Loss of TGF-β Responsiveness in Prostate Stromal Cells Alters Chemokine Levels and Facilitates the Development of Mixed Osteoblastic/Osteolytic Bone Lesions

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Abstract

Loss of TGF-β type II receptor (TβRII, encoded by Tgfb2) expression in the prostate stroma contributes to prostate cancer initiation, progression, and invasion. We evaluated whether TβRII loss also affected prostate cancer bone metastatic growth. Immunohistologic analysis revealed that TβRII expression was lost in cancer-associated fibroblasts in human prostate cancer bone metastatic tissues. We recapitulated the human situation with a conditional stromal Tgfb2 knockout (Tgfb2-KO) mouse model. Conditioned media from primary cultured Tgfb2-KO or control Tgfb2-flox prostatic fibroblasts (koPFCM or wtPFCM, respectively) were applied to C4-2B prostate cancer cells before grafting the cells Tibially. We found that koPFCM promoted prostate cancer cell growth in the bone and development of early mixed osteoblastic/osteolytic bone lesions. Furthermore, the koPFCM promoted greater C4-2B adhesion to type-I collagen, the major component of bone matrix, compared to wtPFCM-treated C4-2B. Cytokine antibody array analysis revealed that koPFCM had more than two-fold elevation in granulocyte colony-stimulating factor and CXCL1, CXCL16, and CXCL5 expression relative to wtPFCM. Interestingly, neutralizing antibodies of CXCL16 or CXCL1 were able to reduce koPFCM-associated C4-2B type-I collagen adhesion to that comparable with wtPFCM-mediated adhesion. Collectively, our data indicate that loss of TGF-β responsiveness in prostatic fibroblasts results in upregulation of CXCL16 and CXCL1 and that these paracrine signals increase prostate cancer cell adhesion in the bone matrix. These microenvironment changes at the primary tumor site can mediate early establishment of prostate cancer cells in the bone and support subsequent tumor development at the metastatic site. Mol Cancer Res; 1–10. ©2012 AACR.

Introduction

Prostate cancer is the most commonly diagnosed cancer and the second leading cause of cancer death in men in the United States. Unfortunately, about 70% of patients with advanced prostate cancer die with bone metastases that are resistant to conventional therapies (1). Bone lesions due to cancer metastases can be classified as osteolytic, osteoblastic, or a mixture of lytic and blastic; prostate cancer in particular causes mainly osteoblastic bone lesions with excessive abnormal bone formation in the bone marrow space (2, 3). Bone metastases can cause a number of skeletal complications, including severe pain, pathologic fracture, spinal cord compression, hypercalcemia, impaired mobility, and bone marrow failure (4). These complications severely impact the quality of life of patients, and researchers have aimed to understand, and ultimately prevent, metastasis from the primary tumor site. Bone metastatic studies have generally focused on examining the interactions of cancer cells with the bone microenvironment due to a lack of appropriate animal models and cells that recapitulate the whole process of metastasis from the orthotopic site. Recently, it has been appreciated that the primary tumor microenvironment not only provides fertile soil for cancer growth but also exerts dominant influences that trigger changes in cancer cells, conferring their selective growth and survival in metastatic sites (5–7).

In prostate cancer and other cancers, TGF-β is one of the key regulators in both the primary and bone microenvironments (8–11). In osteolytic cancer bone metastasis, TGF-β promotes Gli2-induced expression of parathyroid hormone–related protein, destroying the bone, which in turn releases
more TGF-β from the bone matrix—ultimately initiating a "vicious cycle" (12). However, for osteoblastic bone metastasis, the role of TGF-β is less understood. In this study, we investigated the role of TGF-β responsiveness of the primary tumor in prostate cancer osteoblastic bone metastasis. Elucidation of this mechanism may contribute to early prevention and intervention of prostate cancer bone metastasis. The conditional loss of Tgfbr2 (Tgfbr2fspKO) in prostatic stromal cells was previously found to contribute to prostate cancer initiation, progression, and invasion. Elevated Wnt, hepatocyte growth factor, and many other unidentified factors have been shown to be potentially important mediators for ablation of TGF-β signaling in the stromal compartment at the primary tumor site (9, 13–15). It is thought that promotion of invasion likely leads to bone metastasis, but the underlying mechanism of prostate cancer progression in bone is unknown. In this study, we reveal that loss of TGF-β responsiveness in the primary tumor microenvironment promoted C4-2B prostate cancer mixed bone lesion development and correlated with increased cell adhesion on bone matrix components that was mediated by KC (CXCL1) and CXCL16 cytokines.

