CXCL10 Promotes Osteolytic Bone Metastasis by Enhancing Cancer Outgrowth and Osteoclastogenesis

Jong-Ho Lee1, Ha-Neui Kim1, Kyung-Ok Kim2, Won Jong Jin1, Seungbok Lee1, Hong-Hee Kim1, Hyunil Ha3, and Zang Hee Lee1

Abstract

Amplification of the chemokines CXCL10 and RANKL has been suggested to promote osteoclast differentiation and osteolytic bone metastasis, but a function for endogenous CXCL10 in these processes is not well established. In this study, we show that endogenous CXCL10 is critical to recruit cancer cells to bone, support osteoclast differentiation and promote for the formation of osteolytic bone metastases. Neutralizing CXCL10 antibody reduced migration of cancer cells expressing the CXCL10 receptor CXCR3, and loss of CXCR3 or CXCL10 decreased bone tumor burden in vivo. Bone colonization augmented host production of CXCL10, which was required for cancer growth and subsequent osteolysis. Direct interactions between cancer cells and macrophages further stimulated CXCL10 production from macrophages. Growth of bone metastases required CXCL10-stimulated adhesion of cancer cells to type I collagen as well as RANKL-mediated osteoclast formation. Together, our findings show that CXCL10 facilitates trafficking of CXCR3-expressing cancer cells to bone, which augments its own production and promotes osteoclastic differentiation. CXCL10 therefore may represent a therapeutic target for osteolytic bone metastasis. Cancer Res; 72(13); 3175–86. ©2012 AACR.

Introduction

The importance of the interaction between cancer cells and the surrounding environment in organ-specific metastasis has been well characterized in bone metastasis. Bone is one of the most preferential metastatic sites for common human cancers, and bone metastases are a significant cause of morbidity and mortality in patients with cancer, causing severe bone pain, pathologic fractures, and spinal cord compression (1, 2). The development and progression of osteolytic bone metastasis are thought to depend on a complex cycle of bone destruction and cancer outgrowth (3). Colonized cancer cells in bone produce various osteoclastogenic factors that stimulate osteoclast differentiation and activation either directly or indirectly by promoting the ratio of receptor activator of nuclear factor-κB ligand (RANKL) or its decoy receptor osteoprotegerin (OPG) in osteoblasts (4, 5). Increased bone resorption by activated osteoclasts releases growth factors such as transforming growth factor-β and insulin-like growth factors from the degraded bone matrix. These growth factors in turn enhance the growth of cancer cells and further stimulate them to produce the osteoclastogenic factors, resulting in a vicious cycle of bone destruction and cancer outgrowth (4, 5).

The interaction between chemokine receptors expressed on cancer cells and the corresponding chemokines produced by the target organs has emerged as a key mediator of organspecific metastasis (6). For example, the chemokine receptor CXCR4 has been found to be expressed in many tumor types, and CXCR4-expressing cancer cells can metastasize to several organs where CXCL12 is abundantly expressed (7–9). Similarly, the chemokine receptor CXCR3 has also been recently identified in various cancer cells including melanoma, breast cancer, colon cancer, and glioma (10–13). In addition, CXCR3 expression correlates with poor prognosis in breast cancer, colon cancer, and melanoma (11, 12, 14). Furthermore, it has been shown that CXCR3 expression on cancer cells promotes lung metastases of breast cancer and colon cancer as well as lymph node metastases of murine melanoma and colon cancer (10–15, 16). Despite the importance of CXCR3 expression in cancer metastasis to lung and lymph nodes, nothing is known about the contribution of endogenous CXCR3 ligands in the target organs.

