Parathyroid Hormone–Related Protein Drives a CD11b<sup>+</sup>Gr1<sup>+</sup> Cell–Mediated Positive Feedback Loop to Support Prostate Cancer Growth

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Abstract

In the tumor microenvironment, CD11b<sup>+</sup>Gr1<sup>+</sup> bone marrow-derived cells are a predominant source of protumorigenic factors such as matrix metalloproteinases (MMP), but how distal tumors regulate these cells in the bone marrow is unclear. Here we addressed the hypothesis that the parathyroid hormone–related protein (PTHrP) potentiates CD11b<sup>+</sup>Gr1<sup>+</sup> cells in the bone marrow of prostate tumor hosts. In two xenograft models of prostate cancer, levels of tumor-derived PTHrP correlated with CD11b<sup>+</sup>Gr1<sup>+</sup> cell recruitment and microvessel density in the tumor tissue, with evidence for mediation of CD11b<sup>+</sup>Gr1<sup>+</sup> cell–derived MMP-9 but not tumor-derived VEGF-A. CD11b<sup>+</sup>Gr1<sup>+</sup> cells isolated from mice with PTHrP-overexpressing tumors exhibited relatively increased proangiogenic potential, suggesting that prostate tumor–derived PTHrP potentiates this activity of CD11b<sup>+</sup>Gr1<sup>+</sup> cells. Administration of neutralizing PTHrP monoclonal antibody reduced CD11b<sup>+</sup>Gr1<sup>+</sup> cells and MMP-9 in the tumors. Mechanistic investigations in vivo revealed that PTHrP elevated Y418 phosphorylation levels in Src family kinases in CD11b<sup>+</sup>Gr1<sup>+</sup> cells via osteoblast-derived interleukin-6 and VEGF-A, thereby upregulating MMP-9. Taken together, our results showed that prostate cancer–derived PTHrP acts in the bone marrow to potentiate CD11b<sup>+</sup>Gr1<sup>+</sup> cells, which are recruited to tumor tissue where they contribute to tumor angiogenesis and growth. Cancer Res; 73(22); 6574–83. ©2013 AACR.

Introduction

The tumor microenvironment provides primary tumor cells to mix with multiple types of stroma such as endothelium, fibroblasts, and immune cells (1). Such heterogeneity of cell populations presents a major impediment for developing a cure for cancer. Increasing evidence shows that stromal cells in the tumor microenvironment not only occupy a significant fraction of the tumor bulk, but also play critical roles in proliferation, invasion, and/or metastasis of tumor cells (2). In this regard, bone is an essential partner for tumor progression, because bone marrow serves as the supplying organ for numerous critical cells in the tumor microenvironment (3, 4). However, it is unclear how tumor cells co-opt the bone and/or bone marrow to facilitate a favorable tumor microenvironment.

Among the bone marrow–derived cells, CD11b<sup>+</sup>Gr1<sup>+</sup> cells [commonly referred to as myeloid-derived suppressor cells (MDSC)] correlate with tumor progression (5). MDSCs were originally investigated for their roles in evasion of host immune surveillance, especially via suppression of T-cell–dependent antitumor immunity by production of arginase, reactive oxygen species, and inducible nitric oxide synthase (6). Subsequent studies demonstrated that MDSCs are increased in tumor-bearing mice and cancer patients, and infiltrate primary tumor tissue to promote angiogenesis by secreting matrix metalloproteinases (MMP), and also by direct incorporation into tumor endothelium (7, 8). More recently, MDSCs have been shown to play key roles in recovery after radiation therapy (9, 10) and antiangiogenic therapy (11).

In parallel, multiple mechanisms have been proposed to explain the increased recruitment of MDSCs in tumor tissue. Yang and colleagues demonstrated that CXC chemokine ligand (CXCL)-5/CXC receptor (CXCR)-2 and stromal-derived factor-1/CXCR-4 axes recruit circulating MDSCs to tumor tissue (12). More recently, expression of a single integrin (αβ1) promotes MDSC invasion into tumors via activation of phosphatidylinositol 3-kinase (PI3K; ref. 13). However, despite such clear evidence supporting the tumorigenic functions of MDSCs and also the potential mechanisms of recruitment to the tumor tissue, MDSCs are poorly understood about their regulation in the supplying organ (i.e., bone marrow) of the tumor host, and also their potential crosstalk with distant primary tumor cells.

