Use of the Stromal Cell-derived Factor-1/CXCR4 Pathway in Prostate Cancer Metastasis to Bone[1]

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ABSTRACT

Neoplasms have a striking tendency to metastasize or "home" to bone. Hematopoietic cells also home to bone during embryonic development, where evidence points to the chemokine stromal cell-derived factor-1 (SDF-1 or CXCL12; expressed by osteoblasts and endothelial cells) and its receptor (CXCR4) as key elements in these processes. We hypothesized that metastatic prostate carcinomas also use the SDF-1/CXCR4 pathway to localize to the bone. To test this, levels of CXCR4 expression were determined for several human prostate cancer cell lines by reverse transcription-PCR and Western blotting. Positive results were obtained for cell lines derived from malignancies that had spread to bone and marrow. Prostate cancer cells were also observed migrating across bone marrow endothelial cell monolayers in response to SDF-1. In *in vitro* adhesion assays, pretreatment of the prostate cancer cells with SDF-1 significantly increased their adhesion to osteosarcomas and endothelial cell lines in a dose-dependent manner. Invasion of the cancer cell lines through basement membranes was also supported by SDF-1 and inhibited by antibody to CXCR4. Collectively, these results suggest that prostate cancers and perhaps other neoplasms may use the SDF-1/CXCR4 pathway to spread to bone.

INTRODUCTION

Prostate neoplasms have a striking tendency to metastasize to bone. For metastases to occur, the malignant cells must escape the primary tumor, penetrate and circulate through the bloodstream, and subsequently arrest and proliferate in target tissues. The mechanisms that account for bone homing behavior have not yet been elucidated but may include a "direct" vascular pathway, highly permeable marrow sinusoids, chemotactic factors produced by marrow stromal cells, and the synthesis of growth factors by resident cells within the bone and marrow that support the survival, growth, and proliferation of "seeded" cancer cells (1, 2).

It is well known that hematopoietic stem cells also "home" to bone during fetal life and during marrow transplantation (3). In this context, a CXC chemokine SDF-1[3] (or CXCL12) and its receptor, CXCR4 appear to be critical molecular determinants for these events (4, 5). For instance, although normal fetal liver hematopoiesis still occurs in SDF-1 or CXCR4 gene knockouts, marrow engraftment by these hematopoietic cells is not observed (5, 6). In addition, the levels of CXCR4 expression correlate with the ability of human progenitors to engraft into the marrow of nude mice (7). Finally, osteoblasts and

marrow endothelial cells express SDF-1 protein that functions as a chemoattractant for human hematopoietic progenitor cells (8–10). Thus, it appears that SDF-1 and CXCR4 represent at least one of the critical determinants for bone marrow homing by hematopoietic cells.

On the basis of the hematopoietic model, we hypothesize that metastatic prostate carcinomas may use a similar pathway to localize to the bone marrow. In the present investigation, we demonstrate that several human prostate cancer cell lines express functional CXCR4 receptors, and that SDF-1 alters the adherence, migration, and invasion of human prostate cancer cell lines. These data are consistent with a role of SDF-1/CXCR4 in metastatic cascades of prostatic carcinomas and suggest novel targets for therapeutic intervention.

MATERIALS AND METHODS

Primary HOB Cells, Osteosarcoma, Endothelial and Prostate Cancer Cell Lines. Enriched HOB cultures were established as detailed previously (11). MG-63 (CRL1424) and SaOS-2 (ATCC 85-HTB) osteosarcoma cell lines were purchased from the ATCC (Rockville, MD). Bone marrow endothelial cells (HBME) were isolated from a normal Caucasian male and immortalized with SV40 large T-antigen (12).

PC-3 and DU145 prostate cancer cells originally isolated from vertebral and brain metastases from prostate cancer patients were obtained from ATCC. LNCaP cells were isolated from a lymph node of a patient with disseminated bony and lymph node involvement (UroCor, Inc., Oklahoma City, OK). The rat MatLyLu cell line were obtained from Dr. John Isaacs (John Hopkins University, Baltimore, MD). MCF-7 cells were established from a patient with metastatic breast cancer (ATCC).

Prostate cancer cell lines were passaged and allowed to grow to confluence over 5 days. Cells were cultured in RPMI 1640 supplemented with 10% fetal bovine serum, 1% penicillin-streptomycin, and 1% L-glutamine.

RT-PCR. RT-PCR was performed as described previously (9, 11). Sense and antisense primers were prepared to cross intron/exon boundaries including: SDF-1, 5′-CGT CAG CCG CAT TGC CCG CT and 3′-GGT CTA GCG GAA AGT CCT (380 bp); CXCR4, 5′-GGC AGC AGG TAG CAA AGT GA and 3′-TGA TGA CAA AGA GGA GGT CGG (341 bp); glyceraldehyde-3-phosphate dehydrogenase, 5′-GAC AAC AGC CTC AAG ATC ATC AGC and 3′-AAG TCA GAG GAG ACC ACC TGG TGC; and β-actin, 5′-TCC TGT GGC ATC CAT GAA ACT ACA TTC AAT TCC, 3′-GTG AAA ACG CAG CTC AGT AAC AGT CCG CCT AG (347 bp). The samples underwent thermal cycling at 94°C for 1 min and 60°C for 1 min and 72°C for 1 min for 35 cycles for SDF-1, followed by a 10-min extension at 72°C (Perkin-Elmer, Foster City, CA). PCR for CXCR4 was performed at 94°C, 55°C, and 72°C. False positives and DNA contamination were controlled by omitting reverse transcriptase in control reactions.