CXCL10 is a member of the subfamily of IFN-γ-inducible and the non-ELR CXC chemokines and it signals through CXCR3, a common receptor for IFN-γ-inducible chemokines (CXCL9, CXCL10, and CXCL11). The fundamental role of CXCL10/CXCR3 interaction has been well characterized in chronic Th1-inflammatory diseases. In inflamed tissues,
CXCL10 recruits Th1 cells and upregulates the production of IFN-γ. In turn, IFN-γ stimulates CXCL10 expression in various cell types, resulting in a positive feedback to amplify CXCL10 and Th1 responses (17). In line with this, we previously found that reciprocal amplification of CXCL10 and RANKL plays a crucial role in bone destruction in a mouse model of rheumatoid arthritis in which CXCL10 recruits CD4+ T cells and promotes them to produce RANKL, and then RANKL induces CXCL10 expression and osteoclast differentiation from its precursors (18).

These previous reports showing the important role of osteoclast differentiation in bone metastasis, RANKL-induced upregulation of CXCL10, and the participation of CXCR3 in cancer metastasis to target organs led us to explore whether CXCL10 plays a critical role in bone metastasis. To address this possibility, we examined CXCL10 expression in bone and then determined the effects of CXCR3 gene silencing in cancer cells, systemic CXCL10 blockade with a neutralizing antibody, and CXCL10 deficiency in the host on experimental models of bone metastasis. We also investigated the molecular mechanisms underlying CXCL10-mediated osteolytic bone metastasis.

Materials and Methods

Reagents and cells

Mouse CXCL10, human RANKL, human macrophage colony-stimulating factor (M-CSF), anti-mouse CXCL10 antibody (aCXCL10), and control rabbit IgG were obtained from PeproTech. PD98059 was purchased from Calbiochem. AKT Inhibitor VIII was purchased from Santa Cruz Biotechnology. B16F10 murine melanoma cell line, human breast cancer cell lines (MDA-MB-231, MDA-MB-453, and MCF7), MC3T3-E1 murine preosteoblastic cell line, NIH3T3 murine fibroblast cell line, and Raw264.7 murine monocyte cell line were purchased from the American Type Culture Collection and maintained in DMEM (Invitrogen) supplemented with 10% FBS (Hyclone Laboratories) and 1% penicillin–streptomycin (Invitrogen). ST2 murine stromal cell line was kindly provided by Dr. Nacksung Kim (University of Chonnam, Korea) and cultured in α-MEM containing 10% FBS and 1% penicillin–streptomycin. B16-FL cells were generated from B16F10 cells by stable transfection of the firefly luciferase gene.

Animals

Cxcl10−/− mice and Toll-like receptor (Tlr)4−/− mice (C57BL/6 background) were obtained from The Jackson Laboratory. Female BALB/C nude mice were purchased from Charles River Korea. All animal procedures were reviewed and approved by the animal care committee of the Institute of Laboratory Animal Resources of Seoul National University.

Migration assay

Cancer cells were harvested by treatment with cell dissociation solution (Sigma-Aldrich) and then resuspended in serum-free DMEM. Cells were seeded in the upper well of the Boyden chamber (8-μm pore membranes; Corning Costar). Membranes were precoated with 10 μg/mL of fibronectin (Sigma-Aldrich). The lower well was loaded with serum-free DMEM in the presence or absence of used attractants and incubated for 6 hours, and cells attached on the lower surface of the membrane were counted.

Antibody and RANKL injection

Mice (6-week-old C57BL/6 male) were treated intravenously with 6.6 mg/kg αCXCL10 or control IgG the day before tumor intracardiac injection. Soluble RANKL (sRANKL; glutathione S-transferase fusion RANKL protein) was kindly provided by Dr. Hisataka Yasuda (Nagahama Institute for Biochemical Science, Oriental Yeast Company, Shiga, Japan) and used for in vivo experiments only. Mice were injected intraperitoneally with sRANKL (20 μg per mouse), and serum CXCL10 levels were measured after sRANKL injection.

Reverse transcription PCR and real-time PCR analysis

Total RNA isolation, reverse transcription (RT), and real-time PCR were conducted as described previously (19). In some experiments, CXCR3 and RANK transcripts were analyzed by RT-PCR. The primers used for real-time PCR and RT-PCR listed in Supplementary Table S1.