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This study was designed to elucidate how CD11b+Gr1+ cells are regulated in the bone marrow of prostate tumor hosts, contributing to tumor growth and angiogenesis. Prostate cancer provides a unique perspective on this process because of its devastating mortality and morbidity associated with its preferential metastasis to the skeleton (14). Accordingly, prostate cancer cells secrete numerous bone-modulating cytokines, leading to osteoblastic/osteolytic reactions that facilitate growth factor and cytokine release from bone cells and matrix (15). In particular, parathyroid hormone–related protein (PTHrP) is expressed by prostate cancer cells, and stimulates osteoblasts in an endocrine manner to secrete factors such as receptor activator of NF-kB ligand (RANKL), interleukin (IL)-6, C-C chemokine ligand (CCL)-2, and VEGF-A within the bone microenvironment (16–18). Subsequently, PTHrP-induced cytokines have the ability to trigger cascades of unfavorable events (e.g., signaling pathways leading to potentiation of CD11b+Gr1+ bone marrow cells) within the bone marrow, contributing to tumor progression. Overall, the central hypothesis of this study was prostate cancer–derived PTHrP potentiates CD11b+Gr1+ cells within the bone marrow, contributing to angiogenesis and tumor growth.

Materials and Methods

Cells

Two luciferase-labeled PC-3 clones expressing high and low levels of PTHrP were selected from previously established stable-shRNA clones targeting PTHLH (19), designating PTHrP high and PTHrP low, respectively. Ace-1 canine prostate carcinoma cells, expressing undetectable basal levels of PTHrP, were stably transfected with a pcDNA3.1 vector expressing full-length mouse/rat PTHrP (17). An empty-vector transfectant was used as a control. Expression of PTHrP was confirmed from the culture supernatant using an immunoradiometric assay kit (Diagnostic Laboratories). PC-3 clones were regularly authenticated and matched short tandem repeat DNA profiles of the original PC-3 cell line (last tested on August 28, 2012).

Mice and in vivo tumors

All mouse experiments were approved by the Institutional Animal Care and Use Committees of the University of Michigan and Vanderbilt University. For in vivo tumors, 1 × 10⁶ prostate tumor cells were suspended in 100 μL Hank’s balanced salt solution and 1:1 mixed with growth factor–reduced Matrigel (BD Biosciences), followed by subcutaneous injection into male athymic mice (Harlan Laboratories) as previously described (20, 21). Mice were regularly monitored for morbidity or tumor growth, and tumor size was calculated using an equation: Volume = 1/2 × a × b², where a is the long diameter and b is the short diameter measured with a caliper (22). Anti-human PTHrP 1 to 33 monoclonal antibodies (hybridoma 158) were produced and generously gifted by Dr. R. Kremer (McGill University, Montreal, Quebec, Canada; ref. 18). Mice were treated with anti-PTHrP monoclonal antibody (200 μL) or mouse immunoglobulin G (IgG; Sigma-Aldrich) by every other day intraperitoneal injection for the first 3 weeks, followed by daily injection for 1 week before euthanasia.

Flow cytometry

For analyses of CD11b+Gr1+ cells in the tumor tissue, tumors were mechanically dissociated, followed by digestion in complete RPMI-1640 media supplemented with type I collagenase (5 mg/mL; Sigma-Aldrich). Viable cells were counted and resuspended in fluorescence-activated cell sorting buffer containing combinations of antibodies including FITC-conjugated anti-mouse CD11b, PE-conjugated anti-mouse Gr1, or isotype controls. For analyses or sorting CD11b+Gr1+ cells from the bone marrow, the femoral bone marrow was flushed and dissociated, followed by antibody staining and flow cytometry (23). For analyses of phospho-Y418 Src family kinase (SKF), the bone marrow cells were fixed, permeabilized, stained, and analyzed according to the BD PhosFlow Cell Signaling protocols. All materials were from BD Biosciences.

Immunohistochemistry

Tumors were surgically removed and bisected, a portion fixed in formalin and a portion snap-frozen. Murine endothelial cell–specific CD31/PECAM immunostaining (clone MEC13.3; BD Biosciences) was performed according to a previously described method (24). Rat anti-mouse CD11b (clone M1/70; BD Biosciences) and anti-mouse Ly-6G (clone RB6-8C5; eBioscience) were fluorescently labeled and used to detect CD11b+Gr1+ cells in the tumor tissue. Three to five randomly selected microscopic images per sample were obtained, and positively stained cells were counted using ImageJ software.

