopoietic cells themselves, regulates homeostatic leukocyte migration by controlling the availability of chemokines in the extracellular space (30). Our current study shows that CCX-CKR is expressed in breast cancer cell lines and tissues and that CCX-CKR overexpression can inhibit tumor growth, invasion, angiogenesis, and metastasis in vitro and in vivo.

Cytokines and chemokines in the tumor microenvironment may affect each other in the process of tumor progression. We

Fig. 3. CCX-CKR slowed the tumor growth and attenuated lung metastasis in vivo. A and B, average volume of control tumors and CCX-CKR-transfected tumors. In both groups, tumors were significantly larger than CCX-CKR-transfected tumors, **P < 0.05. C, immunohistochemistry examination of CCX-CKR protein (×100), metastasis to lung (H&E, ×40), and CD34 staining (×100, as a marker of microvessels) in the final week of control xenografts and CCX-CKR-transfected xenografts. Red arrows, lung metastasis and microvessels. D and E, quantification of the average metastasis number per lung and average number of microvessel density of the control tumors and CCX-CKR-transfected tumors, **P < 0.05.
speculated that CCX-CKR expression might be linked with cytokines and found that CCX-CKR mRNA expression could be attenuated by interleukin-1β, tumor necrosis factor-α, and IFN-γ. These results provide an initial clue to understand the regulation mechanism of CCX-CKR itself in tumors, although the detailed mechanisms need to be investigated further.

Chemokines and chemokine receptors are reported to be involved in cancer growth and metastasis (4, 8, 31). Despite the most famous CXCR4/CXCL12 axis (5), a lot of investigations indicate that CCR7/CCL19 (CCL21), CCR9/CCL25, and CXCR5/CXCL13 axes (11, 12, 14) can also promote the growth and metastasis of many tumors. Breast cancer cells specifically express functionally active CCR7 (4, 6, 7). CCR7/CCL19 (CCL21) can promote the pathogenesis and progression of breast cancer, melanoma, non-small cell lung cancer, gastrointestinal cancer, head and neck cancer, hematologic cancer, etc.

Table 1. Patient’s clinical characteristics (98 cases)

<table>
<thead>
<tr>
<th></th>
<th>Lymph node negative</th>
<th>Lymph node positive</th>
</tr>
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<tbody>
<tr>
<td>No. cases</td>
<td>47</td>
<td>51</td>
</tr>
<tr>
<td>Median age (y)</td>
<td>56.8 ± 10.8</td>
<td>52.4 ± 11.7</td>
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<tr>
<td>Status of menses</td>
<td></td>
<td></td>
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<tr>
<td>Premenopause</td>
<td>15</td>
<td>23</td>
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<tr>
<td>Menopause</td>
<td>32</td>
<td>28</td>
</tr>
<tr>
<td>Tumor size (cm)</td>
<td>2.51 ± 0.88</td>
<td>2.61 ± 0.98</td>
</tr>
<tr>
<td>Pathologic type</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infiltrating ductal carcinoma</td>
<td>36</td>
<td>46</td>
</tr>
<tr>
<td>Others</td>
<td>11</td>
<td>5</td>
</tr>
<tr>
<td>Status of hormonal receptors</td>
<td></td>
<td></td>
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<tr>
<td>Estrogen receptor</td>
<td></td>
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<tr>
<td>+</td>
<td>25</td>
<td>30</td>
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<tr>
<td>-</td>
<td>17</td>
<td>18</td>
</tr>
<tr>
<td>Progesterone receptor</td>
<td></td>
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<tr>
<td>+</td>
<td>24</td>
<td>28</td>
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<td>-</td>
<td>18</td>
<td>20</td>
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<tr>
<td>Human epidermal growth factor receptor-2</td>
<td></td>
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<td>+</td>
<td>21</td>
<td>27</td>
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<tr>
<td>-</td>
<td>26</td>
<td>24</td>
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</table>
CCR9/CCL25 (12, 13) axis role has been shown in breast carcinoma, prostate cancer, ovarian cancer, and cutaneous melanoma. Overexpression of CXCR5 and CXCL13 is found in B-cell chronic lymphocytic leukemia, non-Hodgkin's lymphomas, primary intraocular lymphoma, metastatic neuroblastoma, and breast cancer (14–16). CXCR5 is expressed by solid carcinoma cells and promotes growth of colon carcinoma in the liver (32) and CXCL13 is expressed by malignant lymphocytes and vascular endothelium in primary central nervous system lymphoma (33). CCX-CKR, acting as a scavenger for cognate chemokines, can bind and clear CCL19, CCL21, CCL25, and CXCL13. We hypothesized that CCX-CKR might be involved in breast cancer cell invasion and metastasis. As we expected, CCX-CKR actually plays a negative role in breast cancer growth and metastasis.