Materials and Methods

Rapid autopsy

Human prostate cancer bone metastatic tissue sections from the University of Washington Prostate Cancer Rapid Autopsy Program (Seattle, WA) were used for histologic determination. Patients with terminal prostate cancer are accrued into the University of Washington Prostate Cancer Rapid Autopsy Program following informed consent (Institutional Review Board approval #39053). Briefly, within 2 to 3 hours following death (typically under hospice care), the patient’s body is transported to the University of Washington. The autopsy is begun immediately and is designed for the acquisition of metastatic foci.

Animals

Tgfrb2flox/loxE2 and Tgfrb2flspKO mice bred on the C57BL/6 background were generated and maintained as previously described (9). Adult male severe combined immunodeficient (SCID) mice and C57BL/6 mice were purchased from Harlan. All animal procedures used in this study were approved by the Vanderbilt Institutional Animal Care and Use Committee (Nashville, TN).

Cell culture and cytokine array analysis

Primary mouse prostate stromal cell cultures were generated from 6- to 8-week-old Tgfrb2flox/loxE2 and Tgfrb2flspKO mice, and conditioned media samples were collected from the cultures, as described previously (14). Prostate stromal fibroblastic cells derived from Tgfrb2flox/loxE2 and Tgfrb2flspKO mice are termed wtPFs and koPFs, respectively. The conditioned media samples from the cultures (wtPFCM and koPFCM, respectively) were analyzed with the Mouse Cytokine Antibody Membrane Array 3 System (RayBiotech, Inc.). Briefly, pooled conditioned media samples from 3 different wtPF or koPF cultures were adjusted with fresh media to contain an equal amount of protein. One milliliter of wtPFCM or koPFCM was added to each membrane. Quantification of the arrays was conducted using ImageJ program and area of interest analysis.

The prostate cancer cell line C4-2B was a generous gift from Dr. Leland Chung (Samuel Oschin Comprehensive Cancer Institute and Department of Medicine, Cedars Sinai Medical Center, Los Angeles, CA). C4-2B cells labeled with GFP were generated by lentivirus transduction of parental C4-2B cells with Em-GFP (Invitrogen) according to the manufacturer’s instructions.

Intratibial injection and radiographic imaging

C4-2B cells were incubated with or without conditioned media from primary cultured wtPFs or koPFs for 48 hours. In the experiments with neutralizing antibodies, either CXCL1 or CXCL16 antibody was added to the koPFCM before incubation. Subsequently, C4-2B cells were injected (1 × 10^6 cells per mouse) into the left tibiae of host SCID mice. For control, PBS was sham-injected into the right tibia of each host mouse.

Mice were radiographically imaged using a Faxitron LX-60 (Faxitron Biopics) weekly beginning 3 weeks after intratibial injection. The lytic, mixed bone lesions were scored in a blinded manner. The number and area of lesions were measured using MetaMorph, a quantitative image analysis software program (Molecular Devices, Inc.) and region of interest analysis.