Quantitative PCR

mRNA samples were prepared from the bone marrow or tumor tissues using TRIzol reagent (Invitrogen), followed by reverse transcription-quantitative PCR (25). All quantitative PCR probes and reagents were from Applied Biosystems.

Statistical analyses

All in vivo data sets were tested for normality by Shapiro–Wilk test. Statistical analyses were performed by GraphPad Prism software. Student t test or Mann–Whitney U test were used to compare 2 groups and all statistical tests were 2-sided.

Results

Reduction of PTHrP in PC-3 prostate tumors decreased CD11b+Gr1+ bone marrow cell recruitment and angiogenesis

As a first approach to investigate the role of PTHrP in the potential crosstalk between tumor and the bone marrow, the PTHLH gene (encoding PTHrP) was targeted via lentiviral shRNA vectors in PC-3, human prostate cancer cells (19). Two clones expressing high and low levels of PTHrP (96.1 ± 12.8 vs. 45.8 ± 4.1 pg/mL; 1 × 10⁶ cells⁻¹ 48 h⁻¹; measured in the culture supernatant by immunoradiometric assays) were selected and designated PTHrP high and PTHrP low, respectively. PTHrP is well known to regulate tumor growth via autocrine, intracrine, and paracrine manners (17–19, 26, 27), hence alterations in the host response (e.g., recruitment of host-derived cells) could simply be secondary to the differences in...
the tumor size, not in PTHrP expression levels. Therefore, PTHrPLo tumors were grown for a longer period until they reached a similar mean tumor volume as PTHrPHi tumors (38 days). B, PTHrPHi and PTHrPLo tumors were surgically dissected on the same day and photographed. Mean tumor volume between the 2 groups was not significantly different (P = 0.68, Student t test). Scale bar, 1 cm. C, percentages of CD11b+Gr1+ double-positive cells in the tumor tissues were analyzed by flow cytometry. D, tumor tissues were sectioned for H&E and murine CD31/PECAM immunohistochemical staining. Original magnification, ×20. Scale bars, 50 μm. E and F, microscopic images were analyzed for tumor mean vessel density (MVD) or CD31+ vascular area with normalization to total nuclear area. G and H, host-derived (i.e., murine) Mmp9 and tumor-derived (i.e., human) VEGFA mRNA levels were measured by quantitative RT-PCR using species-specific probes (n = 9–10 per group). All P values are from Student t test. NS, not significant. Data in all graphs are mean ± SEM.

Figure 1. Reduction of PTHrP in PC-3 prostate tumors decreased CD11b+Gr1+ MDSC recruitment and angiogenesis. A, tumor growth curve of PC-3 clones expressing high or low levels of PTHrP (n = 9 for PTHrPHi and n = 10 for PTHrPLo). PTHrPHi tumors were grown for a longer period (57 days) to reach a similar mean tumor volume as PTHrPLo tumors (38 days). B, PTHrPHi and PTHrPLo tumors were surgically dissected on the same day and photographed. C, percentages of CD11b+Gr1+ double-positive cells in the tumor tissues were analyzed by flow cytometry. D, tumor tissues were sectioned for H&E and murine CD31/PECAM immunohistochemical staining. Original magnification, ×20. Scale bars, 50 μm. E and F, microscopic images were analyzed for tumor mean vessel density (MVD) or CD31+ vascular area with normalization to total nuclear area. G and H, host-derived (i.e., murine) Mmp9 and tumor-derived (i.e., human) VEGFA mRNA levels were measured by quantitative RT-PCR using species-specific probes (n = 9–10 per group). All P values are from Student t test. NS, not significant. Data in all graphs are mean ± SEM.

Ectopic PTHrP increased the recruitment of CD11b+Gr1+ cells in prostate tumor tissue

