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# Stromal Cell-Derived Factor-1$\alpha$ Promotes Melanoma Cell Invasion across Basement Membranes Involving Stimulation of Membrane-Type 1 Matrix Metalloproteinase and Rho GTPase Activities

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## ABSTRACT

**Tissue invasion by tumor cells involves their migration across basement membranes through activation of extracellular matrix degradation and cell motility mechanisms. Chemokines binding to their receptors provide chemotactic cues guiding cells to specific tissues and organs; they therefore could potentially participate in tumor cell dissemination. Melanoma cells express CXCR4, the receptor for the chemokine stromal cell-derived factor-1$\alpha$ (SDF-1$\alpha$). Using Matrigel as a model, we show that SDF-1$\alpha$ promotes invasion of melanoma cells across basement membranes. Stimulation of membrane-type 1 matrix metalloproteinase (MT1-MMP) activity by SDF-1$\alpha$ was necessary for invasion, involving at least up-regulation in the expression of this metalloproteinase, as detected in the highly metastatic BLM melanoma cell line. Moreover, SDF-1$\alpha$ triggered the activation of the GTPases RhoA, Rac1, and Cdc42 on BLM cells, and expression of dominant-negative forms of RhoA and Rac1, but not Cdc42, substantially impaired the invasion of transfectants in response to SDF-1$\alpha$, as well as the increase in MT1-MMP expression. Furthermore, CXCR4 expression on melanoma cells was notably augmented by transforming growth factor-$\beta$1, a Matrigel component, whereas anti-transforming growth factor-$\beta$ antibodies inhibited increases in CXCR4 expression and melanoma cell invasion toward SDF-1$\alpha$. The identification of SDF-1$\alpha$ as a potential stimulatory molecule for MT1-MMP as well as for RhoA and Rac1 activities during melanoma cell invasion, associated with an up-regulation in CXCR4 expression by interaction with basement membrane factors, could contribute to better knowledge of mechanisms stimulating melanoma cell dissemination.**

## INTRODUCTION

During the different steps of metastasis, tumor cells are likely to sense and respond to chemotactic cues that can contribute to their invasion and migration. Chemokines constitute a family of proteins that promote cell migration and activation, exerting these functions on binding to seven transmembrane G-protein-linked chemokine receptors (1–4).

Expression of chemokine receptors on tumor cells and their involvement in metastasis has only recently been addressed, revealing that these receptors can indeed provide migratory directions to tumor cells (5, 6). CXCR4 is a receptor for stromal cell-derived factor-1$\alpha$ (SDF-1$\alpha$; also called CXCL12; Refs. 7, 8), a chemokine expressed in several tissues and organs, including skin, lymph nodes, lung, liver, and bone marrow (5, 9–11), which stimulates cell adhesion, migration, and activation (10, 12–16). CXCR4 has been reported to be expressed on different tumor cell types (5, 6, 17–23), and a key role for CXCR4 has been documented in breast cancer metastasis (5). Expression of CXCR4 and its involvement in cell adhesion, migration, and activation in melanoma was shown recently (5, 20, 24). Moreover, expression of CXCR4 in the B16 murine model of melanoma conferred pulmonary metastatic potential to these cells compared with their CXCR4$^-$ counterparts (25). Therefore, these data provide a reasonable basis to propose that the SDF-1/CXCR4 axis could play an important role in melanoma and breast cancer cell metastasis.

During metastasis, tumor cells activate matrix digestion and locomotion mechanisms to allow their invasion across basement membranes into surrounding connective tissues (26). Degradation of basement membranes by digestion of extracellular matrix (ECM) proteins is accomplished by members of the matrix metalloproteinase (MMP) family, which are multidomain zinc-dependent endopeptidases involved in tissue remodeling and tumor invasion (27–31). Most MMPs are secreted in a latent form and subsequently processed to active species, but they can also be found associated with the cell membrane as membrane-type (MT) MMPs (27, 29, 30). Membrane-type 1 MMP (MT1-MMP; also known as MMP-14) is capable of degrading several ECM proteins, including fibronectin, laminin, collagen, gelatin, and vitronectin, as well as non-ECM substrates such as SDF-1 and several adhesion receptors (29, 32–34). Furthermore, MT1-MMP is an activator of pro-MMP-2 in coordination with tissue inhibitor of metalloproteinase-2 (35–39), and it has been reported to be involved in tumor cell invasion and growth (35, 40–44). MT1-MMP has been found in malignant melanoma specimens (45, 46), raising the possibility that its proteolytic activity might be involved in melanoma cell dissemination.

Cell migration is associated with a reorganization of actin cytoskeleton that triggers changes in cell morphology. Activation of members of the Rho family of GTPases, such as RhoA, Rac1, and Cdc42, controls the dynamics of the actin cytoskeleton; they thus represent key regulatory molecules during cell migration (47, 48). Mutations leading to the expression of constitutively active Rho proteins have not been found in human tumors, but rather a common finding is their overexpression (49, 50). Nevertheless, ectopic expression of dominantly activated and negative mutant forms or overexpression of these GTPases has been crucial in characterizing their important roles in tumor cell invasion (49, 51–55). These mutant forms act as competitors with wild-type endogenous GTPases for binding to RhoGEF (guanine nucleotide exchange factor) for dominant-negative activity or as inhibitors of endogenous GTPase activity for constitutively active mutants (49).

One of the steps in melanoma cell metastasis potentially stimulated on interaction of SDF-1$\alpha$/CXCR4 could be the promotion of cell invasion across basement membranes. As outlined above, matrix degradation and migration mechanisms must be activated during tumor cell invasion; therefore, characterization of these mechanisms represents an important issue of study in cancer science. In the present work we investigated whether MT1-MMP and Rho GTPase functions

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on melanoma cells constitute targets for activation by SDF-1$\alpha$ as well as the consequences of these activations on melanoma cell invasion across basement membranes. The results from this study could contribute to a better knowledge and characterization of the chemotactic cues guiding tumor cell invasion.

## MATERIALS AND METHODS

**Cells and Antibodies.** The human melanoma cells lines BLM, Mel 57, and MeWo were cultured in DMEM (Gibco Invitrogen, Paisley, Scotland) supplemented with 10% fetal bovine serum (BioWhittaker, Verviers, Belgium) and antibiotics. Melanoma lymph node metastases were obtained after informed consent from patients undergoing surgery at the Department of Immuno-Oncology and Surgery, Hospital General Universitario Gregorio Marañón (Madrid, Spain). Histopathological diagnosis was confirmed for each specimen. A homogeneous cell suspension was obtained by mechanic disruption of tumor tissue fragments. Cells were seeded in flasks coated with 0.5% gelatin (Sigma Chemical, St. Louis, MO) and cultured in MCDB 153/L-15 medium (78%:20%; Sigma Chemical) supplemented with 2% fetal bovine serum, 5 $\mu$g/ml bovine insulin (Sigma Chemical), 15 $\mu$g/ml bovine pituitary extract (Clonetics BioWhittaker, Walkersville, MD), 5 ng/ml epidermal growth factor (Sigma), and 1.68 mM calcium chloride (complete medium). After 24 h, nonadherent cells were removed, and adherent cells were detached with 2 mM EDTA in PBS and frozen in complete medium containing 10% DMSO (Merck KgaA, Darmstadt, Germany).