Cell adhesion assay

Concentrated rat-tail type-I collagen in glacial acetic acid was diluted to 500 μg/mL in PBS and coated onto 96-well microplates (Nunc) overnight at 4°C. Microplates were washed twice with serum-free RPMI-1640, blocked with 3% (w/v) bovine serum albumin for 1 hour at 37°C, and again washed twice with serum-free RPMI-1640. GFP-labeled C4-2B cells were incubated with/without wtPFCM or koPFCM for 48 hours. In indicated experiments, neutralizing antibodies of the cytokines found to be upregulated in the koPFCM [CXCL1, CXCL5, CXCL16, and granulocyte colony-stimulating factor (G-CSF)] or their respective IgG controls were added into the koPFCM before incubation. The neutralizing antibodies were all purchased from R&D systems, Inc. (Minneapolis, MN). Each antibody is provided with neutralization dose50 (%) (ND50). After incubation, the C4-2B cells were subcultured, plated on the collagen-coated microplates at 5 × 10^4 cells per 100 μL, and incubated in the plates for 1 hour at 37°C. The microplates were then washed with PBS to remove nonadherent cells, fixed, and stained with crystal violet. Cell adhesion was quantified by measuring the absorbance of the stained cells in each well at 595 nm.

Histology and immunohistochemistry

Mice tibiae were removed at each time point and fixed in 10% neutral-buffered formalin (Fisher Scientific) for 48 hours at room temperature. Bone specimens were then decalcified in 10% EDTA for 2 weeks at 4°C and embedded in paraffin.
Bone sections (5-µm thickness) were stained with hematoxylin & eosin (H&E), orange G, and phloxine.

Immunohistochemical staining of paraffin-embedded tissue sections (5-µm) was carried out using a primary antibody against phosphorylated-histone H3 (p-HH3; 1:1,000; Millipore).

Statistical analysis
The Fisher exact test was used to analyze TβRII expression in prostate cancer bone metastatic tissues. Fisher exact test or logistic regression was applied to the analysis of the incidence of mixed bone lesions in host SCID mice tibiae among the different groups. Mixed-effect model was used to examine the differences in the average mixed bone lesion areas among the mice groups. One-way ANOVA with Bonferroni adjustment was applied to examine the phosphorylated-histone H3 staining among the 3 different C4-2B groups. Pair-wise t-tests with Bonferroni adjusted P-values were applied in the analysis of cell adhesion. All statistical analyses were carried out using R software (16).

Results
TβRII is lost in the stroma of primary and secondary prostate adenocarcinomas
Cancer-associated fibroblasts (CAF) were shown to promote tumor cell growth, progression, and metastasis in various cancer types, including prostate cancer (8, 17, 18). We previously reported a loss of TβRII in CAFs in nearly 70% of human prostate cancer tissues (14). To determine whether similar changes occur in the bone marrow microenvironment after prostate cancer cells metastasize, we conducted immunohistochemistry (IHC) for α-smooth muscle actin (α-SMA; a marker of myofibroblastic cells), androgen receptor (a marker of androgen-responsive stromal and epithelial cells), and TβRII in human bone tissue both associated with and not associated with prostate cancer bone metastasis (Fig. 1). Neither α-SMA nor androgen receptor expression was detected in the bone marrow, but TβRII was highly expressed in marrow cells of naive bone tissues. However, in the bone tissues associated with prostate cancer, α-SMA was detected in CAFs. Androgen receptor was detected in prostate cancer cells and CAFs, indicating that the prostate cancer cells exert a strong influence on cells within the bone microenvironment. Similar to prostate cancer at the primary site, TβRII expression was detected in cancer epithelial cells but was lost in CAFs in 7 of 9 bone metastatic tissues examined (P < 0.005; Fisher exact test). The loss of TβRII in the stromal compartment of prostate cancer is therefore a characteristic of both primary prostate cancer and bone metastatic sites. These results support the notion that communication between tumor cells and the surrounding microenvironment can significantly affect their behavior.