Immunohistochemistry. Indirect immunohistochemistry was performed for CXCR4 on cells grown in eight-well tissue slides (Costar Corp). Cells fixed in 2% paraformaldehyde at 25°C for 30 min with Triton X-100 were incubated with either 10 μg/ml of a murine antihuman CXCR4 monoclonal antibody (BD PharMingen, San Diego, CA) in PBS with 10% normal goat serum or an isotype-matched control at 25°C (Sigma Chemical Co., St. Louis, MO), followed by a goat antimurine FITC-conjugated serum at a 1:50 dilution (Sigma Chemical Co.).

SDF-1 ELISA. For determination of SDF-1 levels in conditioned medium, primary human osteoblasts and osteosarcoma cell lines were plated to an initial density of 2.0×10^5 cells/cm^2 in Ham's F-12/DMEM (1:1, v/v) medium

containing 10% FBS, antibiotics, 10 mM β-glycerol phosphate, and 10 μg/ml L-ascorbate in 24-well plates (Life Technologies, Inc., Grand Island, NY). Medium was changed on days 3 and 5, then on day 7; after the cells had reached confluence, cells were washed twice in PBS, medium was replaced, and conditioned medium was collected and stored at −80°C. Medium was analyzed by antibody sandwich ELISA (R&D Systems, Minneapolis, MN) with a detection range of 62.5–5000 pg/ml SDF-1.

Western Blot Analysis. Prostate cancer cells were cultured to confluence, and cells were washed and then serum-starved in RPMI with 0.1% BSA for 48 h. SDF-1 stimulation was performed with 0–200 ng/ml SDF-1 in PBS containing 0.1% BSA or vehicle (R&D Systems) for 5–60 min or 24 h. Cells were lysed by freeze-thawing in ice-cold lysis buffer (50 mM Tris-HCl, 1% NP40, 120 mM NaCl, 1 mM EDTA, 25 mM NaF, 40 mM β-glycerol phosphate, 0.1 mM sodium orthovanadate, 0.5 mM phenylmethylsulfonyl fluoride, and 1.0% mammalian protease inhibitor mixture; Sigma Chemical Co.). The nuclei and cellular debris were removed by centrifugation at $16,000 \times g$ for 15 min at 4°C. Normalized lysates (30 μg) in Laemmli buffer were electrophoresed on 10% polyacrylamide gels under reducing conditions and transferred to polyvinylidene difluoride membranes. For CXCR4 detection, the membranes were either blocked in 3% BSA in PBS-0.1% Tween 20, and a mouse antihuman monoclonal antibody (1 μg/ml; clone 12G5; PharMingen, San Diego, CA) was used in conjunction with goat antimurine horseradish peroxidase, or membranes were blocked in 5% Blotto in PBS-0.1% Tween 20 and a rabbit anti-CXCR4 antiserum (1:1000 dilution; AB2074; Abcam Corp., Cambridge, United Kingdom) was used in conjunction with goat antirabbit horseradish peroxidase (Sigma Chemical Co.). Final detection was by chemiluminescence (Amersham Pharmacia, Inc., Piscataway, NJ). ERK detection was similarly performed in 5% Blotto with a mouse monoclonal reactive to Tyr-204-phosphorylated ERK1, ERK2, and total ERK1 and ERK2 (Santa Cruz Biotechnology, Inc., Santa Cruz, CA).

Cell-Cell Adhesion Assay. Prostate cancer cell lines were labeled with 3′-O-acetyl-2′,7′-bis(carboxyethyl)-4-5-carboxyfluorescein ester (Molecular Bioprobes, Inc., Eugene, Oregon) for 30 min. Labeled cells (1×10^5) were deposited directly onto osteosarcoma MG-63 or SaOS-2 or human bone marrow endothelial cell (labeled as endo) monolayers. SDF-1 pretreatment was performed by incubating the prostate cancer cells with 0–200 ng/ml SDF-1 (or 200 ng/ml SDF-1 that had been boiled for 15 min as a negative control) for 30 min at 37°C. Cell-to-cell adhesion was allowed to proceed for 30 min at 37°C. Adherence was quantified in a 96-well fluorescent plate reader (IDEXX Research Products, Westbrook, ME). Data are presented as raw fluorescent counts.

Transendothelial Migration. HBME cells were seeded onto 12-μM TransWell microporous membrane (Corning Costar Corp., Cambridge, MA) 24-well plates. Prostate cancer cell lines were placed in the upper chamber. Transmigration supported by a SDF-1 gradient (0–200 ng/ml) was achieved by adding various concentrations of SDF-1 in the lower chamber. To evaluate random migration (chemokinesis), SDF-1 was added to both upper and lower chambers. After a 24–30 h incubation, the number of transmigrated cells in the lower chambers were enumerated by direct microscopic counts. Spontaneous transendothelial migration was compared with transmigration supported by a SDF-1 gradient (13).

Invasion of Prostate Cancer Cells. Cell invasion was examined using a reconstituted extracellular matrix membrane (Matrigel, Beckman Coulter Labware, Franklin Lakes, NJ) or type I collagen (Collagen Corp., Palo Alto, CA). Cell invasion chambers were prepared by placing 40 μl of the extracellular matrix into the top chamber of Transwells, which were incubated for 2 h at 37°C. Test cells were placed in the upper chamber (1×10^5 cells/well) in serum-free medium, and 0–400 ng/ml SDF-1 were added to the lower chamber. Spontaneous invasion was compared with invasion supported by a SDF-1 gradient (13). Invasion into the matrix was assayed after 24–30 h by visual quantification of the cells that had migrated into matrix. The effect of 1 μg/ml CXCR4 blocking antibody (12G5 PharMingen) added to the top chamber of the Transwell was used to provide additional verification that observed responses are dependent on CXCR4 receptor binding.