For flow cytometry and invasion assays, frozen cells were thawed and grown to subconfluency in complete medium and used without any passage. Monoclonal antibody (mAb) to CXCR4 (44717.111) was purchased from R&D Systems (Minneapolis, MN), and anti-$\alpha$5-integrin and antipaxillin mAbs were from BD Biosciences PharMingen (San Diego, CA). The anti-MT1-MMP mAb LEM-2/15 has been described previously (56). The anti-CXCR4–01 mAb was kindly provided by Drs. Mario Mellado and José Miguel Rodríguez-Frade (Centro Nacional de Biotecnología, Madrid, Spain), and integrin anti-$\beta$1 mAbs Lia 1/2.1 and anti-$\alpha$2 TEA1/4.1, as well as control P3X63 mAb, were gifts of Dr. Francisco Sanchez-Madrid (Hospital de la Princesa, Madrid, Spain). Anti-transforming growth factor-$\beta$ (TGF-$\beta$) antibody 1D11.16.8 was obtained from the American Type Culture Collection (Rockville, MD), and antimacrophage colony-stimulating factor antibody was from R&D Systems. Antifibroblast AS02-clon was purchased from Dianova (Hamburg, Germany), anti-HMB-45 mAb was from Signet Laboratories Inc. (Dedham, MA), anti-HMW-MAA was from BD Biosciences PharMingen, and anti-S100 and anti-Melan A were from Novocastra Laboratories Ltd. (Newcastle upon Tyne, United Kingdom).

**Flow Cytometry.** Melanoma cell lines or melanoma cells from lymph node metastases were detached with 2 mM EDTA in PBS, and after washing, samples were resuspended in ice-cold PBS. Cells were incubated on ice for 30 min with primary antibodies, followed by incubation with FITC-conjugated antimouse or antirabbit secondary antibodies (DAKO A/S, Copenhagen, Denmark) and analysis in a Coulter Epics XL or a FACScan cytofluorometer. For S-100, Melan A, and HMB-45 intracellular staining, cells were fixed and permeabilized with the Cytofix/Cytoperm TM kit (PharMingen) according to the instructions of the manufacturer.

**Invasion Assays.** For Matrigel invasion assays, 15–30 $\times$ $10^3$ BLM, Mel 57, MeWo, transfected BLM, or melanoma cells isolated from lymph node metastases were resuspended in invasion medium (serum-free DMEM containing 0.4% BSA) and loaded onto 8 $\mu$m pore-size filters coated with 35–50 $\mu$l of 1:3 dilution of Matrigel (BD Biosciences) in Transwells (Costar, Cambridge, MA). For collagen invasion assays, 20 $\times$ $10^3$ BLM cells were loaded on filters coated with 60 $\mu$l of a 300 $\mu$g/ml solution of type I collagen (ICN Biomedicals Inc., Costa Mesa, CA). The lower compartments of invasion chambers were filled with medium alone or with medium containing different concentrations of SDF-1$\alpha$ (R&D Systems). After 22 h of incubation at 37°C, noninvading cells were removed from the upper surface of the filter, and cells that had migrated through the filter were fixed with 4% paraformaldehyde (Sigma), stained with crystal violet for Matrigel or toluidine blue for collagen invasions, and counted under a microscope. When invasion of transfected cells was assessed, green fluorescent protein (GFP)-positive cells on the lower surface of the filters were counted by fluorescence microscopy. When metastatic melanoma samples were used, invasive cells beneath the filters were fixed, permeabilized with 0.5% Triton X-100 (Sigma) in Tris-buffered saline, blocked with Tris-buffered saline containing 0.5% blocking reagent (Boehringer Mannheim GmbH, Mannheim, Germany), and incubated in the same solution for 30 min with anti-S-100, anti-Melan-A, or control antibodies. After washing, cells were incubated for 10 min with Alexa 546-conjugated antirabbit (Molecular Probes Inc., Eugene, OR) or FITC-conjugated antimouse (DAKO A/S) secondary antibodies. Finally, filters were washed, and melanoma cells were counted by fluorescence microscopy.

**Transfections.** BLM cells were transfected with expression vectors (pEGFP-C1) coding for GFP alone; GFP-fused forms of wild-type RhoA, Rac1, and CDC42; dominant-negative N19-RhoA, N17-Rac1, and N17-Cdc42; activated V14-RhoA and V12-Rac1 (gifts of Dr. Francisco Sanchez-Madrid); wild-type full-length MT1-MMP or MT1-MMP mutant lacking its cytoplasmic domain (MT1$\Delta$cyt-GFP; Ref. 57); or wild-type full-length CXCR4 (a gift of Dr. Antonio Serrano, Centro Nacional de Biotecnología, Madrid, Spain) by use of Lipofectamine reagent (Invitrogen) according to the manufacturer's instructions. In brief, cells were incubated for 3.5 h with 1.5 $\mu$g of vectors in the presence of Lipofectamine in serum-free Optimem medium (Invitrogen), followed by incubation for 18 h in the same medium supplemented with 10% FCS (BioWhittaker). After 36 h, cells were analyzed by flow cytometry to assess transfection efficiency, which was consistently >85%.

**Zymography Assays.** BLM cells incubated with or without SDF-1$\alpha$ or BLM cells transfected with MT1-MMP-GFP or MT1$\Delta$cyt-GFP were washed twice with PBS and lysed directly in Laemmli buffer. Samples were resolved under nonreducing conditions on SDS-PAGE gels embedded with 1 mg/ml fibrinogen (Calbiochem-Novabiochem Co., Darmstadt, Germany). Gels were rinsed three times with 2.5% Triton X-100, followed by incubation for 12 h at 37°C in 50 mM Tris-HCl (pH 7.5), 10 mM $CaCl_2$, and 200 mM NaCl. Gels were stained with Coomassie Blue, and areas of fibrinolytic activity were visualized as transparent bands.

**GTPase Activity Assays.** The glutathione *S*-transferase (GST)-PAK-CD and GST-C21 fusion proteins were generated as described previously (58). Melanoma cells were starved for 2 h in DMEM without serum, and after detachment they were washed and resuspended at 2.5 $\times$ $10^6$ cells/ml in DMEM containing 0.4% BSA. SDF-1$\alpha$ (150 ng/ml) was added to the cell suspensions and incubated at 37°C for different times followed by rapid washing with ice-cold PBS. Cells were lysed at 4°C in 300 $\mu$l of lysis buffer, as reported previously (20). Lysates were centrifuged; 15 $\mu$l of the supernatant were kept for total lysate controls, and the remaining volume was mixed with fusion proteins in the presence of glutathione-agarose beads. The mixtures were incubated for 16 h at 4°C, beads were pelleted and washed, and bound proteins were eluted in Laemmli electrophoresis buffer. Proteins were resolved by SDS-PAGE on 12% polyacrylamide gels and transferred to polyvinylidene fluoride membranes (Hybond-P; Amersham Pharmacia Biotech), which were incubated with antibodies to Rac1 or Cdc42 (BD Biosciences PharMingen) or to RhoA (Santa Cruz Biotechnology, Inc., Santa Cruz, CA). Recombinant C3 transferase was expressed and purified as described previously (59).