Retroviral gene transduction

Short hairpin RNAs (shRNA) targeting luciferase (shLuci; CAAATCACAGAATCGTCGT), mouse CXCR3 (mouse shCXCR3; CAGGCGCCTTGTTCAACAT), and human CXCR3 (human shCXCR3; CACACCAACCAGACCGCAG) were inserted into the pSUPER.retro.puro vector (OligoEngine). Retrovirus packaging, cell infection, and selection were conducted as described previously (19).

In vitro osteoclast formation assay

In vitro osteoclast formation from mouse bone marrow macrophages (BMM) and from coculture of mouse calvarial osteoblasts and bone marrow cells (BMC) was done as described previously (19). Tartrate-resistant acid phosphatase (TRAP)-positive multinucleated cells containing 3 or more nuclei were counted as osteoclasts.

ELISA

The protein levels of mouse RANKL, mouse OPG, mouse CXCL10, human CXCL10, and mouse CXCL12 in samples collected as indicated were measured using the corresponding ELISA kits (R&D Systems). The detailed procedures were described in Supplementary Materials and Methods.

Bone metastasis models

For intracardiac injections, mice were anesthetized and injected with 1 × 10^7 tumor cells in 100 μL PBS into the left cardiac ventricle, a route of administration that allows for bone metastasis rather than lung infiltration of injected cells. For intrafemoral injections, 1 × 10^7 tumor cells in 5 μL PBS were injected into the left femur using a 28-gauge Hamilton syringe. After metastases, mice were sacrificed and underwent blinded necropsy.
Bioluminescence imaging and analysis

Mice (C57BL/6) were intraperitoneally injected with 150 mg/kg n-luciferin (Xenogen) in PBS 12 minutes before bioluminescence imaging (BLI). Imaging was conducted using a charge-coupled device camera (IVIS 100, Xenogen; exposure time of 3 minutes, binning of 8, field of view of 15 cm, f/stop of 1, and no filter). Mice were anesthetized with isoflurane (2% vaporized in O2) and shaved to minimize attenuation of signal by pigmented black hair. For analysis, total photon flux (photons per second) was measured from a fixed region of interest in the hind limbs and the mandible/maxilla using Living Image software (Xenogen). Bioluminescent signals within the fixed region of interest were normalized to the background luminescence taken over the same region of interest from animals not injected with n-luciferin.

Radiographs and measurement of osteolytic lesion area

X-ray images of the mouse hind limbs were taken using a cabinet-style soft X-ray unit (Softex, SOFTEX Co.; 30 KVp, 2 mA, exposure time: 90 seconds), scanned, and evaluated without knowledge of the experimental groups. The area of osteolytic lesions, defined as a radiolucent lesion in bone, was measured using image analysis system (Image J) and expressed as a percentage of the total tissue area.

Histology and histomorphometry

Paraffin-embedded sections of decalcified bones were prepared as described previously (20). Sections were stained with hematoxylin and eosin (H&E) or separately for TRAP activity to visualize osteoclasts. Histomorphometric analysis of tumor burden, osteoclast number, and total bone including both trabecular and cortical bones was conducted using VECTASTAIN Elite ABC kit (Vector Laboratories), followed by 3,3′-diaminobenzidine (Vector Laboratories) incubation and nuclear staining with hematoxylin. Ki67 and CD31 staining were quantified on 400× fields per slide were analyzed and averaged.

Immunohistochemistry

Immunohistochemical analysis was conducted on decalcified paraffin-embedded tissue sections. Antibodies against Ki67 and CD31 were purchased from Dako and Santa Cruz Biotechnology, respectively. Detection of the primary antibody was conducted using VECTASTAIN Elite ABC kit (Vector Laboratories), followed by 3,3′-diaminobenzidine (Vector Laboratories) incubation and nuclear staining with hematoxylin. Ki67 and CD31 staining were quantified by the percentage of positively stained nuclei per 400× field and the number of vessels per 200× field, respectively. Three or more randomly chosen fields per slide were analyzed and averaged.