Statistical Analysis. Statistical differences between the means for the different groups were evaluated with Instat 4.0 (GraphPAD software) using one-way ANOVA, with the level of significance at $P < 0.05$. All experiments were repeated two to three times with triplicate samples, and similar results were obtained.

RESULTS

To determine whether SDF-1 and its receptor (CXCR4) help to define the bone specific metastasis of prostate carcinomas, we first identified which elements in the bone marrow express SDF-1 (11, 14). Using RT-PCR, we observed that the majority of the normal primary human osteoblastic cells express mRNA for SDF-1 (Fig. 1A), and SDF-1 levels were easily detected in the conditioned medium of four primary osteoblastic cell isolates with levels ranging from 138 ± 36 to 787 ± 48 pg/ml/96 h/10^4 cells (Fig. 1B). In addition, several human osteosarcoma cell lines, including MG-63 and HOS TE85 (not presented), also express SDF-1 mRNA, murine bone marrow stromal cells that express an osteoblast phenotype, as well as human osteoblasts *in situ,* express SDF-1 (9) but not the SaOS-2 cell line.

Several human prostate cancer cell lines were evaluated for the expression of the SDF-1 receptor by RT-PCR. Positive controls for CXCR4 expression included RNA collected from hematopoietic (HL-60) and breast cancer (MCF-7) cell lines (15). RNA collected from the SaOS-2 osteosarcoma cell line served as a negative control. Expres-
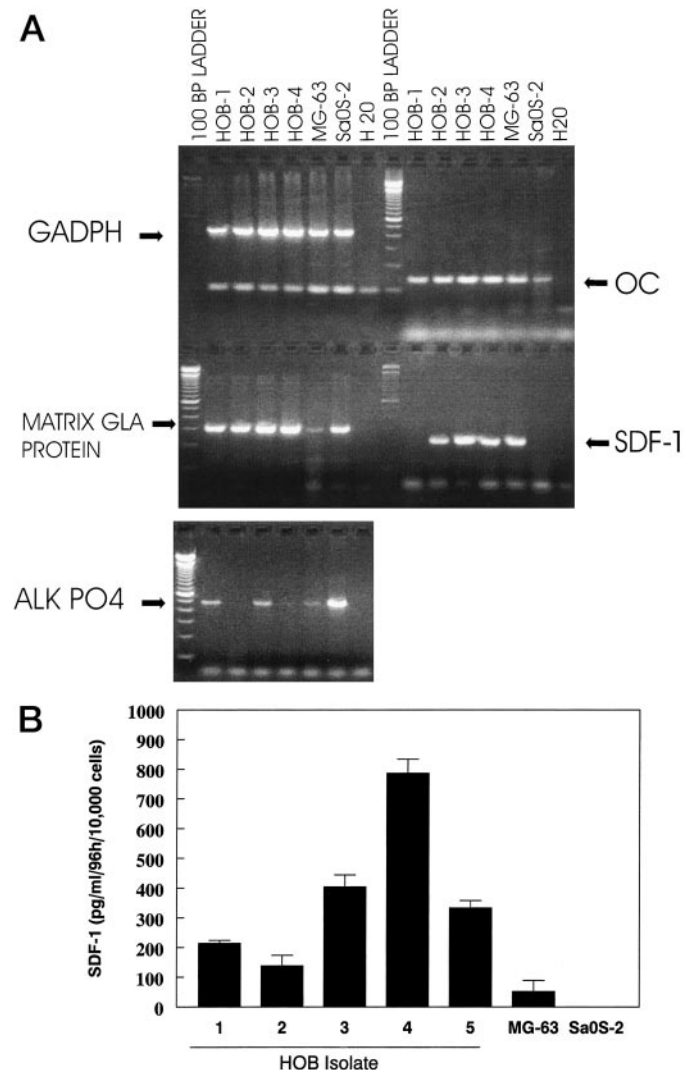