**Western Blotting and Reverse Transcription-PCR Analyses.** BLM cells (2.5 $\times$ $10^5$) incubated in the presence or absence of SDF-1$\alpha$, or 5 $\times$ $10^5$ transfected BLM cells expressing different GFP-fused proteins were solubilized at 4°C in lysis buffer (20). Proteins were resolved by SDS-PAGE and electrotransferred to polyvinylidene difluoride membranes (Amersham Pharmacia Biotech Inc., Piscataway, NJ). Membranes were blocked with 5% skim milk in PBS; incubated with LEM 2/15 anti-MT1-MMP, anti-RhoA, anti-Rac1, or anti-Cdc42 antibodies; washed with 0.1% Tween 20 in PBS; and then incubated with horseradish peroxidase-conjugated secondary antibodies. Proteins were visualized by use of SuperSignal chemiluminescent substrate (Pierce, Rockford, IL). After stripping and blocking, the same blots were reprobed with antipaxillin antibodies (BD Biosciences) to test for total protein content or with anti-GFP antibodies (Molecular Probes, Leiden, the Netherlands). For reverse transcription-PCR analysis, BLM cells (2 $\times$ $10^6$) treated under different conditions were lysed in TriReagent (Sigma-Aldrich, St. Louis, MO), and RNA was extracted and reverse-transcribed with use of Superscript reverse transcriptase (Gibco Invitrogen) and random hexadeoxynucleotide primers. Amplification of MT1-MMP cDNA was performed by PCR using AmpliTaq DNA polymerase (Roche Applied Science, Indianapolis, IN) with primers and profile conditions as described previously (60). Aliquots of each sample were amplified under the same conditions with the following glyceraldehyde-3-phosphate dehydrogenase primers as cDNA loading

control: 5′-GGCTGAGAACGGAAGCTTGTCA-3′ and 5′-CGGCCATCACGCCACAGTTTC-3′.

## RESULTS

**SDF-1α Promotes Melanoma Cell Invasion across Reconstituted Basement Membranes.** Matrigel is a basement membrane matrix extract rich in laminin, type IV collagen, heparan sulfate proteoglycans, and growth factors that when reconstituted retains structural and functional characteristics resembling those of basement membranes *in vivo* and constitutes a valuable *in vitro* basis for assessing the invasive potential of tumor cells (61). Only cells expressing or responding to invasive stimuli digest the Matrigel matrix and are capable of moving across this basement membrane. To investigate the potential of SDF-1α to promote melanoma cell invasion through basement membranes, we used the highly metastatic $CXCR4^+$ BLM melanoma cell line (20) in transwell invasion assays across Matrigel-coated filters. BLM cells displayed no or minimal invasiveness through Matrigel when no SDF-1α was present in the lower chamber of transwells. Instead, SDF-1α promoted substantial invasiveness of these cells in a dose-dependent manner (Fig. 1, *left panel*). Anti-CXCR4 mAb, as well as T134, a small molecule known to specifically block SDF-1/CXCR4 interactions (62), abolished the invasiveness, whereas binding of control anti-α5 integrin mAb to α5β1 on BLM cells (not shown) did not inhibit it. In addition, pertussis toxin also blocked BLM cell invasiveness in response to SDF-1α, suggesting the participation of $G\alpha_i$-dependent downstream signaling events. Two other melanoma cell lines positive for $CXCR4^+$ expression, Mel 57 (as detected by flow cytometry and immunocytochemistry; not shown) and MeWo (20), also displayed specific invasiveness in response to SDF-1α, but to a lesser extent than BLM cells (Fig. 1, *middle* and *right panels*). These results indicate that interaction of SDF-1α with CXCR4 on melanoma cells provides these cells with a remarkable potential to invade across reconstituted basement membranes.

**SDF-1α-Promoted Invasion by BLM Melanoma Cells: Role of MT1-MMP.** The membrane-type metalloproteinase MT1-MMP is capable of degrading several ECM proteins, including laminin, a main constituent of Matrigel basement membranes. MT1-MMP is expressed by BLM melanoma cells (60) and has been detected in malignant melanoma specimens (46). Because tumor cell invasion involves a remodeling of basement membrane layers in addition to cell locomotion, we first investigated whether promotion of SDF-1α-dependent BLM cell invasion through Matrigel involved the activity of MT1-MMP. SDF-1α-triggered BLM invasion was blocked to basal levels by the anti-MT1-MMP mAb LEM-2/15 (56), but not by control antibodies (Fig. 2*A*). Moreover, anti-MT1-MMP mAb abolished SDF-1α-dependent invasion of BLM cells across type I collagen, an additional MT1-MMP substrate that is found in dermal connective tissue and bones (Fig. 2*B*). These data indicate that the activity of MT1-MMP is necessary during SDF-1α-promoted invasion of BLM cells through Matrigel and type I collagen.

To investigate whether SDF-1α could influence MT1-MMP expression, we incubated BLM cells with this chemokine in the absence of serum and performed Western blot analysis with cell lysates using the LEM-2/15 mAb. The data revealed a 3–4-fold enhancement in the accumulation of 60-kDa active form of MT1-MMP after exposure for 24 h to SDF-1α compared with untreated BLM cells (Fig. 2*C*). On the other hand, when BLM cells were incubated with Matrigel, up-regulation of 60-kDa MT1-MMP expression was detected only when SDF-1α was present, and not in incubations with Matrigel alone (not shown), indicating that regulation of expression of this MMP was dependent on SDF-1α action. Furthermore, reverse transcription-PCR analyses revealed that the increase in MT1-MMP protein expression by SDF-1α was correlated with an enhancement in MT1-MMP mRNA levels on serum-starved, SDF-1α-treated BLM cells, compared with untreated controls (Fig. 2*D*). Whether enhanced MT1-MMP mRNA amounts reflect transcriptional or post-transcriptional regulatory events is not known at present. In addition to controlling MT1-MMP expression, lysates from SDF-1α-treated BLM cells displayed higher intrinsic MT1-MMP fibrinogenolytic activity compared with untreated cells (Fig. 2*C*).

Expression in BLM cells of GFP-fused full-length MT1-MMP (MT1-MMP-GFP) on top of the endogenous MT1-MMP (Fig. 3*A*) resulted in higher potential invasiveness across Matrigel in response to SDF-1α compared with GFP-mock-transfected counterparts (Fig. 3*B*). To test the specificity of the increased invasion after enhance-