Immunoblotting

Immunoblot analysis was conducted as described previously (19). Specific antibodies against phospho-ERK1/2 (Thr202/Tyr204), ERK, phospho-AKT (Thr308 and Ser473), and AKT (Cell Signaling Technology) were used.

Statistical analysis

All quantitative data is presented as the mean ± SD. Two-group comparison was conducted by using 2-sided, 2-sample Student’s t test. Simultaneous comparison of more than 2 groups was conducted by 1-way ANOVA (SPSS statistical package, version 12; SPSS Inc.). Values of P < 0.05 were considered significant.

Results

CXCL10 is produced in bone and its neutralization reduces bone metastasis

To determine whether RANKL induces CXCL10 production in vivo, mice were injected intraperitoneally with sRANKL. Serum CXCL10 levels were elevated on day 1 by sRANKL and sustained until day 3 (Fig. 1A). Because CXCL10 has been shown to promote directional migration and invasion of several cancer cells (10, 21, 22), we postulated that CXCL10, induced by RANKL during bone remodeling process, may be implicated in bone metastasis via recruiting cancer cells to the bone. CXCL10 induced the chemotactic migration of several cancer cells capable of developing bone metastasis, including human MDA-MB-231 breast cancer cells and mouse B16F10 melanoma cells (data not shown). In addition, blocking CXCL10 activity with αCXCL10 reduced mouse bone marrow fluid–induced migration of B16F10 cells, suggesting the biologic relevance of CXCL10 in recruitment of B16F10 cells to the bone (Fig. 1B).

When B16F10 cells are injected into the left cardiac ventricle of syngeneic C57BL/6 mice, they result in metastases into several organs, such as bones, lungs, and adrenal glands (23). Bone metastasis of B16F10 cells causes black pigmentation in bone lesions as a result of the melanin-pigment-producing B16F10 cells (23). To test whether CXCL10 is involved in cancer bone metastasis, mice were treated with 6.6 mg/kg αCXCL10 or control IgG the day before injection of B16F10 cells. Black pigmentation and histomorphometric analysis revealed that although all mice in both groups developed bone metastasis in their hind limbs at 14 days after injection of B16F10 cells, αCXCL10 reduced the number of bone lesions and tumor size in the hind limbs compared with control IgG (Fig. 1C and D). Similar to these results, the number of lesions and tumor size in lungs and adrenal glands were also decreased by αCXCL10 (Supplementary Fig. S1A and S1B).

Reduction of CXCR3 expression in cancer cells decreases bone metastasis

To investigate whether CXCL10-induced signaling in cancer cells plays a crucial role in bone metastasis, we stably reduced CXCR3 expression in B16F10 cells by retrovirus-mediated gene transfer of shRNA. Compared with control (shLuci), shCXCR3 reduced CXCR3 mRNA levels and impaired chemotaxis in response to CXCL10 but not RANKL in B16F10 cells (Fig. 2A and B). Under the normal cell culture condition, shCXCR3 had no effect on the proliferation of B16F10 cells (data not shown). The bone metastasis by intracardiac injection of B16F10 cells carrying shLuci or shCXCR3 was analyzed by black pigmentation and histomorphometric analysis. Consistent with the results of the αCXCL10 experiment, shCXCR3 decreased the number of lesions and tumor size in the hind limbs compared with shLuci (Fig. 2C and D).
The effect of CXCR3 knockdown in cancer cells on bone metastasis was not restricted to the melanoma model but was also observed in a human breast cancer cell model. MDA-MB-231 cells stably expressing shLuci (MDA-shLuci) or shCXCR3 targeting human CXCR3 (MDA-shCXCR3) were generated, and the functional knockdown of CXCR3 was confirmed by CXCL10-induced chemotaxis (data not shown). When MDA-shLuci cells were injected into the left ventricles of nude mice, 6 of 8 mice developed severe osteolytic bone lesions in the hind limbs 7 weeks after injection, as determined by X-ray imaging. In contrast, 4 of 8 mice injected with MDA-shCXCR3 cells showed relatively smaller bone lesions in the hind limbs (Fig. 2E). Histomorphometric analysis further revealed that bone tumor burden is dramatically reduced in mice-bearing MDA-shCXCR3 cells compared with mice bearing MDA-shLuci cells (Fig. 2F).