Fig. 1. SDF-1 is expressed by marrow-derived HOBs. In *A,* ethidium bromide-stained gel of RT-PCR performed using RNA obtained from HOBs (*HOB1* to *HOB4*) for glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*), SDF-1, matrix Gla protein, osteocalcin (*OC*), and alkaline phosphatase (*ALK PO4*). In *B,* SDF-1 protein levels were measured in 96-h conditioned medium from five additional HOB cell isolates (*B*). The data indicate that three of four primary HOBs and MG-63 express SDF-1 mRNA and protein, whereas HOB1 and SaOS-2 do not.
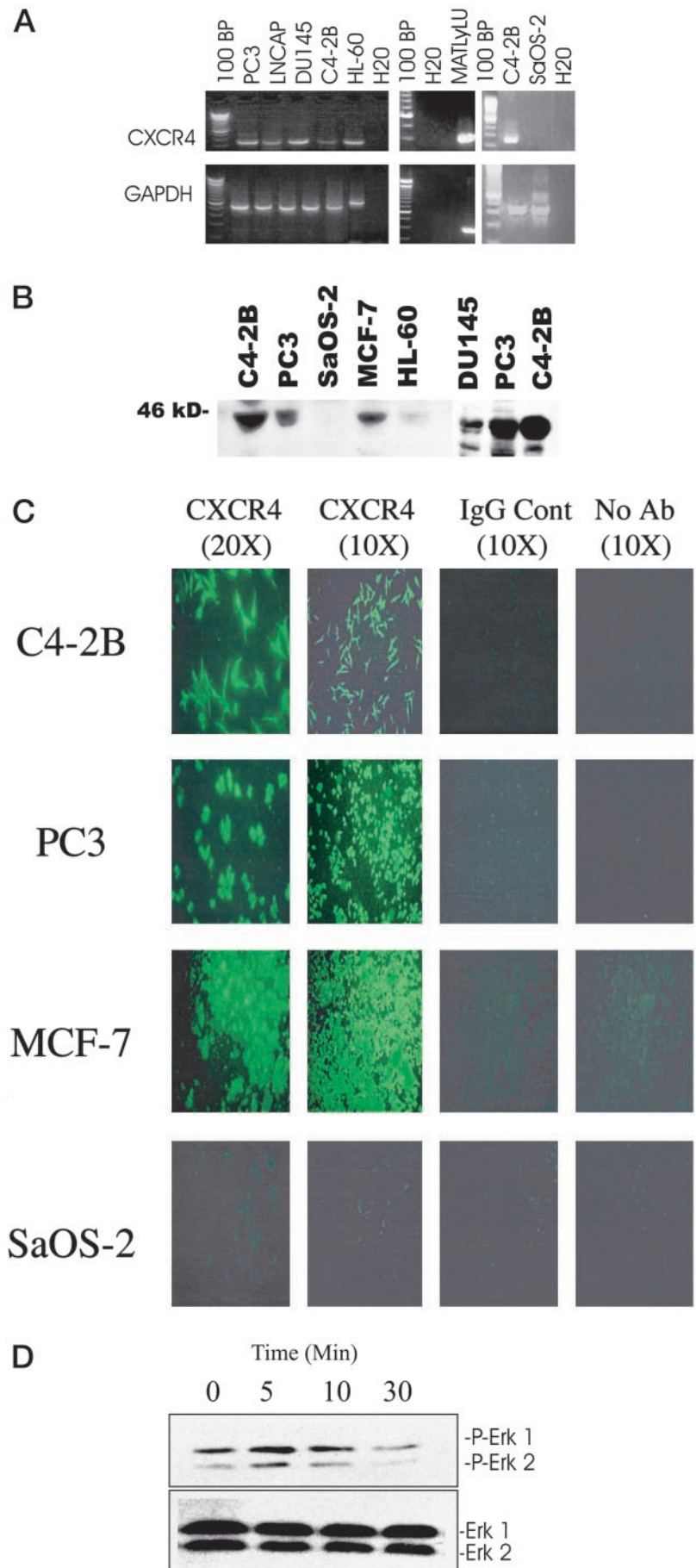
Fig. 2. Functional CXCR4 receptors are expressed by prostate cancer cell lines. In *A,* ethidium bromide-stained gel of RT-PCR performed using RNA recovered from human (DU145, PC3, LNCAP, and C4-2B) and rat (MatLyLu) prostate cancers, with SaOS-2 and HL-60 cells, as negative and positive controls, respectively. CXCR4 mRNA was observed for all cell types evaluated except SaOS-2. *B,* Western blot probed directly with a monoclonal anti-CXCR4 (*B, right*) or a polyclonal antibody (*B, left*), demonstrating a M_r 46,000 band corresponding to the CXCR4 receptor. Controls included protein isolated from MCF-7, HL-60 (positive), and SaOS-2 cells (negative) cells. *C,* immunohistochemistry with an isotype-matched control or CXCR4 antibody demonstrating CXCR4 expression by C4–2B, PC3, and MCF-7 (positive control) but not SaOS-2 (negative control). *D,* Western blots of SDF-1-stimulated PC-3 cells using antibodies that detect total (*bottom*) and phosphorylated (*P*) Erk-1 and Erk-2 proteins (*top*). The data demonstrate that SDF-1 stimulates phosphorylation of ERK proteins in PC3 cells.

sion of CXCR4 was observed in the PC-3 and DU145 cell lines, derived from malignancies that had spread to bone marrow and brain, respectively. Hormone-refractory prostate carcinoma cell lines cloned from a lymph node (LNCaP) and bone marrow (C4–2B) and the rat MatLyLu prostate carcinoma cell line also expressed the CXCR4 mRNA but not the SaOS-2 osteosarcoma cell line (Fig. 2*A*). Confirmation that CXCR4 is expressed by prostate carcinoma cells was obtained using Western blotting using monoclonal and polyclonal antibodies (Fig. 2*B*). The data demonstrate a M_r 46,000 band corresponding to CXCR4 for both MCF-7, HL-60, and the prostate carcinomas but not for the SaOS-2 cell line. Further conformation that prostate cancer cell lines express CXCR4 was obtained using immunohistochemistry to the receptor (Fig. 2*C*).

To verify that the CXCR4 receptors are functional in prostate cancer cell lines, the cells were examined to determine whether ERK-1/ERK-2 pathways are activated upon ligand binding, as has been demonstrated in hematopoietic cells. Rapid activation of phosphorylated ERK-1/ERK-2 proteins was observed within 5 min of SDF-1 stimulation, with ERK-1/ERK-2 levels returning to baseline within 30 min after receptor engagement (Fig. 2*D, top*). No changes in the total Erk protein expression were observed over the course of the investigation (Fig. 2*D, bottom*).

Prostate cancer cells may use CXCR4 receptors to adhere to cells and/or extracellular matrix components in the bone marrow. We carried out experiments to test this possibility by determining whether SDF-1-treated cells adhere preferentially to osteosarcoma cell lines or human bone marrow endothelial cells than untreated controls. SDF-1 pretreatment enhanced the binding of prostate cancer cell lines to human osteosarcoma cells (Fig. 3, *A* and *B*). Similarly, SDF-1 enhanced the binding of several prostate cancer cell lines to human endothelial cells, although the total percentage of bound cells was considerably less (Fig. 3*C*).

Once bound to endothelium, the metastatic tumor cells must migrate across the microvascular barrier to invade and colonize target tissues. As an *in vitro* analogue, we explored whether prostate cancer cells could migrate across endothelial cell monolayers in response to SDF-1. Confluent layers of bone marrow endothelial cells (HBME) were seeded onto Transwell microporous membranes. Migrating cells were introduced into the upper chamber, and a gradient of SDF-1 (0 or 200 ng/ml) was established by placing the chemokine in the lower chamber. Our data support this concept because SDF-1 stimulated the directed movement of the prostate cancer cell lines (Fig. 4).