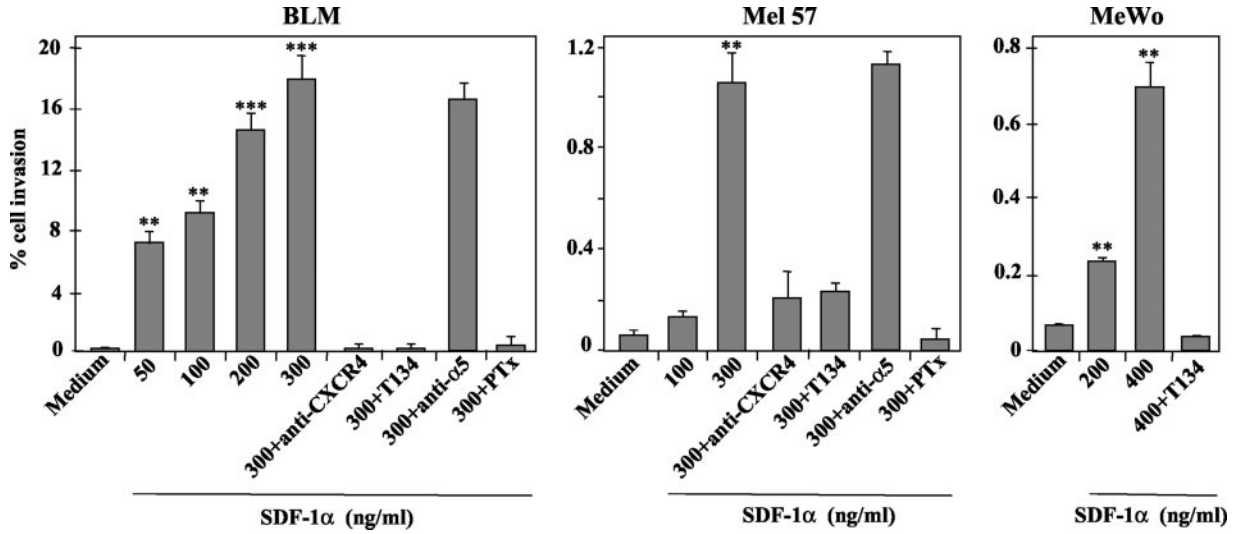


Fig. 1. Stromal cell-derived factor-1α (*SDF-1α*) promotes melanoma cell invasion across basement membranes. BLM, Mel 57, and MeWo melanoma cells were suspended in invasion medium and loaded on Matrigel-coated filters in invasion chambers, and invasion assays were performed with the stated concentrations of SDF-1α or medium alone (*Control*) as described in the "Materials and Methods." Where indicated, invasion was in the presence of anti-CXCR4 or anti-α5 integrin (15 μg/ml) monoclonal antibody, T134 (50 nM), or pertussis toxin (*PTx*; 100 ng/ml). The percentage of cell invasion (*Y axis*) represents the mean ± SD (*bars*) of at least three independent experiments done in duplicate for each cell line. The results of Student's two-tailed *t* test analysis for significant stimulation of invasion were as follows: ***, $P < 0.001$; **, $P < 0.01$.

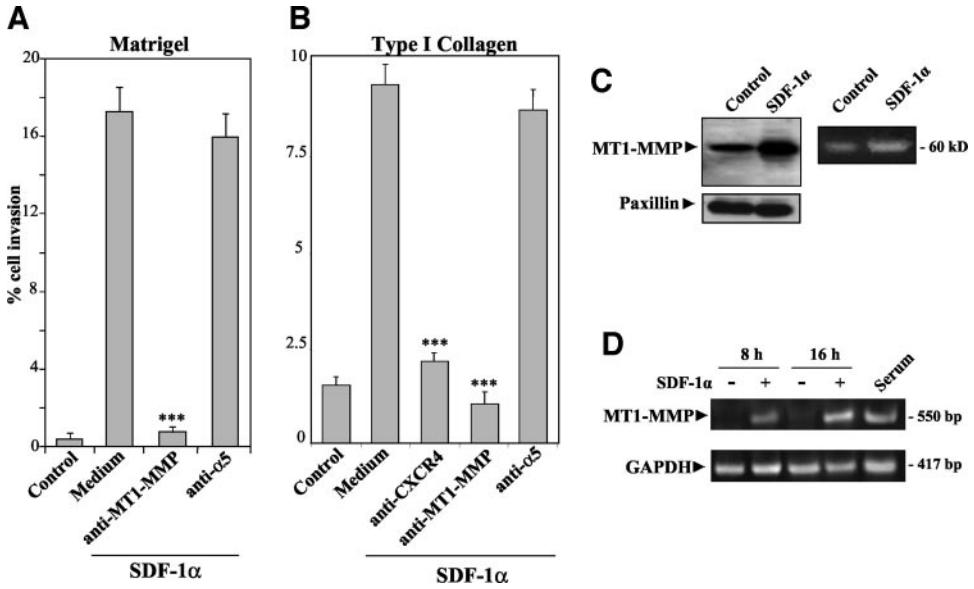


Fig. 2. Role of membrane type-1 matrix metalloproteinase (*MT1-MMP*) on stromal cell-derived factor-1$\alpha$ (*SDF-1$\alpha$*)-stimulated melanoma cell invasion. SDF-1$\alpha$-promoted invasion of BLM cells through Matrigel (*A*) or type I collagen (*B*) was assessed in the presence of anti-MT1-MMP LEM-2/15 (10 $\mu$g/ml), anti-CXCR4, or control anti-$\alpha$5 monoclonal antibody or in the absence of antibody (*Medium*). *Control* denotes invasion performed in the absence of SDF-1$\alpha$. The percentage of invasive cells (*Y axis*) represent the mean $\pm$ SD (*bars*) of five (*A*) or three (*B*) independent experiments done in duplicate. ***, invasion was significantly inhibited ($P < 0.001$) according to the Student's two-tailed *t* test. *C* and *D*, SDF-1$\alpha$ up-regulates MT1-MMP expression and activity on BLM melanoma cells. *C*, cells were incubated in the absence of serum for 24 h with or without SDF-1$\alpha$ (300 ng/ml). Cells were then lysed, and extracts were subjected to immunoblotting with anti-MT1-MMP monoclonal antibody (*left*) or to fibrinogenolytic zymography (*right*). After stripping and blocking, the same blot was reprobed with antipaxillin monoclonal antibody to check for total protein content. *D*, BLM cells were incubated for the indicated times with or without SDF-1$\alpha$ in the absence of serum or for 16 h with culture medium (*Serum*). RNA from cell lysates was reverse-transcribed, and PCR amplification of MT1-MMP mRNA was performed with MT1-MMP-specific primers. Also shown is control amplification using glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*)-specific primers. *kD*, kilodaltons.

ment of MT1-MMP expression, we overexpressed in BLM cells a MT1-MMP mutant form lacking its cytoplasmic region (MT1$\Delta$cyt-GFP), a domain shown to be required for efficient melanoma cell invasion (63). The MT1$\Delta$cyt-GFP transfectants displayed Matrigel invasion levels in response to SDF-1$\alpha$ close to those achieved by GFP-mock-transfected controls (Fig. 3*B*), although they retained fibrinogenolytic activity similar to that achieved by MT1-MMP-GFP transfectants (Fig. 3*A*). Together, these results indicate that promotion by SDF-1$\alpha$ of BLM melanoma cell invasion across basement membranes involves MT1-MMP activity and suggest that increased expression and activity of MT1-MMP induced by SDF-1$\alpha$ could represent a potential mechanism contributing to the invasion.