**Host deficiency of CXCL10 reduces bone metastasis**

To further validate the effects of CXCL10 on bone metastasis, the bone metastasis in wild-type (WT) and Cxcl10−/− mice after intracardiac injection of B16-FL cells stably expressing firefly luciferase was monitored by BLI. This analysis revealed that the tumor burden in the hind limbs and the mandible/maxilla was significantly less in Cxcl10−/− mice than in WT mice (Fig. 3A and B), which was further confirmed by histologic and histomorphometric analyses (Fig. 3C and F).
Because it has been suggested that osteoclastic bone resorption is implicated in cancer bone metastasis (3), we analyzed the bone phenotype of \(\text{Cxcl10}^{-/-}\) mice. There were no significant differences in bone volume and osteoclast number between WT and \(\text{Cxcl10}^{-/-}\) femoral bones. However, metastasis of B16-FL cells to femoral bones resulted in a 3.5-fold increase in osteoclast number with a decrease in bone volume in WT mice, whereas only a 1.3-fold increase in osteoclast number was observed in \(\text{Cxcl10}^{-/-}\) mice (Fig. 3D and E). These differences were more clearly observed in the mandibular alveolar bone (Fig. 3F).

**CXCL10 expression increases during bone colonization of B16F10 cells**

Serum CXCL10 levels were markedly elevated after intracardiac injection of B16F10 cells, whereas CXCL12 levels were not affected (Fig. 4A). Thus, we next investigated whether bone metastasis affects CXCL10 production. We directly inoculated B16F10 cells into the femur of syngeneic mice and monitored the mRNA expression levels of the 3 CXCR3 ligands (CXCL9, CXCL10, and CXCL11). Among the CXCR3 ligands, CXCL10 showed the highest basal gene expression. Although the mRNA expression levels of all 3 CXCR3 ligands were augmented after inoculation of B16F10 cells, the increase of CXCL10 mRNA levels was the most rapid and highest (Fig. 4B). The intrafemoral inoculation of B16F10 cells also increased CXCL10 protein levels in serum and bone marrow fluid in a time-dependent manner (Fig. 4C).

**Cancer cells interact with macrophages to promote CXCL10 production**

We next investigated the mechanism of CXCL10 upregulation by bone colonization of B16F10 cells. Although B16F10 cells secreted CXCL10 into the culture medium, synergistic augmentation of CXCL10 release was observed in the coculture of B16F10 cells with BMCs (Fig. 5A). Such synergistic CXCL10 release was also observed when B16F10 cells were cocultured with BMMs or RAW264.7, a monocyte/macrophage cell line.
Prevention of direct contact between B16F10 cells and BMMs by membrane filters abrogated the augmentation of CXCL10 release (Fig. 5C).

To determine the origin of enhanced CXCL10 release, B16F10 cells were cocultured with BMMs from WT or Cxcl10−/− mice. A marked reduction of CXCL10 release was observed in coculture with Cxcl10−/− BMMs (Fig. 5D). To clarify the issue further, mouse BMMs were cocultured with human breast cancer cell lines (MDA-MB-231, MDA-MB-453, and MCF-7), and the production of mouse and human CXCL10 was differentially measured by each specific ELISA (Fig. 5E). These breast cancer cells secreted undetectable levels (MDA-MB-231 and MDA-MB-453) or very low levels (MCF-7) of human CXCL10. Cocultures of mouse BMMs with these human breast cancer cells led to augmentation of mouse CXCL10 release without changes in human CXCL10 release in all the cocultures.