Once tumor cells have adhered to and moved through the endothelium, they must invade through the extracellular matrix. The ability of SDF-1 and CXCR4 to influence prostate carcinoma invasion were studied using a reconstituted extracellular matrix (Matrigel; Beckman Coulter Labware, Franklin Lakes, NJ) in porous chambers. SDF-1 supported the invasion of PC3 and C4–2B cells into the reconstituted matrix (Fig. 4). Addition of SDF-1 to both chambers of the assay abrogated the invasion. Similar findings were made for the DU145, and PC3 invasion into type I bovine collagen (Collagen Corp.). Serum-free osteoblast conditioned medium also supported invasion, but not medium derived from the SaOS-2 cell line, which is consistent with our RT-PCR data (Fig. 1 and data not presented). Addition of antibody to CXCR4 the top chamber of the culture but not an isotype-matched control significantly reduced the number of invading cells (Fig. 4). As a whole, these data support the role of SDF-1 and CXCR4 in the development of metastasis of prostate carcinomas.

DISCUSSION

Prostate cancer is a common neoplasm and the second leading cause of cancer deaths in American males (16). The high mortality
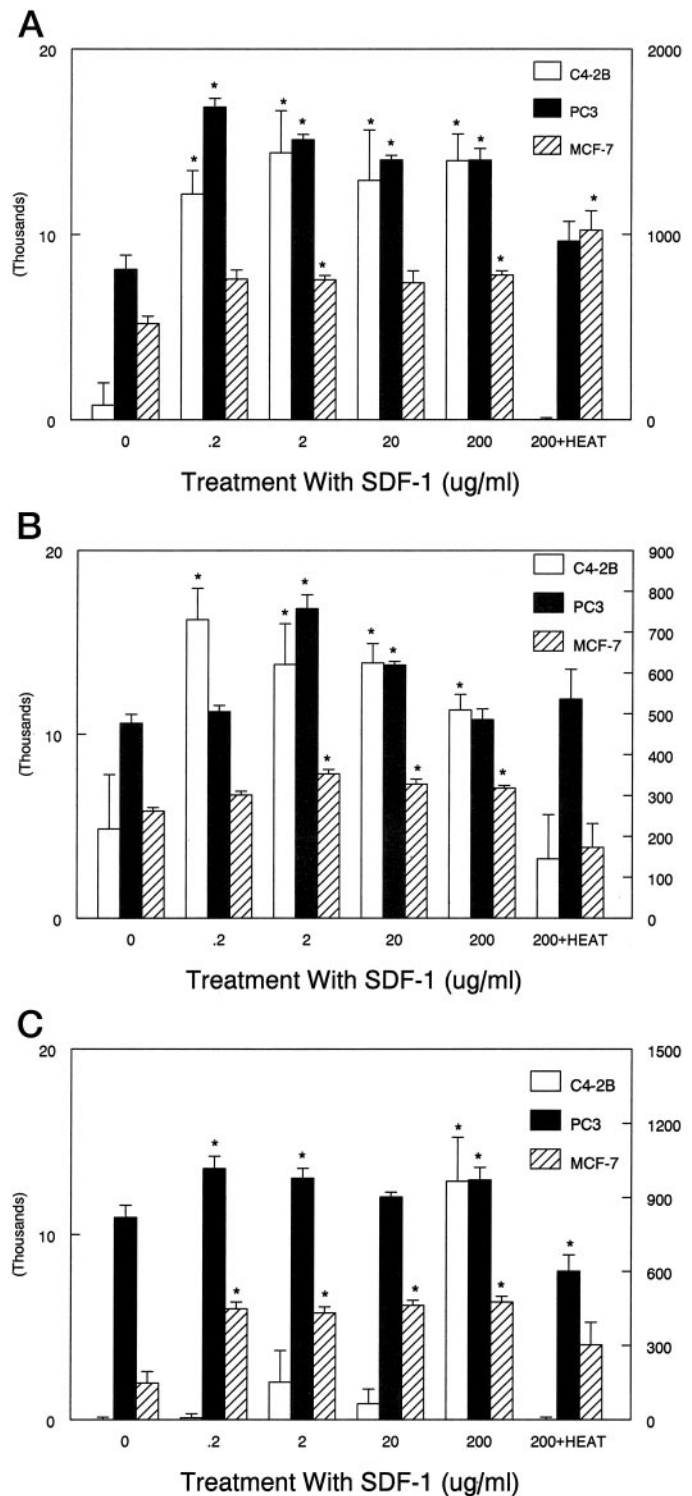