**Melanoma Cell Exposure to Matrigel and TGF-$\beta$1 Up-Regulates CXCR4 Expression.** Because BLM cells express low to moderate levels of surface CXCR4 (20), we tested the possibility that its expression could be modulated during the process of Matrigel invasion, given the substantial invasiveness of these cells (see Fig. 1). Flow cytometric analyses revealed a substantial up-regulation of cell surface CXCR4 expression on BLM cells on incubation with Matrigel, whereas expression of $\beta$1-integrin (Fig. 4) and $\alpha$2-integrin (not shown) was not significantly affected. In addition, incubation of these cells with type I collagen also promoted an increase in CXCR4 but not in $\beta$1-integrin expression. Moreover, exposure of BLM cells to TGF-$\beta$1 (2.25 ng/ml), a component of Matrigel, notably increased expression of CXCR4 (Fig. 4), but not CXCR3 (not shown). Instead, another component of this basement membrane matrix, such as epidermal growth factor, did not influence expression of CXCR4. Furthermore, anti-TGF-$\beta$, but not control, antibodies partially inhibited the enhancement in CXCR4 expression observed after BLM cell incubation with Matrigel (Fig. 4).

Enhanced CXCR4 expression on BLM cells was clearly detected at TGF-$\beta$1 concentrations of 0.5–2.25 ng/ml and to a lesser extent at 10 ng/ml, whereas addition of anti-TGF-$\beta$ antibodies reversed the increase in CXCR4 expression induced by TGF-$\beta$1 (Fig. 5*A*). Time kinetics analyses revealed that the increase in cell surface CXCR4 expression induced by TGF-$\beta$1 was optimally seen around 25 h of incubation with the cytokine and started to decrease by 30 h (Fig. 5*B*). No significant alterations in CXCR4 expression on the surface of BLM cells were detected before exposure for 12 h to TGF-$\beta$1 (not shown). These results indicate that CXCR4 expression on BLM melanoma cells is subject to regulation by interaction with Matrigel components, including TGF-$\beta$1, and by type I collagen.

To gain insight into the role of TGF-$\beta$1 on SDF-1$\alpha$-dependent BLM cell invasion, we performed Matrigel invasion assays in the presence of anti-TGF-$\beta$ antibodies. The results showed that these antibodies partially inhibited invasion of BLM cells in response to SDF-1$\alpha$, whereas invasions carried out with control antibodies were not significantly affected (Fig. 5*C*). To test whether increased CXCR4 expression could influence the invasiveness of BLM cells in response to SDF-1$\alpha$, we overexpressed CXCR4 in BLM cells by transfection of CXCR4-GFP forms (Fig. 5*D*) and studied the Matrigel invasion potential of transfectants in response to SDF-1$\alpha$. These transfectants exhibited 3–4-fold higher invasion levels in response to SDF-1$\alpha$ compared with their mock-transfected counterparts (Fig. 5*E*). Invasion was completely blocked by anti-CXCR4 antibodies, confirming the specificity of the assay, as well as by anti-MT1-MMP but not by control antibodies. Anti-TGF-$\beta$ antibodies inhibited SDF-1$\alpha$-promoted invasion of mock transfectants and decreased that of CXCR4-GFP transfectants ($P < 0.05$). Together these data suggest that at least a portion of SDF-1$\alpha$-dependent BLM invasion across reconstituted basement membranes may be attributable to enhanced CXCR4 expression by TGF-$\beta$1.

**SDF-1$\alpha$ Activates RhoA, Rac1, and Cdc42 on Melanoma Cells: Role in Invasion across Basement Membranes.** Coordinately with the degradation of basement membranes, tumor cells must also activate their locomotion mechanisms to migrate across these matrix layers. Cell locomotion is dependent on cell morphology changes that are based on reorganization of the actin cytoskeleton. Rho GTPases

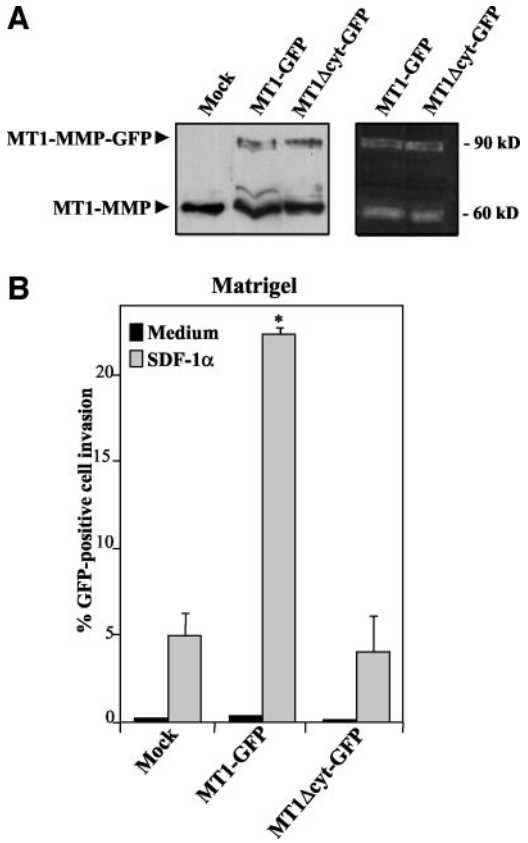


Fig. 3. Enhancement in membrane type-1 matrix metalloproteinase (*MT1-MMP*) expression results in up-regulation of stromal cell-derived factor-1$\alpha$-promoted melanoma cell invasion. *A*, BLM cells were transfected with expression vectors coding for green fluorescent protein (GFP)-fused full-length wild-type MT1-MMP (*MT1-GFP*), truncated MT1-MMP lacking the cytoplasmic domain (*MT1*$\Delta$*cyt-GFP*), or GFP alone (*Mock*). Expression and enzymatic activity of fusion proteins were checked in transfected cell lysates by immunoblotting with anti-MT1-MMP monoclonal antibody (*left*) and by fibrinogenolytic zymography (*right*), respectively. *kD*, kilodaltons. B, the same transfectants were subjected to Matrigel invasion assays in the presence of stromal cell-derived factor-1$\alpha$ (*SDF-1*$\alpha$; ▨) or medium alone (*Control*; ■). Invasive GFP-positive cells were counted under a fluorescence microscope, and the percentage of invasive cells (*Y axis*) represents the mean ± SD (*bars*) of two independent experiments done in duplicate. *, invasion was significantly stimulated with respect to Mock transfectants ($P < 0.05$), according to the Student's two-tailed *t* test.

such as RhoA, Rac1, and Cdc42 are key regulators of the dynamics of the actin cytoskeleton, and their activation controls cell migration (47, 48). Upstream events leading to the exchange of GDP for GTP bound to Rho GTPases activate them so they can interact with downstream targets to produce different biological responses. We investigated whether SDF-1$\alpha$ was capable of activating Rho GTPases on BLM cells and whether this activation could be involved in SDF-1$\alpha$-promoted BLM invasion through reconstituted basement membranes. To test for Rho GTPase activation by SDF-1$\alpha$, we performed GTPase assays with lysates from SDF-1$\alpha$-stimulated cells, using GST fusion proteins containing domains derived from Rho GTPase targets. Low or minimal levels of RhoA, Rac1, and Cdc42 activation were detected in unstimulated, serum-starved BLM cells. Incubation with SDF-1$\alpha$ rapidly promoted the activation of these cells, as detected with the GST-C21 fusion protein for RhoA or GST-PAK-CD for Rac1 and Cdc42 activation (58; Fig. 6), whereas treatment with C3 transferase, a specific inhibitor of RhoA activation, blocked the activation of this GTPase (Fig. 6*A*).