An additional prostate tumor model was utilized to establish the causal relationship between PTHrP and CD11b+Gr1+ cells. Ace-1 prostate cancer cells produce predominantly osteoblastic lesions in vivo, a phenotype that recapitulates human prostate cancer more realistically than the majority of currently available prostate cancer cell lines (17, 29). Ace-1 cells, expressing undetectable basal levels of PTHrP, were stably transfected with PTHrP overexpression (designated PTHrP OE) or empty control (designated pcDNA) vectors. In the same approach as the PC-3 tumor model (i.e., growth in differential periods), 2 groups of similarly sized tumors, PTHrP OE and pcDNA control, were produced. To directly examine the effects of systemic PTHrP on CD11b+Gr1+ cell recruitment, one group of mice carrying pcDNA control tumors was treated with recombinant PTHrP, whereas the 2nd group received recombinant PTHrP OE. The 2 groups received 7 days of recombinant PTHrP before harvest (Fig. 2A and Supplementary Fig. S1). Both PTHrP OE and recombinant PTHrP-treated groups had significantly increased CD11b+Gr1+ cells in the tumor tissue compared with pcDNA control tumors (Fig. 2B). Although mice burdened with PTHrP OE tumors had
significantly increased percentages of CD11b+Gr1+ cells in the bone marrow (Fig. 2C), recombinant PTHrP treatment failed to show such an increase in the bone marrow. This may be explained by either the different modes of PTHrP administration (i.e., intermittent injection vs. continuous expression) or the reduced duration (7 days) of PTHrP treatment compared with tumor burden (21 days). Immunohistochemical analyses of tumor tissue showed that both PTHrP OE and recombinant PTHrP tumors had significantly increased evidence of angiogenesis (Fig. 2D and Supplementary Fig. S1B). In addition, host-derived Mmp9 expression was significantly increased in the tumor tissues or in the bone marrow (Fig. 2E), potentially because increased MMP-9 and angiogenesis were associated with the altered recruitment of CD11b+Gr1+ cells, suggesting that altered tumor size in Figs. 1 and 2 were secondary to the altered recruitment of CD11b+Gr1+ cells in the tumor tissue.

Tumor-derived PTHrP confers increased angiogenic potential to CD11b+Gr1+ cells

To examine whether tumor-derived PTHrP regulates CD11b+Gr1+ cells within the bone marrow of tumor hosts, CD11b+Gr1+ bone marrow cells were isolated from 2 groups of mice bearing either PTHrP-overexpressing or pcDNA control tumors for 3 weeks, resulting in 2 fractions of CD11b+Gr1+ cells (i.e., PTHrP-activated vs. control). Parental Ace-1 tumor cells were mixed with the isolated CD11b+Gr1+ cells and xenografted into male athymic mice (Fig. 4A). Tumor cells coimplanted with PTHrP-activated CD11b+Gr1+ cells were significantly larger than the tumors with control CD11b+Gr1+ cells (Fig. 4B), potentially because increased MMP-9 and angiogenesis as determined by immunohistochemistry (Fig. 4C and D and Supplementary Fig. S2).

PTHrP increased expression of phospho-[Y418] Src family kinases in CD11b+Gr1+ cells

The molecular mechanism for the observed PTHrP-dependent CD11b+Gr1+ cell potentiation was subsequently investigated. Recently, Liang and colleagues demonstrated that dasatinib, an SFK inhibitor, suppressed prostate tumor growth as well as the numbers of CD11b+ myeloid cells in tumor tissues (32). Accordingly, the effects of PTHrP administration...
on SFK in CD11b^+Gr1^+ cells were investigated. A single administration of PTHrP (1–34) to male athymic mice significantly increased the activating phosphorylation of Tyr-418 residue of SFK in CD11b^+Gr1^+ cells (Fig. 5A). As SFK activation requires intramolecular conformational changes and interaction with activated receptor kinases via the SH-2 domain, phosphorylation of [Y418] in the SH-2 domain indicates the status of full activation. However, because CD11b^+Gr1^+ cells do not express receptors for PTHrP (as determined by quantitative reverse transcription (RT)-PCR for Pthr1; Supplementary Fig. S3), phosphorylation of [Y418] SFK was reasoned to be indirect through cytokines from osteoblasts, the predominant cells expressing the PTH/PTHrP receptor (PTH1R) in the bone marrow. Potential candidate cytokines from PTHrP-stimulated osteoblasts included IL-6, VEGF-A, C-C chemokine ligand (CCL)-2, and RANKL (16, 33–35). Therefore, CD11b^+Gr1^+ cells were isolated from femoral bone marrow and treated with these osteoblastic cytokines. Although all 4 cytokines (IL-6, VEGF-A, CCL-2, and RANKL) have been shown to upregulate SFKs (36–38), only IL-6 and VEGF-A increased the expression of phospho-[Y418] SFK in MDSCs (Fig. 5B).

**Phospho-[Y418] SFK by osteoblastic VEGF-A and IL-6 increased MMP-9 expression in CD11b^+Gr1^+ cells**

To further investigate the functional significance of phospho-[Y418] SFKs in MDSCs, several published markers of
CD11b\(^+\)Gr1\(^+\) cell activation were examined in combination with PTHrP-dependent osteoblastic cytokines and an SFK selective inhibitor, PP2 (5, 13, 28). Only VEGF-A and IL-6 increased Mmp9 gene expression, whereas Cxcr2, Cxcr4, or Itgb1 expression remained unaffected in CD11b\(^+\)Gr1\(^+\) cells, and this increase was reversed by PP2 treatment (Fig. 6A–D).