CXCL10 promotes tumor outgrowth in the bone microenvironment

The ability of cancer cells to bind type I collagen, the most abundant extracellular matrix molecule (ECM) within the bone, is closely related to cancer outgrowth within the bone (24). We found that CXCL10 promotes adhesion of B16F10 cells to fibronectin and type I collagen (Supplementary Fig. S2A). Furthermore, CXCL10 significantly enhanced survival of B16F10 cells in the serum-free condition but did not affect cell proliferation under the normal (10%) and low (0.1%) serum

(Fig. 5B). Prevention of direct contact between B16F10 cells and BMMs by membrane filters abrogated the augmentation of CXCL10 release (Fig. 5C).
condition (Supplementary Fig. S2B). These results prompted us to investigate the possibility that the augmented CXCL10 production during bone metastasis provides a growth advantage for B16F10 cells within the bone. B16-FL cells were inoculated directly into the femoral cavity of WT and Cxcl10−/− mice and the progression of bone metastasis was monitored by BLI analysis. There was no significant difference in bone tumor burden between WT and Cxcl10−/− mice on day 7. On day 14, however, tumor burden was significantly lower in Cxcl10−/− mice than in WT mice (Fig. 6A and B). The reduction of tumor burden in Cxcl10−/− mice was confirmed by histomorphometric analysis (Fig. 6C) and was accompanied by a marked decrease in osteoclast number and bone destruction (Fig. 6D and E). Notably, Ki67 staining revealed a significant decrease in the number of proliferating tumor cells in Cxcl10−/− mice (Fig. 6F). In contrast, there was no significant difference in tumor angiogenesis between WT and Cxcl10−/− mice as determined by CD31 staining (Fig. 6G).

CXCL10 stimulates osteoclast differentiation

Because CXCL10 deficiency dramatically reduced osteoclast number induced by bone metastasis of B16F10 cells, we next examined whether CXCL10 stimulates osteoclast differentiation. CXCL10 did not impact RANKL-induced osteoclast differentiation from its precursors, BMMs. However, CXCL10 induced osteoclast formation in coculture of osteoblasts and BMCs in a dose-dependent manner (Fig. 7A and B). We found that CXCL10 increases RANKL mRNA and protein levels in osteoblasts in a dose-dependent manner with no significant change in OPG levels (Fig. 7C and D). Interestingly, CXCR3 knockdown had no effect on CXCL10-induced RANKL mRNA expression in osteoblasts. In contrast, CXCR3 knockdown impaired the slight increase in RANKL mRNA expression in response to 2 other CXCR3 agonists, CXCL9 and CXCL11 (Fig. 7E). Next, we sought to identify the CXCL10 receptor that mediates RANKL induction in osteoblasts. LPS, a TLR4 ligand, increases RANKL expression in osteoblasts (25), and CXCL10 has been shown to activate TLR4 signaling by binding to TLR4 (26). Indeed, CXCL10-induced RANKL mRNA expression was impaired in Tlr4−/− osteoblasts (Fig. 7F). In addition, CXCL10-stimulated osteoclast formation was dramatically reduced in the coculture of WT BMCs and Tlr4−/− osteoblasts (Fig. 7G).

We then studied the molecular mechanism underlying TLR4-mediated RANKL expression in response to CXCL10. Treatment of WT osteoblasts with CXCL10 increased ERK and AKT phosphorylation within 5 minutes, which was sustained for at least 1 hour. In contrast, these increases were not observed in Tlr4−/− osteoblasts (Fig. 7H). TLR4-mediated activation of ERK and AKT was linked to CXCL10-induced RANKL expression. Pretreatment of WT osteoblasts with either the ERK kinase inhibitor (PD98059) or the AKT inhibitor (AKT inhibitor VIII) blocked CXCL10-induced RANKL expression (Fig. 7I).

Discussion

Evidence is accumulating for a positive role of CXCR3 expression on cancer cells in organ-specific metastasis (10–12, 15, 16). However, CXCR3 ligands including CXCL10 have exhibited opposing effects on cancer cells and on stromal cells in the tumor microenvironment. CXCL10 has been shown to increase migration, invasion, proliferation, survival, and adhesion to ECM of diverse cancer cells (12, 13, 21, 22). In contrast to
the direct effects on cancer cells, it has also been reported that CXCL10 exerts antitumor activities by inducing immune-stimulating and angiostatic effects (27–30). However, the role of endogenous CXCL10 in the development of primary cancers and their metastatic foci has not been addressed. Here we show that host-derived CXCL10 plays an important role in osteolytic bone metastasis by promoting cancer outgrowth and osteoclast differentiation in bone as well as by trafficking CXCR3-expressing cancer cells to bone.