To investigate whether SDF-1$\alpha$-promoted BLM cell invasion across Matrigel was dependent on the activation of RhoA by this chemokine, we initially performed the invasion assays with C3 transferase-treated BLM cells. The results showed that C3 abolished the invasiveness of these cells in response to SDF-1$\alpha$ (Fig. 7*A*), suggesting that inhibition of RhoA activation could interfere with the invasion process. To further analyze the involvement of RhoA activation in SDF-1$\alpha$-dependent BLM invasion, we expressed by transfection GFP-fused wild-type, dominant-negative (N19-RhoA) or active (V14-RhoA) RhoA forms, as well as GFP alone (mock), and measured the

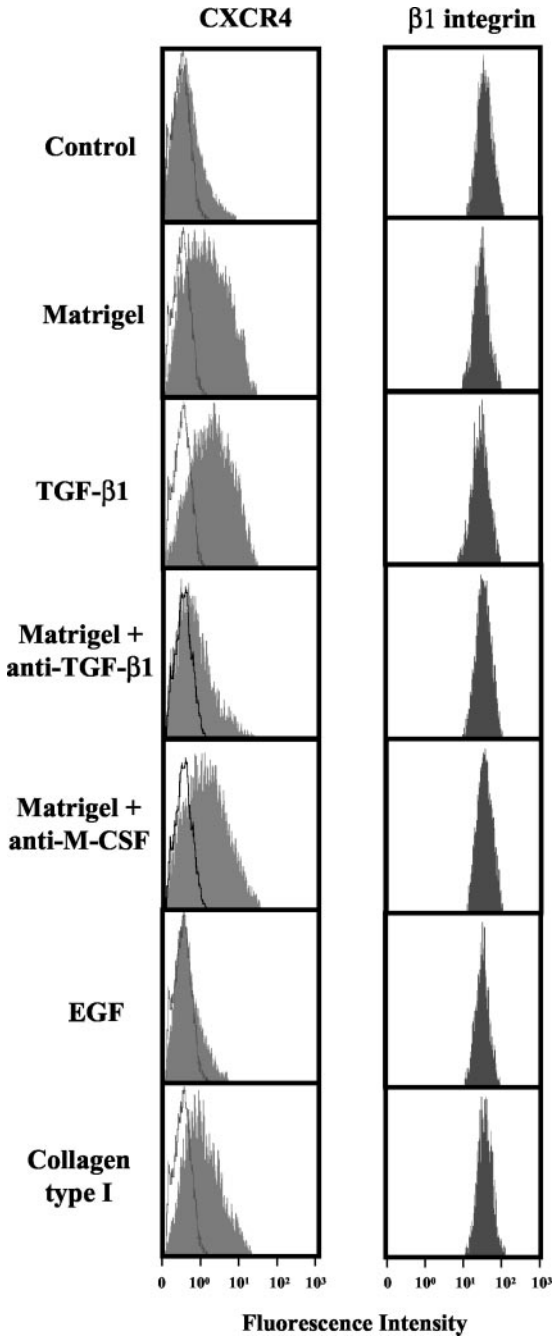


Fig. 4. Melanoma cell exposure to Matrigel up-regulates CXCR4 expression. BLM cells were resuspended in serum-free DMEM and cultured for 20 h in this medium alone (*Control*); in plates coated with Matrigel (0.4 $\mu$g/mm$^2$) in the absence (*Matrigel*) or presence of anti-transforming growth factor-$\beta$1 antibody (*Matrigel + anti-TGF-*$\beta$*1*) or control antimacrophage colony-stimulating factor antibody (*Matrigel + anti-M-CSF*; 30 $\mu$g/ml), or with type I collagen (*Collagen type I*; 0.12 $\mu$g/mm$^2$), or were incubated in the presence of transforming growth factor-$\beta$1 (*TGF-*$\beta$*1*; 2.25 ng/ml) or epidermal growth factor (*EGF*; 0.6 ng/ml). After incubation, cells were detached, washed, and incubated with anti-CXCR4 or Lia 1/2.1 anti-$\beta$1-integrin (10 $\mu$g/ml; *shaded curves*) monoclonal antibody or with control P3X63 (10 $\mu$g/ml; *unshaded curves*) monoclonal antibody, followed by incubation with FITC-conjugated secondary antibody and analysis by flow cytometry.