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Figure 5. PTHrP phosphorylated [Y418] Src family kinases in CD11b\(^+\)Gr1\(^+\) cells. A, male athymic mice (n = 3 per group) were stimulated with a single administration of PTHrP (1–34) or saline control, 8 hours before sacrifice and flow cytometric analyses of phospho-[Y418] Src family kinase expression levels in CD11b\(^+\)Gr1\(^+\) bone marrow cells. Representative histograms from the control group (shaded) and the PTHrP-stimulated group (open) were overlapped to show the intensity of phospho-[Y418] Src expression. CD11b\(^+\)Gr1\(^+\) cells expressing high levels of phospho-[Y418] Src family kinases (indicated by a bracket [M]) were quantified and plotted. Data are mean ± SEM. *P < 0.01, Student t test. B, CD11b/Gr1 double positive cells were sorted from the femoral bone marrow of male athymic mice, followed by treatment with saline, IL-6, VEGF-A, CCL-2, RANKL, or PTHrP (all 100 ng/mL for 0.5 × 10\(^6\) cells) for 1 hour at 37°C (n = 3 per group). Representative histograms (open) were overlapped onto unstimulated controls (shaded) to show the intensity of staining.

Figure 6. Phosphorylation of Src family kinases by osteoblastic VEGF-A and IL-6 increased MMP-9 expression in CD11b\(^+\)Gr1\(^+\) cells. A–D, CD11b\(^+\)Gr1\(^+\) cells were sorted from the femoral bone marrow of male athymic mice via flow cytometry, followed by treatment with saline (control), VEGF-A, IL-6, PTHrP, or CCL-2 (100 ng/mL for 0.5 × 10\(^6\) cells) in combination with PP2 (an SFK inhibitor; 100 nmol/L) or dimethyl sulfoxide (DMSO) control for 1 hour at 37°C (n = 3 per group). mRNA levels of Mmp9, Cxcr4, Cxcr2, and Itgb1 were determined via quantitative RT-PCR. Data are mean ± SEM. *P < 0.01 compared with the DMSO control group. No other combination had statistical significance (P > 0.05, Student t test). E–G, conditioned media were collected from primary calvarial osteoblasts stimulated with saline (control) or PTHrP (1–34). Femoral bone marrow CD11b\(^+\)Gr1\(^+\) cells were treated with the control or PTHrP-conditioned media in combination with neutralizing antibodies against VEGF-A and/or IL-6, followed by quantitative PCR (E) for Mmp9 gene expression (n = 3 per group) or zymography (F). Data are mean ± SEM. *P < 0.05; NS, not significant by Student t test. G, CD11b\(^+\)Gr1\(^+\) cells were treated with control or PTHrP-conditioned media in combination with PP2 or DMSO control, followed by zymography.
Furthermore, to confirm the requirement of osteoblasts in PTHrP-dependent potentiation of CD11b+Gr1+ cells, primary osteoblasts were established from murine calvaria and treated with PTHrP (1–34) or saline for 24 hours and conditioned media harvested (39). CD11b+Gr1+ cells were isolated from femoral bone marrow and stimulated with osteoblast-derived control- or PTHrP-conditioned media in combination with neutralizing antibodies against VEGF-A and/or IL-6. Consistent with the previous data, PTHrP-conditioned media from osteoblast cultures increased \( Mmp9 \) gene expression (Fig. 6E) and functional MMP-9 (Fig. 6F) in the MDSCs, and these effects were blocked by anti-VEGF-A and/or anti-IL-6 neutralizing antibodies. Furthermore, the effect of PTHrP-conditioned media on MMP-9 expression was suppressed by PP2 (Fig. 6G).