Fig. 3. SDF-1 enhances binding of prostate cancer cells to human osteosarcoma cell lines and human bone marrow endothelium. 3′-*O*-Acetyl-2′,7′-bis(carboxyethyl)-4-5-carboxyfluorescein ester-labeled C4–2B, PC3, or MCF-7 cells (1×10^5) were labeled and deposited directly on to osteosarcoma MG-63 (*A*), SaOS-2 cells (*B*), or human bone marrow endothelial cell (*C*) monolayers. Pretreatment was performed by incubating the prostate cancer cells with 0–200 ng/ml SDF-1 or heat-inactivated 200 ng/ml SDF-1. Cell-to-cell adhesion was allowed to proceed for 30 min at 37°C, and adherence was quantified in a fluorescent plate reader (IDEXX Research Products, Westbrook, ME; $n = 5$). Data for the C4–2B cell line use the right *Y axis*, and PC3 and MCF-7 (positive control) use the *left*, presented as a raw fluorescent counts/well of the mean; *bars*, SD. *, significant from nontreatment control ($P < 0.05$).
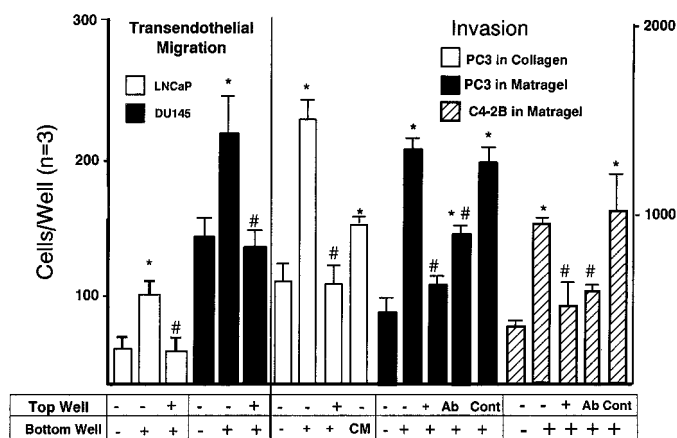
Fig. 4. SDF-1 supports transendothelial migration and invasion of prostate cancer cells. Confluent HBME cells were established in Transwell plates. LNCaP or PC3 cells were suspended in the top well (5×10^4 cells/0.1 ml), and SDF-1 (0 or 200 ng/ml) was placed into the bottom chambers. Chemokinesis was studied by adding SDF-1 to both the upper and lower chambers. Transmigrated cells in the lower chambers were enumerated at 24 h (*left*). Invasion activity (*right*) was examined using a reconstituted extracellular matrix membrane or type I collagen. PC3 or C4–2B cells were placed in the upper chamber in serum-free medium, control (0), or 200 ng/ml SDF-1 (or 50% primary osteoblast conditioned medium collected at 72 h, *CM*) were added to the lower chamber. CXCR4 function-blocking antibody (*Ab*) or isotype-matched control (*Cont*) were used to evaluate specificity. Data are presented as mean migrating cells/well; *bars,* SD ($n = 3$). ∗, significant difference from nontreatment control; #, significant from invasion or transendothelial migration mediated by SDF-1 (*top,* 0; *bottom,* 200 μg/ml; $P < 0.05$).

rate is principally attributable to the spread of malignant cells to many tissues including bone (17). As a result, there is a growing interest in the early detection and screening of men for prostate cancer and for a greater understanding of the mechanisms that lead to metastasis.

We hypothesized that SDF-1 and its CXCR4 receptor would help to define the bone-specific metastasis of prostate carcinomas. To address this hypothesis, we first identified osteoblasts and endothelial as key elements in the bone marrow that express SDF-1 (9). We observed that primary HOBs produce a wide range of SDF-1 levels. Although we have not attempted to optimize the conditions for SDF-1 synthesis, these data are in keeping with those we reported previously for other cells types including marrow stromal and murine osteoblastic cells (9) and thyroid-derived fibroblasts and adenomas (18) but considerably less than gingival fibroblasts (19). We next examined the expression of CXCR4 in several human prostate cancer cell lines by RT-PCR and by Western blotting and immunohistochemistry. Expression of CXCR4 was observed for PC-3 and DU145 cell lines, derived from malignancies that had spread to bone and brain, respectively. Hormone-refractory prostate carcinoma cell lines cloned from a lymph node (LnCaP) and marrow (C4–2B) also expressed CXCR4.

For prostate cancers to exit the vasculature, they must first adhere to the endothelium and subsequently move through the endothelial monolayer and underlying connective tissues. We observed that in *in vitro* adhesion assays, pretreatment of the prostate cancer cells with SDF-1 significantly increased their adhesion to several osteosarcomas and bone marrow-derived endothelial cells in a dose-dependent manor, suggesting that prostate carcinomas migrate across endothelial cell monolayers in response to SDF-1. Finally, we were able to demonstrate that SDF-1 supported the invasion of prostate carcinoma cell lines into reconstituted basement membranes, and this activity could be blocked by either antibody to the CXCR4 receptor or by using a specific synthetic inhibitor of CXCR4. Collectively, our results suggest this possibility that prostate cancers and perhaps other neoplasms (*i.e.,* breast) use the SDF-1/CXCR4 pathway during their spread to bone and other tissues.

In the bone marrow, SDF-1 is constitutively produced by osteo-

blasts, fibroblasts, and endothelial cells (9). It is important to point out that vascular endothelial cells in other tissues, such as those lining pulmonary channels, do not secrete SDF-1 (20). Together, selective expression of SDF-1 by endothelial cells and other resident cells in specific tissues may provide a mechanism to localize hematopoietic cells to these tissue compartments. More important than secretion, SDF-1 must be biologically active. SDF-1 is known to bind heparin, heparin sulfated proteoglycans, and fibronectin, which may change the activity of the ligand (21, 22). Indeed, this has been demonstrated recently by Peled *et al.* (23), who showed that heparin-bound SDF-1 was able to arrest CD34$^+$ cells rolling on marrow vascular endothelium. Subsequently, firm adhesions were established by CD34$^+$ cells on endothelium using VLA-4 and LFA-1 receptors, ultimately culminating in the extravasation of CD34$^+$ progenitors into the marrow. As cancer cells also produce humoral factors (including interleukin 1 and tumor necrosis factor) that facilitate the expression of cell adhesion molecules on endothelial cells [*e.g.,* E-selectin, P-selectin, and hyaluronate (ligand for CD44; Refs. 24, 25)], we are currently exploring whether the synthesis of SDF-1 by marrow endothelium and osteoblasts can be altered by prostate carcinoma cells and whether this then further enhances tumor cell adhesions (26, 27).