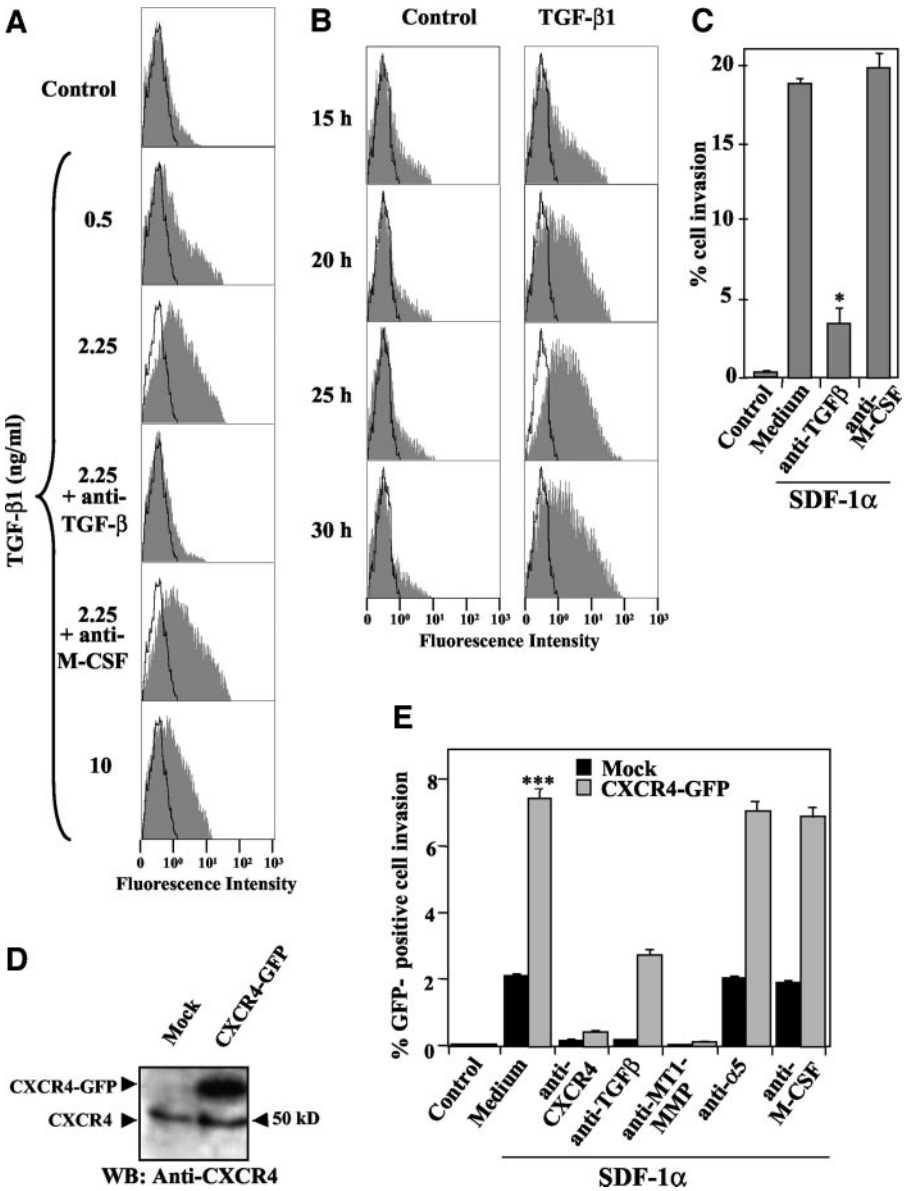


Fig. 5. Transforming growth factor-$\beta$1 (*TGF-$\beta$1*) controls CXCR4 expression on melanoma cells: role in stromal cell-derived factor-1$\alpha$ (SDF-1$\alpha$)-dependent invasion. *A*, BLM cells were kept in serum-free DMEM (incubation medium) for 24 h with the indicated concentrations of TGF-$\beta$1 in the absence or in the presence of anti-TGF-$\beta$ or control antimacrophage colony-stimulating factor (*anti-M-CSF*) antibodies. CXCR4 expression on cell surfaces was analyzed by flow cytometry using anti-CXCR4 (*shaded*) or control P3X63 (*unshaded*) monoclonal antibody. *B*, BLM cells were incubated for the indicated times in incubation medium with or without (*Control*) TGF-$\beta$1 (2.25 ng/ml), followed by analysis of CXCR4 expression by flow cytometry as above. *C*, BLM cells were subjected to Matrigel invasion assays in response to SDF-1$\alpha$ or medium alone (*Control*) in the presence or absence of anti-TGF-$\beta$ or control anti-M-CSF antibodies. *D*, BLM cells were transfected with vectors coding for CXCR4-green fluorescent protein (*CXCR4-GFP*) or GFP alone (*Mock*), and cell lysates were analyzed by immunoblotting with anti-CXCR4–01 monoclonal antibody. *E*, the same transfectants were subjected to Matrigel invasion assays in medium alone (*Control*) or with SDF-1$\alpha$ in the absence (*Medium*) or presence or of anti-CXCR4, anti-TGF-$\beta$, antimembrane type-1 matrix metalloproteinase (*anti-MT1-MMP*), or control anti-$\alpha$5 or anti-M-CSF antibodies. Percentage of invasive cells (*Y axis*) represents the mean $\pm$ SD (*bars*) of two independent experiments for each panel done in duplicate. In *C*, invasion was significantly inhibited (*, $P < 0.05$), and in *E* invasion was significantly stimulated with respect to mock transfectants (***, $P < 0.001$), according to the Student's two-tailed *t* test. *WB*, Western blotting; *kD*, kilodaltons.

invasion of transfectants across Matrigel in response to SDF-1$\alpha$. Overexpression of wild-type or activated RhoA did not significantly influence SDF-1$\alpha$-promoted invasion of BLM transfectants compared with mock transfectants (Fig. 7*B*, *top*). Instead, a substantial decrease in invasiveness in response to SDF-1$\alpha$ was obtained with transfectants expressing dominant-negative RhoA forms (Fig. 7*B*, *top*).

Likewise, expression of dominant-negative Rac1 (N17-Rac1) forms in BLM cells resulted in a 60–70% reduction in their invasiveness in response to SDF-1$\alpha$ compared with their mock-transfected or wild-type RhoA-transfected counterparts (Fig. 7*C*, *top*). In addition, transfectants expressing active forms of Rac1 (V12-Rac1) displayed a marked up-regulation in SDF-1$\alpha$-triggered Matrigel invasion. In contrast to the above results, BLM cells expressing GFP-fused dominant-negative forms of Cdc42 did not display significant alterations in their invasive potential in response to SDF-1$\alpha$ compared with transfectants expressing wild-type Cdc42 (Fig. 7*D*, *top*). Western blot control experiments using anti-GFP, anti-RhoA, anti-Rac1, or anti-Cdc42 antibodies confirmed the expression of the different GTPase forms in

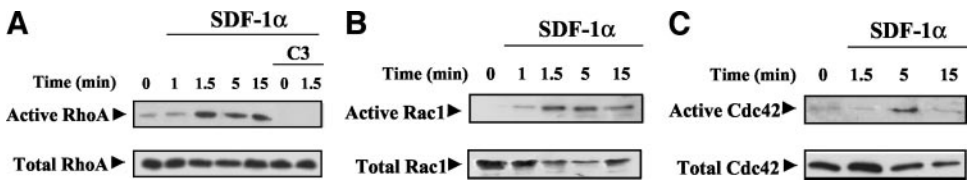


Fig. 6. Stromal cell-derived factor-1$\alpha$ (*SDF-1$\alpha$*) activates RhoA, Rac1, and Cdc42 on BLM melanoma cells. For RhoA (*A*), BLM cells were first exposed to C3 exozyme (50 $\mu$g/ml for 16 h) or serum-free DMEM alone. Cells were incubated for the indicated times in the presence or absence of SDF-1$\alpha$, followed by solubilization and incubation of cell extracts with glutathione-agarose-bound GST-C21 to detect active RhoA or with GST-PAK-CD for active Rac1 and Cdc42 (*B* and *C*, respectively). After elution in Laemmli sample buffer, bound RhoA, Rac1, and Cdc42 were analyzed by Western blotting using anti-RhoA, anti-Rac1, or anti-Cdc42 monoclonal antibody. Aliquots from each lysate were kept aside for measurement of total protein.