Chemokines play multifaceted roles in malignancy. Angiogenesis, one of the major aspects of chemokine activities, is regulated by angiogenic and angiostatic chemokines. CCL19 and CCL21 are known as angiostatic chemokines. They can mediate potent antitumor responses in vivo leading to significant tumor reduction through the specific G protein-coupled seven-transmembrane domain chemokine receptor CCR7. Gene modification or local treatment with CCL19 can enhance antitumor immunity in mice breast cancer (34), ovarian cancer (35), and lung cancer (36). Intratumoral administration of CCL21 can slow the growth of orthotropic mammary tumors and prolong the survival of tumor-bearing mice (37). Opposing voice also emerges. CCL21 promotes migration and adhesion of highly lymph node metastatic human non-small cell lung cancer Lu-99 in vitro (38). More importantly, it has been shown that, in many solid tumors, CCR7 and its ligands (CCL19, CCL21) play a prominent role in lymph node metastasis (4, 8–11). In our study, CCX-CKR can decrease these two chemokines and significantly inhibit the growth and lung metastasis of xenograft tumors. We noticed that the concentration of CCL19 and CCL21 in vitro and in vivo were extremely lower, suggesting that the down-regulation may involve in the antitumor effect of CCX-CKR. In other words, in this cell and animal model of breast cancer, CCL19 and CCL21 may actually play a tumor promotion role. We think that the relationship among chemokines, chemokine functional receptors such as CCR7, atypical chemokine binders including CCX-CKR, and breast cancer is complicated and needs to be investigated further. It is possible that the general and ultimate role of CCL19 and CCL21 depends on their concentrations and interaction with their receptor in tumor microenvironment. Besides CCL19 and CCL21, a significant decrease in CCL25 and CXCL13 protein levels in CCX-CKR-transfected xenografts is also observed. It must be very interesting to identify the precise role of CXCL13, a unique known chemokine for B lymphocyte, in breast cancer. All the ligand gene expression in CCX-CKR-transfected cells has no significant change, but their protein levels in conditioned medium and xenograft tumor were markedly reduced, suggesting that CCX-CKR-transfected cells have already obtained a high clearance capability for target chemokines in post-translational but not translational level.

It is widely accepted that MMPs, vascular endothelial growth factor, and basic fibroblast growth factor usually correlate with cancer growth, invasion, and metastasis. However, in our study, there was no mRNA change of MMPs, vascular endothelial growth factor, and basic fibroblast growth factor in transfected...
cancer cells and control cells. Despite the study in vitro and in vivo, we also performed a study in human breast cancer samples. In our 98-case breast cancer study, a significant correlation between CCX-CKR expression and lymph node metastasis was observed and CCX-CKR was found to be associated with long patient survival. In multivariate analysis by Cox risk proportion model, CCX-CKR status was an independent prognostic factor for disease-free survival in breast patients. In view of the fact that CCR7/CCL19 (CCL25) promotes the metastasis of many tumors including breast cancer, our observations add strong clinical support for the effect that CCX-CKR can negatively regulate the breast cancer development and progression by sequestrating their cognate chemokines.

In summary, CCX-CKR is differentially expressed in breast cancer cell lines and the expression could be regulated by some inflammatory cytokines. CCX-CKR plays an antitumor role both in vitro and in vivo. Higher CCX-CKR expression is negatively correlated with lymph node metastasis and better survival. In multivariate analysis, CCX-CKR was an independent prognosis factor for disease-free survival in the breast cancer patients. This finding may lead to a new therapeutic strategy against breast cancer.

### Table 2. Multivariate analyses of disease-free survival by Cox proportional hazards models

<table>
<thead>
<tr>
<th>Patients</th>
<th>( P )</th>
<th>Hazard ratio (95% confidence interval)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.707</td>
<td>—</td>
</tr>
<tr>
<td>Tumor size, ( T_1 ) vs ( T_2 ) vs ( T_3 )</td>
<td>0.349</td>
<td>—</td>
</tr>
<tr>
<td>Nuclear grade, 0-1 vs II vs III</td>
<td>0.872</td>
<td>—</td>
</tr>
<tr>
<td>Axilla lymph nodes, positive vs negative</td>
<td>0.015</td>
<td>2.356 (1.174-4.728)</td>
</tr>
<tr>
<td>Estrogen receptor status, positive vs negative</td>
<td>0.945</td>
<td>—</td>
</tr>
<tr>
<td>Progesterone receptor status, positive vs negative</td>
<td>0.036</td>
<td>0.247 (0.067-0.914)</td>
</tr>
<tr>
<td>Human epidermal growth factor receptor-2, positive vs negative</td>
<td>0.078</td>
<td>—</td>
</tr>
<tr>
<td>CCX-CKR status, positive vs negative</td>
<td>0.015</td>
<td>4.117 (1.311-12.932)</td>
</tr>
</tbody>
</table>

### References


### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

### Acknowledgments

We thank the studied women for willingness to cooperate with our study.
Involvement of a Novel Chemokine Decoy Receptor CCX-CKR in Breast Cancer Growth, Metastasis and Patient Survival


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