In addition to chemoattraction, SDF-1 may also help to establish metastases in bone by serving as a growth factor or to prevent apoptosis of the tumor cells. To evaluate this possibility, we cultured for several prostate carcinoma cell lines in serum and serum-free medium in the presence of increasing amounts of SDF-1. SDF-1 alone failed to modulate proliferation of any of the cell lines evaluated (data not presented). It should be noted that SDF-1 does not stimulate proliferation of early hematopoietic cells but synergizes in combination with other growth factors (28). Furthermore, SDF-1 did not the preserve colony formation upon serum starvation, nor did it prevent anoikis of PC3 and C4–2B cells. Thus, although it may be premature to conclude that SDF-1 is without effect on tumor cell growth or survival alone, we have no evidence that SDF-1 in osteoblast or mixed stromal cell-conditioned medium supported prostate cancer cell growth.

Although both blood cells and prostate cancer cells home to bone marrow, we are not aware of any investigation that addresses whether SDF-1/CXCR4 is operating in the pathogenesis of prostate cancer metastasis. This possibility is reinforced by virtue of the fact that the *CXCR4* gene is expressed in normal prostate tissues, albeit at low levels (29). Moreover, both ligand and receptor are overexpressed in several neoplasms that invade the marrow (*e.g.,* breast cancers, Burkitt's lymphoma, several leukemias, and neuroblastomas; Refs. 15, 29–32). On the basis of these considerations, it is reasonable to ask whether CXCR4 receptors are up-regulated in malignant prostate cancer cells, and if they are, do these receptors function to direct malignant prostate cancers to the bone marrow? Moreover, Muller *et al.* (33) recently reported similar findings in a breast cancer model. These authors demonstrated that normal breast tissues express little CXCR4, whereas breast neoplasms express high levels of CXCR4. Furthermore, antibody to CXCR4 blocks the metastatic spread of the tumors to the lung and lymph nodes. Together, these investigations suggest a role of SDF-1/CXCR4 in metastatic cascades of prostate carcinoma and thereby suggest novel targets for therapeutic intervention to prevent prostate cancer metastasis.

REFERENCES

1. Geldof, A. A. Models for cancer skeletal metastasis: a reappraisal of Batson's plexus. Anticancer Res., 17: 1535–1539, 1997.

2. Diel, I. Historical remarks on metastasis and metastatic bone disease. *In:* I. Diel, M. Kaufmann, and Bastert G. (eds.), Metastatic Bone Disease. Fundamental and Clinical Aspects, pp. 1–11. New York: Springer-Verlag, 1994.

3. Hardy, C. Homing of hematopoietic stem cells to the marrow. Am J. Med. Sci., 309: 260–266, 1995.

4. Kim, C. H., and Broxmeyer, H. E. SLC/exodus2/6Ckine/TCA4 induces chemotaxis of hematopoietic progenitor cells: differential activity of ligands of CCR7, CXCR3, or CXCR4 in chemotaxis *vs.* suppression of progenitor proliferation. J. Leuk. Biol., 66: 455–461, 1999.

5. Aiuti, A., Tavian, M., Cipponi, A., Ficara, F., Zappone, E., Hoxie, J., Peault, B., and Bordignon, C. Expression of CXCR4, the receptor for stromal cell-derived factor-1 on fetal and adult human lympho-hematopoietic progenitors. Eur. J. Immunol., 29: 1823–1831, 1999.

6. Nagasawa, T., Hirota, S., Tachibana, K., Takakura, N., Nishikawa, Kitamura, Y., Yoshida, N., Kikutani, H., and Kishimoto, T. Defects of B-cell lymphopoiesis and bone-marrow myelopoiesis in mice lacking the CXC chemokine PBSF/SDF-1. Nature (Lond.), 382: 635–638, 1996.

7. Peled, A., Petit, I., Kollet, O., Magid, M., Ponomaryov, T., Byk, Nagler, A., Ben-Hur, H., Many, A., Shultz, L., Lider, O., Alon, R., Zipori, D., and Lapidot, T. Dependence of human stem cell engraftment and repopulation of NOD/SCID mice on CXCR4. Science (Wash. DC), 283: 845–848, 1999.

8. Aiuti, A., Webb, I. J., Bleul, C., Springer, T., and Gutierrez-Ramos, J. C. The chemokine SDF-1 is a chemoattractant for human CD34+ hematopoietic progenitor cells and provides a new mechanism to explain the mobilization of CD34+ progenitors to peripheral blood. J. Exp. Med., 185: 111–120, 1997.

9. Ponomaryov, T., Peled, A., Peled, I., Taichman, R. S., Habler, L., Sandbank, J., Arenzana-Seisdedos, G., Nagler, A., Lahav, M., Szyper-Kravitz, M., Zipori, D., and Lapidot, T. Increased production of SDF-1 following treatment with DNA damaging agents: relevance for human stem cell homing and repopulation of NOD/SCID mice. J. Clin. Investig., 106: 1331–1339, 2000.

10. Hamada, T., Mohle, R., Hesselgesser, J., Hoxie, J., Nachman, R. L., Moore, M. A., and Rafii, S. Transendothelial migration of megakaryocytes in response to stromal cell-derived factor 1 (SDF-1) enhances platelet formation. J. Exp. Med., 188: 539–548, 1998.

11. Taichman, R. S., and Emerson, S. G. Human osteoblasts support hematopoiesis through the production of granulocyte colony-stimulating factor. J. Exp. Med., 179: 1677–1682, 1994.

12. Lehr, J. E., and Pienta, K. J. Preferential adhesion of prostate cancer cells to a human bone marrow endothelial cell line. J. Natl. Cancer Inst. (Bethesda), 90: 118–123, 1998.