In this study, CXCR3 gene silencing in cancer cells inhibited bone metastasis by intracardiac injection of cancer cells. Among the CXCR3 ligands, CXCL10 is likely to be a major contributor to the effects of CXCR3 gene silencing on bone metastasis, because CXCL10 expression is much higher than that of the other CXCR3 ligands, CXCL9 and CXCL11, in bone tissues. Our finding of RANKL-induced CXCL10 production may provide a possible explanation for the high expression of CXCL10 in bone tissues. Loss of CXCL10 by neutralizing antibody or genetic knockout also suppressed bone metastasis by intracardiac injection of cancer cells. These observations collectively indicate that CXCL10 plays an important role in bone metastasis, at least in part, by directing CXCR3-expressing cancer cells to the bone. In addition to bone metastasis, the CXCL10/CXCR3 axis may also be implicated in cancer metastasis to other organs such as lung. In our study, CXCL10 neutralization reduced the number of lesions and tumor size.
only in bones but also in lungs and adrenal glands, and CXCR3 gene silencing and a pharmacologic CXCR3 antagonist were shown to inhibit lung metastases of breast cancer cells and colon cancer cells (11, 15, 16).

Notably, CXCL10 production in bone was augmented by bone colonization of cancer cells. In accordance with the importance of host-derived CXCL10 in bone metastasis, cancer cells stimulated CXCL10 production from macrophages in a cell–cell contact manner. Tumor-associated macrophages are the major leukocyte population present in tumors and provide many trophic factors that promote tumor progression and metastasis (31). In addition, macrophages have been shown to transmit survival signals in breast cancer cells during lung metastasis (32) and provide prostate cancer cells with resistance to selective androgen receptor antagonists/modulators (33) through direct interaction with cancer cells. Also,
Macrophages mainly produce TNF-α in tumor microenvironment (34). TNF-α was shown to increase CXCL10 production in bone marrow-derived mesenchymal stem cells (21). We also observed that TNF-α increases CXCL10 expression in osteoblasts and in macrophages (data not shown). These observations suggest that macrophages can contribute to the augmented production of CXCL10 by bone metastasis via direct interaction with cancer cells as well as via TNF-α production.

Our findings may thus provide new insight into the role of macrophages in bone metastasis.

The CXCL10 augmentation by bone metastasis raises the possibility that CXCL10 might do much more than simply recruit CXCR3-expressing cancer cells to bone. Indeed, it has been reported that when colon cancer cells are inoculated in the rectum of nude mice, forced expression of CXCR3 on cancer cells does not affect the initial dissemination of cancer cells to lymph nodes but facilitates expansion of cancer cells in lymph nodes (12). In line with these findings, we found that the augmented production of CXCL10 is required for cancer outgrowth within the bone. It has been suggested that osteoclastic bone resorption facilitates successful outgrowth of breast cancer cells in bone marrow by providing growth factors such as transforming growth factor-β stored in bone matrix (3).

However, it is worth noting that osteoclast-defective Src−/− mice are resistant to melanoma-induced bone destruction but not melanoma metastasis to bone after intracardiac injection of B16F10 melanoma (35). Those findings suggest that functional osteoclasts or osteoclastic bone resorption is not required for cancer entry into and growth in bone marrow in case of B16F10 melanoma. Therefore, the reduced cancer outgrowth observed in Cxcl10−/− mice is unlikely because of the decreased bone destruction. The success of solid tumor
growth is dependent on angiogenesis (36), and CXCL10 has been suggested to inhibit tumor angiogenesis (27, 28). However, host-deficiency of CXCL10 did not affect tumor angiogenesis by intrafemoral injection of B16F10 melanoma. Given the importance of CXCR3 expression in metastatic outgrowth in lymph nodes (12), it is likely that augmented CXCL10 facilitates cancer outgrowth by directly acting on cancer cells.