Figure 1. Expression of α-SMA and androgen receptor (AR) with a loss of TβRII was observed in human prostate cancer (PCa) bone metastatic tissues. In the bone tissues without prostate cancer cells, expression of α-SMA and AR were not detectable by IHC, but TβRII was expressed in all cell types of the bone microenvironment. In prostate cancer bone metastatic tissues, α-SMA was in the stromal cells, and AR was detected in both cancer and stromal cells, as shown with arrows. TβRII was expressed in cancer epithelial cells, but loss of expression was observed in CAFs in 7 of 9 bone metastatic tissues examined (N = 9 different patients; P < 0.005; shown with arrows). Scale bar, 100 µm.
alter the expression patterns of key signaling molecules and change the cellular phenotype.

Tgfbr2fspKO mouse prostate fibroblasts promote early mixed bone lesion development in a tibial injection model

To determine whether the loss of TβRII in CAFs at the primary site influenced the ability of the prostate cancer cells to establish secondary lesions within the bone, we used a mouse model involving tibial injection of human C4-2B prostate cancer cells which had been exposed to conditioned media of prostatic fibroblasts isolated from Tgfbr2floxE2/foxE2 or Tgfbr2fspKO mice (wtPFs and koPFs, respectively) for 48 hours. The injection of C4-2B cells into the tibiae of SCID mice produced mixed osteolytic and osteoblastic bone lesions that were detectable after 4 weeks and resembled lesions found in human prostate cancer that had metastasized to bone in previous studies (Fig. 2; refs. 19–21). Mixed bone lesions were observed in all 5 mice injected with C4-2B cells that had been incubated with koPFCM (C4-2B/koPFCM). In contrast, only osteolytic lesions were observed in one mouse in the control C4-2B group (C4-2B), and in 2 mice injected with C4-2B cells that were incubated with wtPFCM (C4-2B/wtPFCM; Fig. 2). All other mice in these 2 groups had no detectable bone lesions by X-ray. Tibially injected mice were imaged by Faxitron weekly from 5 to 10 weeks postinjection. At 5 weeks, mixed bone lesions were detected in all 6 mice in the group injected with C4-2B cells incubated in koPFCM, but only in one mouse in the group injected with untreated C4-2B cells, as well as in 2 mice in the group injected with C4-2B incubated in wtPFCM (Fig. 2). Thus, the incidence of mixed bone lesion detection was significantly higher in the C4-2B/koPFCM group than in both the C4-2B and C4-2B/wtPFCM groups (P = 0.001 and P < 0.05, respectively) at 4 and 5 weeks. By 6 to 10 weeks, however, lesions were detectable in almost all mice and no statistically significant differences were observed between the groups as shown in Table 1. Bone lesion areas detectable at 5 to 10 weeks were measured with MetaMorph software. The average mixed lesion areas in the C4-2B/koPFCM group were consistently larger than those of the other 2 groups, but statistical analysis showed a significant difference only at 5 weeks (P < 0.05; Fig. 2). The histology of the injected tibiae confirmed the apparent tumor growth of the experimental groups (Fig. 3). IHC of phosphorylated-histone H3 indicated 1.7- and 2.2-fold increase in mitotic cells in the C4-2B/koPFCM group at 4 weeks compared with C4-2B/wtPFCM and C4-2B groups, respectively (Fig. 3A). The positively stained cells were counted from 6 independent fields of each group. ANOVA analysis identified a significant difference between the C4-2B/koPFCM group and the C4-2B/wtPFCM or C4-2B group (P < 0.001; Fig. 3A). At 10 weeks postinjection, the tumor size in the bone was similar among the 3 groups, as revealed by optical imaging of GFP-labeled C4-2B cells obtained postmortem (Supplementary Fig. S1B). Histologic analysis of the tibiae also revealed no difference at later time points (Supplementary Fig. S1C and S1D). Taken together, these findings indicate that ablation of TGF-β responsiveness in prostate stromal cells resulted in an early paracrine effect, which promoted the establishment and growth of C4-2B cells in the tibiae and the development of mixed bone lesions.