**Anti-PTHrP monoclonal antibody treatment decreased MDSC recruitment in PC-3 tumors**

Finally, to more rigorously determine the causal relationship between PTHrP and MDSC recruitment, mice bearing PTHrPHi PC-3 tumors were treated with nonspecific control IgG or anti-human PTHrP monoclonal antibodies. Anti-PTHrP antibodies significantly suppressed tumor growth, but not to the level of PTHrPLo tumors (Fig. 7A and B). As anti-PTHrP monoclonal antibodies potentially suppress tumor growth via inhibition of autocrine PTHrP effects on tumor cells (Supplementary Fig. S4), tumor tissues were analyzed for MDSC recruitment by immunofluorescence colocalization of CD11b+Gr1+ cells (Fig. 7C and D). Numbers of CD11b+Gr1+ cells were decreased in anti-PTHrP antibody-treated or PTHrPLo tumor tissues, suggesting that reduced PTHrP is causal to decreased MDSCs found in tumor tissues. Serum calcium levels were correlated with PTHrP levels, indicating the functional activity of PTHrP (Fig. 7E). Quantitative RT-PCR analysis in tumor tissues revealed that shRNA-mediated PTHrP knockdown was stable in PC-3 tumor cells during in vivo tumor growth, and the correlation between PTHrP and \( Mmp9 \) gene expression (Fig. 7F and G).

![Figure 7](image_url)
Discussion

This study provides new evidence that distant tumors stimulate the bone marrow to increase critical component cells in the tumor microenvironment. In brief, prostate cancer–derived PTHrP circulates to potentiate CD11b+Gr1+ cells within the bone marrow via upregulation of IL-6 and VEGF-A in osteoblasts, contributing to tumor growth and angiogenesis (Fig. 8). As a proposed mechanism of CD11b+Gr1+ cell potentiation, these data demonstrate that PTHrP increased activating phosphorylation of SFKs that subsequently increased Ampi9 gene expression in CD11b+Gr1+ cells, supporting that CD11b+Gr1+ cell-dependent tumor growth is, at least in part, mediated by MMP-9 expression and angiogenesis.

Increasing evidence now clearly supports the critical functions of CD11b+Gr1+ MDSCs in the tumor microenvironment (40). However, the majority of previous studies have been focused either on the roles of MDSCs within the tumor microenvironment (i.e., immune suppression and angiogenesis) or the mechanism of MDSC recruitment to the tumor (41, 42). Given that bone is an essential partner for tumor progression by supplying numerous bone marrow–derived stromal cells, primary tumor cells are speculated to have active mechanisms to interact with the bone/bone marrow (43, 44). The data in this study demonstrate that PTHrP serves as a messenger between the primary tumor and the bone marrow, conferring MDSCs with increased angiogenic potential. PTHrP is a potent bone-modulating cytokine expressed by multiple types of tumor cells such as prostate, breast, lung, and colorectal cancers (45–48). In addition, PTHrP is a key regulator of the "vicious cycle" hypothesis of metastatic tumor–bone interactions (49). However, given that MDSCs are currently considered universally essential components of the tumor microenvironment, not all tumor types express PTHrP, suggesting that PTHrP is not the only factor mediating the interactions between tumor and bone.

The molecular mechanisms of MDSC activation, expansion, and/or mobilization, and ultimately therapeutic approaches targeting the key signaling mechanisms, warrant extensive further investigation. Interestingly, the preliminary studies shown in Supplementary Fig. S5 suggest that PTHrP induces a series of alterations in the bone marrow to mobilize and/or expand MDSCs. Still, questions remain about whether and how PTHrP stimulates differentiation of MDSCs from bone marrow precursors. Nevertheless, this work provides a biological rationale for the clinical application of SFK inhibitors in targeting 2 compartments (i.e., tumor and the microenvironment) simultaneously, of which the mechanism requires further studies. The data in this study demonstrate that activation of SFKs is one of the key signal transduction mechanisms of MDSCs’ angiogenic potential, in addition to two other factors, STAT3 and PI3K, that have previously been shown to be implicated in MDSC functions (13, 31). Indeed, SFKs mediate crucial regulatory functions in both tumor cells and stromal cells (e.g., endothelial cells and osteoclasts), suggesting that SFKs are promising therapeutic targets for the suppression of tumor as well as stromal compartments (24, 50).

In conclusion, this study provides evidence that prostate cancers positively regulate the bone marrow microenvironment via PTHrP, IL-6, VEGF-A, and SFKs, thereby increasing the angiogenic potential of CD11b+Gr1+ MDSCs, leading to increased tumor growth.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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Figure 8. Proposed model of CD11b+Gr1+ cell activation within the bone marrow of prostate tumor hosts via PTHrP. Prostate tumor–derived PTHrP circulates to stimulate VEGF-A and IL-6 expression by osteoblasts, leading to SFK phosphorylation of CD11b+Gr1+ MDSCs. Activation of SFK confers angiogenic potential of MDSCs via increased MMP-9 expression, contributing to prostate cancer growth and angiogenesis.
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