It has been shown that bone metastatic prostate cancer cells preferentially adhere to type I collagen compared with prostate cancer cells that form only visceral metastases (24). In addition, selection for collagen adhesion confers outgrowth within the bone to prostate cancer (LNCaP) cells, which routinely fail to bind to collagen and do not have bone metastatic potential (24). Thus, our finding that CXCL10 stimulates adhesion of cancer cells to ECM including type I collagen might provide a possible mechanism underlying CXCL10-induced cancer outgrowth within the bone. Interestingly, it has been shown that blockage of CXCR3 on cancer cells inhibits lung colonization of murine mammary cancer cells, which is compromised in mice depleted of NK cells or with mutation in IFN-γ (11, 15). These previous observations imply that CXCR3 activation confers cancer cells resistance to the antitumor effects of NK cells and IFN-γ during cancer metastasis. Therefore, this might provide another possible mechanism for CXCL10-induced cancer outgrowth within the bone.

In this study, host deficiency of CXCL10 markedly decreased bone metastasis-induced osteoblastic bone destruction. Our in vitro study revealed that CXCL10 stimulates osteoclast formation in coculture of osteoblasts and BMCs by inducing RANKL expression in osteoblasts, which is reminiscent of our previous observations about the role of CXCL10 in osteoblast differentiation in coculture of CD4+ T cells and BMCs (18). Here we additionally found that CXCL10 increases RANKL expression through TLR4-mediated activation of ERK and AKT in osteoblasts. These results suggest that CXCL10 mediates bone metastasis-induced osteoclastic bone destruction through stimulating RANKL expression.

In summary, our findings show that CXCL10 facilitates trafficking of CXCR3-expressing cancer cells to bone, which augments its own production in bone and then promotes cancer outgrowth and osteoclastic differentiation. These results underscore the importance of CXCL10 in mediating osteolytic bone metastasis and suggest that targeting CXCL10 may provide a novel therapeutic opportunity for osteolytic bone metastasis.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors’ Contributions

Conception and design: J.-H. Lee, H. Ha, Z.H. Lee
Development of methodology: H. Ha
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): J.-H. Lee, W.-J. Jin, H. Ha
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): J.-H. Lee, H.-N. Kim, K.-O. Kim, H. Ha, Z.H. Lee
Writing, review, and/or revision of the manuscript: J.-H. Lee, K.-O. Kim, S. Lee, H.-H. Kim, H. Ha, Z.H. Lee
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): K.-O. Kim, H. Ha
Study supervision: J.-H. Lee, H. Ha, Z.H. Lee

Grant Support

This work was supported by the National Research Foundation of Korea (NRF, Grant No. 2010-0015131) and a Science Research Center grant through the Bone Metabolism Research Center funded by the Korea government (MEST, Grant No. 2011-0001023). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received February 7, 2012; revised April 16, 2012; accepted April 30, 2012; published OnlineFirst May 4, 2012.

References


34. Miles DW, Happerfield LC, Naylor MS, Bobrow LG, Rubens RD, Balmwill FR. Expression of tumour necrosis factor (TNF alpha) and its receptors in benign and malignant breast tissue. Int J Cancer 1994;56:777–82.


CXCL10 Promotes Osteolytic Bone Metastasis by Enhancing Cancer Outgrowth and Osteoclastogenesis

Jong-Ho Lee, Ha-Neui Kim, Kyung-Ok Kim, et al.


Updated version
Access the most recent version of this article at:
doi: 10.1158/0008-5472.CAN-12-0481

Supplementary Material
Access the most recent supplemental material at:
http://cancerres.aacrjournals.org/content/suppl/2012/05/04/0008-5472.CAN-12-0481.DC1

Cited articles
This article cites 36 articles, 17 of which you can access for free at:
http://cancerres.aacrjournals.org/content/72/13/3175.full.html#ref-list-1

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.