Tgfbr2fspKO mouse prostate fibroblasts secrete cytokines and promote cancer cell adhesion to collagen

Antibody-based cytokine arrays were conducted to identify secreted factors in the koPFCM that may have been
involved in stimulating C4-2B cells to grow in the bone microenvironment. Figure 4 shows the expression of 62 cytokines in both koPFCM and wtPFCM, CXCL16, G-CSF, CXCL1, and CXCL5 were expressed more than 2-fold higher in koPFCM than in wtPFCM, and the differences were significant between koPFCM and wtPFCM (Table 2). Most prominently, CXCL1 (KC) was expressed to nearly 10-fold greater in koPFCM than in wtPFCM. (Full array quantitation can be found in Supplementary Table S1.)

A contribution of koPFCM compared with wtPFCM in bone lesion establishment of exposed C4-2B cells may be promoting C4-2B cells to adhere to bone. Indeed, we found that koPFCM significantly promoted the adhesion of C4-2B cells to type-I collagen—the major component of bone matrix—relative to adhesion of untreated cells or those incubated with wtPFCM (Supplementary Fig. S2). The organic component of the bone extracellular matrix consists of approximately 95% type-I collagen (22). Previous studies showed that collagen I serves as a major adhesive substrate for the retention of various metastatic cells, including prostate cancer cells, in skeletal tissues (23–25). To further investigate whether the cytokines that were differentially expressed

### Table 1. Lists of the incidence of mixed bone lesions in different groups of mice as shown in Fig. 2

<table>
<thead>
<tr>
<th></th>
<th>4 wk</th>
<th>5 wk</th>
<th>6–10 wk</th>
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<tbody>
<tr>
<td></td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>C4-2B</td>
<td>0</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>C4-2B/wtPFCM</td>
<td>0</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>C4-2B/koPFCM</td>
<td>5</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td><em>P</em></td>
<td>0.001</td>
<td>0.024</td>
<td>0.451</td>
</tr>
</tbody>
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Figure 3. Histologic analysis of tumor-bearing mouse tibiae. A, IHC of phosphorylated-histone H3 in tissues indicated more mitotic cells in the C4-2B/koPFCM group at 4 weeks. The inserted numbers represent the average count ± SD of 6 different fields of view for each group. B, H&E staining of the tumor-injected mice tibiae. Scale bar, 100 μm.
in koPFCM and wtPFCM play a role in promoting adhesion to collagen I, we incubated C4-2B cells with koPFCM containing neutralizing antibodies against CXCL16, G-CSF, CXCL1, or CXCL5. After normalization to their respective IgG controls, the elevated adhesion of C4-2B cells on collagen mediated by koPFCM was reduced significantly by neutralizing antibodies to CXCL16 and CXCL1 (P < 0.0001 and P < 0.05, respectively) but not by those to G-CSF or CXCL5 (Fig. 5).

We carried out an in vivo experiment after incubating C4-2B cells for 48 hours with koPFCM with or without neutralizing antibodies to CXCL1 or CXCL16 (or an IgG control). The mixed bone lesion development induced by koPFCM was reduced by the addition of CXCL16 antibodies to the level observed with wtPFCM, and, although not statistically significant at the classically set 95% confidence level, it was statistically significant at the 90% confidence level. Neutralizing antibodies to CXCL1 had a modest effect that reached statistical significance only when evaluated at the approximately 80% confidence level (Fig. 6 and table 3).

### Table 2. Lists of cytokines from the cytokine array analysis with an intensity ratio (koPFCM/wtPFCM) of more than 2 that was significantly different for Fig. 4

<table>
<thead>
<tr>
<th>Position</th>
<th>Cytokines</th>
<th>Ratio (ko/wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>d3, d4</td>
<td>G-CSF</td>
<td>8.7</td>
</tr>
<tr>
<td>j5, j6</td>
<td>KC (CXCL1)</td>
<td>10.7</td>
</tr>
<tr>
<td>m1, m2</td>
<td>CXCL16</td>
<td>3.3</td>
</tr>
<tr>
<td>m5, m6</td>
<td>LIX (CXCL5)</td>
<td>2.3</td>
</tr>
</tbody>
</table>

**Discussion**

In this study, we showed that TGF-β signaling in prostatic fibroblasts contributed to subsequent prostate cancer bone metastasis through paracrine interactions. Conditioned medium prepared from koPFs promoted the early development and growth of prostate cancer mixed bone lesions in the tibiae of mice. This effect was, in part, mediated by elevated expression of cytokines CXCL16 and CXCL1 present in the koPFCM, which stimulated prostate cancer cell adhesion to the bone matrix and induced proliferation at an early stage.