13. Mohle, R., Bautz, F., Rafii, S., Moore, M. A., Brugger, W., and Kanz, L. The chemokine receptor CXCR-4 is expressed on CD34+ hematopoietic progenitors and leukemic cells and mediates transendothelial migration induced by stromal cell-derived factor-1. Blood, 91: 4523–4530, 1998.

14. Taichman, R. S., and Emerson, S. G. The role of osteoblasts in the hematopoietic microenvironment. Stem Cells, 16: 7–15, 1998.

15. Sehgal, A., Ricks, S., Boynton, A. L., Warrick, J., and Murphy, G. P. Molecular characterization of CXCR-4: a potential brain tumor-associated gene. J. Surg. Oncol., 69: 239–248, 1998.

16. Pienta, K. J., and Esper, P. S. Risk factors for prostate cancer. Ann. Int. Med., 118: 793–803, 1993.

17. Jacobs, S. C. Spread of prostatic cancer to bone. Urology, 21: 337–344, 1983.

18. Aust, G., Steinert, M., Kiessling, S., Kamprad, M., and Simchen, C. Reduced expression of stromal-derived factor 1 in autonomous thyroid adenomas and its regulation in thyroid-derived cells. J. Clin. Endocrinol. Metabol., 86: 3368–3376, 2001.

19. Fedyk, E. R., Jones, D., Critchley, H. O., Phipps, R. P., Blieden, T. M., and Springer, T. A. Expression of stromal-derived factor-1 is decreased by IL-1 and TNF and in dermal wound healing. J. Immunol., 166: 5749–5754, 2001.

20. Imai, K., Kobayashi, M., Wang, J., Shinobu, N., Yoshida, H., Hamada, J., Shindo, M., Higashino, F., Tanaka, J., Asaka, M., and Hosokawa, M. Selective secretion of chemoattractants for haemopoietic progenitor cells by bone marrow endothelial cells: a possible role in homing of haemopoietic progenitor cells to bone marrow. Br. J. Haematol., 106: 905–911, 1999.

21. Mbemba, E., Gluckman, J. C., and Gattegno, L. Glycan and glycosaminoglycan binding properties of stromal cell-derived factor (SDF)-1α. Glycobiology, 10: 21–29, 2000.

22. Sbaa-Ketata, E., Vasse, M., Lenormand, B., Schneider, P., Soria, C., and Vannier, J. P. Fibronectin increases the migration induced by stromal cell-derived factor-1α (SDF-1α) in pre-B acute lymphoblastic leukemia cells. Eur. Cytokine Network, 12: 223–230, 2001.

23. Peled, A., Grabovsky, V., Habler, L., Sandbank, J., Arenzana-Seisdedos, F., Petit, I., Ben-Hur, H., Lapidot, T., and Alon, R. The chemokine SDF-1 stimulates integrin-mediated arrest of CD34(+) cells on vascular endothelium under shear flow. J. Clin. Investig., 104: 1199–1211, 1999.

24. Mohamadzadeh, M., DeGrendele, H., Arizpe, H., Estess, P., and Siegelman, M. Proinflammatory stimuli regulate endothelial hyaluronan expression and CD44/HA-dependent primary adhesion. J. Clin. Investig., 101: 97–108, 1998.

25. Iademarco, M. F., Barks, J. L., and Dean, D. C. Regulation of vascular cell adhesion molecule-1 expression by IL-4 and TNF-α in cultured endothelial cells. J. Clin. Investig., 95: 264–271, 1995.

26. Holzmann, B., Gosslar, U., and Bittner, M. α4 integrins and tumor metastasis. Curr. Top. Microbiol. Immunol., 231: 125–141, 1998.

27. Coffey, D. S. Do bone marrow endothelial cells have specific house numbers, and how is this involved in metastasis? J. Natl. Cancer Inst., 90: 84–85, 1998.

28. Hodohara, K., Fujii, N., Yamamoto, N., and Kaushansky, K. Stromal cell-derived factor-1 (SDF-1) acts together with thrombopoietin to enhance the development of megakaryocytic progenitor cells (CFU-MK). Blood, 95: 769–775, 2000.

29. Gupta, S. K., and Pillarisetti, K. Cutting edge: CXCR4-Lo: molecular cloning and functional expression of a novel human CXCR4 splice variant. J. Immunol., 163: 2368–2372, 1999.

30. Mohle, R., Failenschmid, C., Bautz, F., and Kanz, L. Overexpression of the chemokine receptor CXCR4 in B cell chronic lymphocytic leukemia is associated with increased functional response to stromal cell-derived factor-1 (SDF-1). Leukemia (Baltimore), 13: 1954–1959, 1999.

31. Slanicka, K. M., Nissen, C., Manz, C. Y., Toksoz, S. D., Lyman, and Wodnar-Filipowicz, A. The membrane-bound isoform of stem cell factor synergizes with soluble flt3 ligand in supporting early hematopoietic cells in long-term cultures of normal and aplastic anemia bone marrow. Exp. Hematol., 26: 365–373, 1998.

32. Geminder, H., Sagi-Assif, O., Goldberg, L., Meshel, T., Rechavi, G., Witz, I. P., Ben-Baruch, A. A possible role for CXCR4 and its ligand, the CXC chemokine stromal cell-derived factor-1, in the development of bone marrow metastases in neuroblastoma. J. Immunol., 167: 4747–4757, 2001.

33. Muller, C. A., Homey, B., Sato, H., Ge, N., Catron, D., Buchanan, M., McClanahan, T., Murphy, E., Yuan, W., Wagners, S., Barrera, J., Mohar, A., Verastegui, E., and Zlotnik, A. Involvement of chemokine receptors in breast cancer metastasis. Nature (Lond.), 410: 50–56, 2001.