It is well established that stromal TGF-β signaling is important for prostate tissue development as well as prostate cancer initiation and progression (9, 26–29). TGF-β is abundant in the bone matrix, and that the release of TGF-β from the matrix is a critical component for the "vicious cycle" of osteoclastic bone metastasis (12). Inhibition of TGF-β with small-molecule inhibitors or neutralizing antibodies has previously been shown to decrease osteolytic bone lesion development and reduce tumor burden in both breast cancer and melanoma tibial injection models (30, 31). In contrast, patients with advanced prostate cancer often develop osteoblastic bone metastasis. Both primary prostate cancer CAFs and bone stroma have been known to induce prostate cancer growth and confer castration resistance and metastatic potential (5, 32–34). However, the role of TGF-β signaling in osteoblastic lesion development in bone remains largely unclear, further the effect of TGF-β signaling in the primary site on eventual prostate cancer bone metastasis is unknown.

One finding in this study that links primary growth and bone metastasis in prostate cancer is the parallel expression of α-SMA and androgen receptor with the loss of stromal TβRII expression both in the primary prostate cancer site and the prostate cancer cells in the bone (Fig. 1). Our previous studies suggested that the loss of TGF-β sensitivity...
in prostatic fibroblasts promote human LNCaP cell proliferation and invasion in a tissue recombination xenograft model 

(13, 14). In the present study, we wanted to further investigate whether the Tgfbr2-KO fibroblasts also contribute to osteoblastic bone lesion development. Interestingly, although the cell origin of CAFs of prostate cancer in the bone is unknown, they may arise from resident fibroblasts, immune cells, pericytes, or endothelial cells in the bone marrow (35–37). There is no evidence for CAFs from the primary site metastasizing with prostate cancer cells to the bone. Yet, as a clear example of stromal coevolution, we observed phenotypic changes in the bone marrow stroma associated with prostate cancer, same as found at the primary site (e.g., gain of α-SMA expression and loss of Tgfbr2 expression). However, before tumor establishment in the secondary bone site, we hypothesized that the paracrine effect of the microenvironment in the primary tumor site would influence prostate cancer growth at the secondary site. The primary tumor microenvironment may also support the predisposition of prostate cancer to predominantly metastasize to the bone. Indeed, upon incubating prostate cancer cells with conditioned media from the different prostatic fibroblasts, we found that prostate cancer mixed bone lesion development was promoted in the C4-2B/koPFCM group compared with the C4-2B/wtPFCM and C4-2B groups by 4 and 5 weeks postinjection (Figs. 2 and 3). Although lesion development was equal at 5+ weeks, the different rates of bone lesion development in this experiment could be used to further understand the biology of tumor dormancy and metastatic colonization. To our knowledge, this is the first in vivo evidence that showed that the primary prostate cancer microenvironment could influence osteoblastic bone metastasis. Although intratibial injection cannot fully address the complete metastatic process, we are using this model to depict the latter steps in bone lesion development. Models that reliably develop spontaneous mixed or osteoblastic metastatic lesions are currently not available. The development of prostate cancer cell lines that can metastasize from the orthotopic site to the bone and elicit osteoblastic bone lesions will be critical for overcoming this limitation in bone metastasis research.

This is the first in vivo evidence that TGF-β signaling in the primary microenvironment may affect prostate cancer osteoblastic bone lesion development. We reasoned that the effect of koPFCM on C4-2B cells may be mediated through promotion of cell proliferation, inhibition of apoptosis, or induction of cell adhesion to the bone microenvironment. However, we found no significant increase in cell proliferation or reduction in apoptosis in C4-2B cells after they were incubated with koPFCM, relative to cell behavior after incubation with either wtPFCM or without conditioned media in vitro (data not shown). Our previous studies using tissue recombination xenograft model revealed that koPF promotes LNCaP cell proliferation and invasion. Increased Wnt ligand expression, particularly Wnt3a by the koPF, accounts for the increased proliferation of LNCaP (13, 14). Although LNCaP are the parental cells of C4-2B, they are genotypically and phenotypically different (19, 38). The present study shows that the differences between C4-2B cells and the parental LNCaP cells extend to their response to koPFCM-containing factors in terms of their ability to grow in the bone and at the primary tumor site, respectively. Promotion of C4-2B cell adhesion to the bone matrix was the mechanism by which koPFCM induced mixed bone lesion development and that the secreted factors in the koPFCM that played a role in cell adhesion to bone matrix were the cytokines CXCL1 or CXCL16. Although the in vitro assay showed a significant increase of C4-2B cell adhesion on collagen after incubation with wtPFCM (Fig. 5), there was no significant increase of bone lesion development of C4-2B/wtPFCM in vivo (Fig. 2). The binding effect in vitro...
could be due to the type-I collagen used in the assay, although as the major component of the bone matrix, it does not fully represent the complexity of bone microenvironment in vivo. Currently, this is the best available in vitro assay to quantify this step in metastasis.

There is increasing evidence that chemokines and their receptors play a key role in cancer metastasis. In particular, CXCL1, CXCL5, and CXCL16 have all been shown to promote prostate cancer tumorigenesis and metastasis in prostate cancer cell line models (39–41). CXCL1 is also named CXCL8 or interleukin 8 (IL8) in human. Increased expression of CXCL1 was consistently observed in cancer and the microenvironment of multiple cancers, correlating with advanced stages, metastases, and poor clinical outcomes (42–44). There is considerable evidence from studies on patient samples supporting the role of CXCL1/CXCL8/IL8 in promoting prostate cancer progression (40, 45, 46). Human prostate CAFs have increased level of CXCL1/ CXCL8/IL8 by quantitative reverse transcriptase-PCR (47, 48). By IHC, CXCL1/CXCL8/IL8 and their receptors have shown to be increased in epithelia and neuroendocrine compartments of the prostate cancer in patient samples (49, 50). CXCL16 and CXCL5 are also reportedly associated with human prostate cancer progression and metastasis (41, 51). Our present study suggested an additional mechanism whereby these cytokines induce adhesion of prostate cancer cells to the bone matrix to promote cancer metastasis.

Elevated proliferation of C4-2B cells in the C4-2B/koPFCM mice at an early time point in vivo, but not in vitro, suggests that this was not a direct effect but might be the result of more C4-2B cells in the bone. In this study, the expression levels of G-CSF, CXCL1, CXCL16, and CXCL5 were higher in the koPFCM than in the wtPFCM (Table 2). The increase of these cytokines was associated with increased prostate cancer proliferation in the bone at early stages in vivo and increased adhesion to type-I collagen in vitro. Neutralizing CXCL1 or CXCL16 in the koPFCM abolished the increased adhesion of C4-2B cells to collagen, which confirmed that the augmented cell adhesion was mediated by these cytokines. Ablation of these cytokines from the koPFCM also repressed koPFCM-promoted mixed bone lesion development from C4-2B cells in vivo.

Taken together, our results indicate that loss of TBRII expression occurred in the stromal compartments of human primary prostate cancer and prostate cancer bone metastatic tissues. Our findings suggest that the loss of stromal TGF-β responsiveness in the primary site promoted prostate cancer mixed bone metastasis, which was mediated through increased expression of cytokines CXCL1 and CXCL16 promoting prostate cancer cell adhesion to the bone matrix.
Disclosure of Potential Conflicts of Interest
No potential conflicts of interests were disclosed.

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