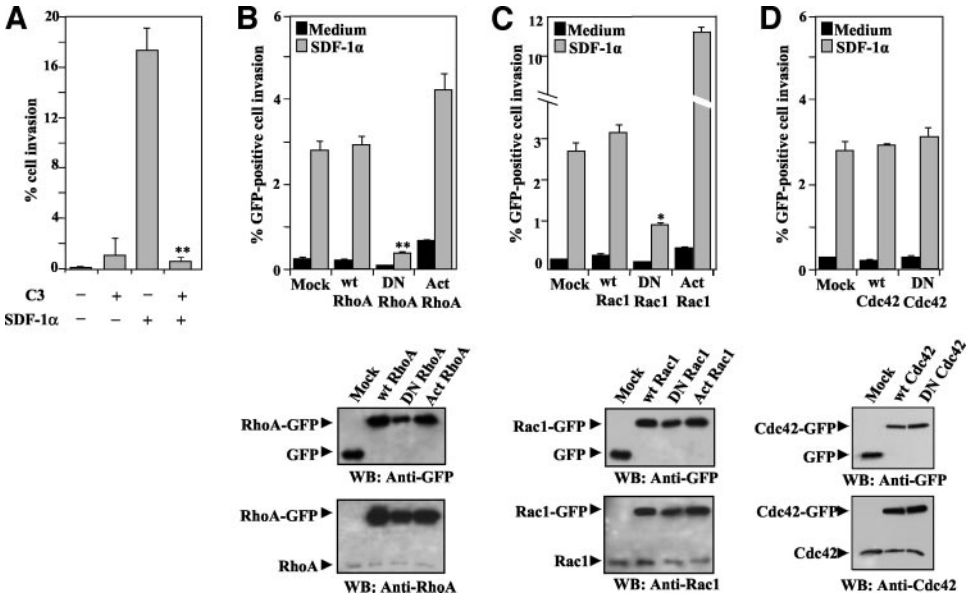


Fig. 7. Role of stromal cell-derived factor-1α (*SDF-1α*)-triggered RhoA and Rac1 activation on BLM melanoma cell invasion. *A*, BLM cells were subjected to invasion assays across Matrigel in the presence of SDF-1α (300 ng/ml) or medium alone, in the absence (−) or in the presence (+) of C3 transferase (50 μg/ml). The percentage of cell invasion (*Y axis*) was determined as in Fig. 1. *B–D*, BLM cells were transfected with expression vectors coding for the wild-type (*wt*), dominant-negative (*DN*), or active (*Act*) forms of RhoA-, Rac1-, or Cdc42-green fluorescent protein (GFP) fusion proteins or with GFP alone (*Mock*). Two days after transfection, $3 \times 10^4$ cells/condition were subjected to Matrigel invasion assays in the presence of SDF-1α or medium alone (*Control*); only invasive GFP-positive cells were counted by fluorescence microscopy. The rest of the cells ($10^6$/condition) were lysed to assess expression of fusion proteins by immunoblotting with anti-RhoA, anti-Rac1, anti-Cdc42, or anti-GFP monoclonal antibody. The percentage of invasive cells (*Y axis*) represents the mean ± SD (*bars*) of at least three independent experiments done in duplicate for each panel. Results of analysis with the Student's two-tailed *t* test to determine whether invasion was significantly inhibited were as follows: **, $P < 0.01$; *, $P < 0.05$. *WB*, Western blotting.

BLM transfectants (Fig. 7, *B–D*, *bottom*). The viability of dominant-negative, activated, and wild-type RhoA, Rac1, and Cdc42 BLM transfectants was assessed 48 h post-transfection by trypan blue exclusion, which showed only 5–7% cell death and no significant variations among the different transfectants. Altogether, these data indicate that activation of endogenous RhoA and Rac1 by SDF-1α on BLM cells plays an important role in their invasion across basement membranes promoted by this chemokine.

**Role of SDF-1α-Promoted RhoA and Rac1 Activation on MT1-MMP Expression on Melanoma Cells.** To investigate whether there was a functional relationship between Rho-GTPase and MT1-MMP activation promoted by SDF-1α on BLM melanoma cells, we first tested the effect of C3 treatment on the expression of MT1-MMP. Up-regulation of MT1-MMP expression by SDF-1α was significantly impaired on exposure of BLM cells to C3 (Fig. 8*A*). Moreover, Western blot and reverse transcription-PCR analyses revealed that expression of dominant-negative RhoA and Rac1 forms on BLM cells resulted in a blockade of increased MT1-MMP expression in response to SDF-1α compared with the enhancement detected in transfectants expressing wild-type RhoA and Rac1 (Fig. 8, *B–E*), whereas expression of dominant-negative forms of Cdc42 did not influence this increase (not shown). These results suggest that RhoA- and Rac1-promoted signaling originating in response to stimulation with SDF-1α controls the subsequent expression of MT1-MMP and that it therefore could represent a mechanism regulating SDF-1α-triggered invasion of BLM melanoma cell across basement membranes.

**SDF-1α Promotes Invasion of Metastatic Lymph Node Melanoma Cell across Basement Membranes.** Melanoma cells isolated from lymph node metastases were thawed and cultured for 24–48 h before use in Matrigel basement membrane assays in the presence of SDF-1α. Four of five samples exhibited SDF-1α-dependent invasion levels ranging from 0.65 to 1% of the cell input that was blocked by anti-CXCR4 mAb and by T134 ($P < 0.01$), implicating the involvement of SDF-1α/CXCR4 interactions in this invasion. In addition, anti-MT1-MMP mAb ($P < 0.05$), but not control antibodies, interfered with SDF-1α-promoted invasion (Fig. 9*A*). Moreover, treatment of melanoma cells with C3 also abolished their invasiveness in response to SDF-1α. To characterize the invaded cells, we performed immunocytochemistry with a combination of the melanoma markers S100 and Melan-A. The results showed that >99% of β1-integrin-positive cells expressed both markers (not shown), indicating that the invading cell population corresponded to melanoma cells.

Melanoma cells isolated from lymph node metastases and incubated in the presence of Matrigel or TGF-β1 exhibited an enhancement in CXCR4 cell surface expression (Fig. 9*B*) similar to the increase detected in BLM melanoma cells. These data indicate that CXCR4 on metastatic melanoma cells is capable of mediating SDF-1α-dependent invasion across basement membranes and that MT1-

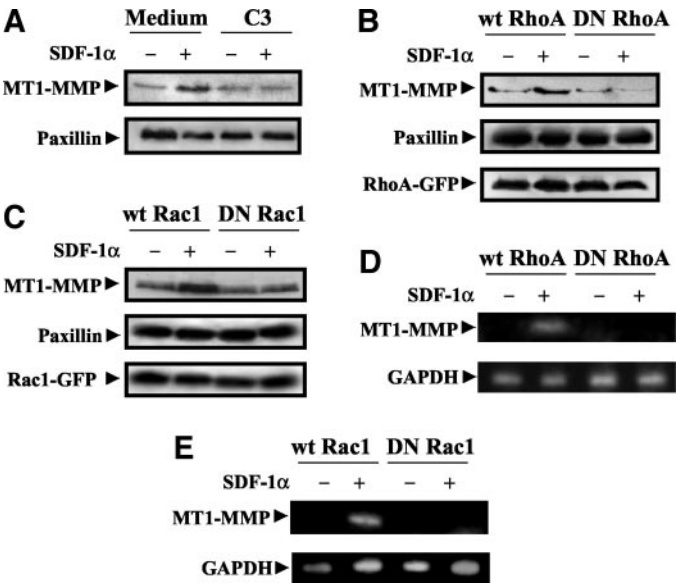


Fig. 8. Role of stromal cell-derived factor-1α (*SDF-1α*)-promoted RhoA and Rac1 activation on membrane type-1 matrix metalloproteinase (*MT1-MMP*) expression on melanoma cells. *A*, BLM cells were treated for 24 h with C3 transferase or in serum-free DMEM alone in the absence (−) or presence (+) of SDF-1α (300 ng/ml). *B–E*, BLM cells were transfected with expression vectors coding for wild-type (*wt*) or dominant-negative (*DN*) RhoA- or Rac1-green fluorescent protein (*GFP*) fusion proteins, and 2 days after transfection cells were incubated for 24 h (*B* and *C*) or 16 h (*D* and *E*) with (+) or without (−) SDF-1α. After incubation with this chemokine, cells were lysed, and extracts were subjected to immunoblotting with anti-MT1-MMP monoclonal antibody. After stripping and blocking, the same blots were reprobed with antipaxillin (*A*, *B*, and *C*) or anti-RhoA (*B*) or anti-Rac1 mAb to check for total protein content. RNA from cell lysates was reverse-transcribed, and amplification of MT1-MMP mRNA was performed by PCR using MT1-MMP-specific primers (*D* and *E*). Also shown is control amplification using glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*)-specific primers.

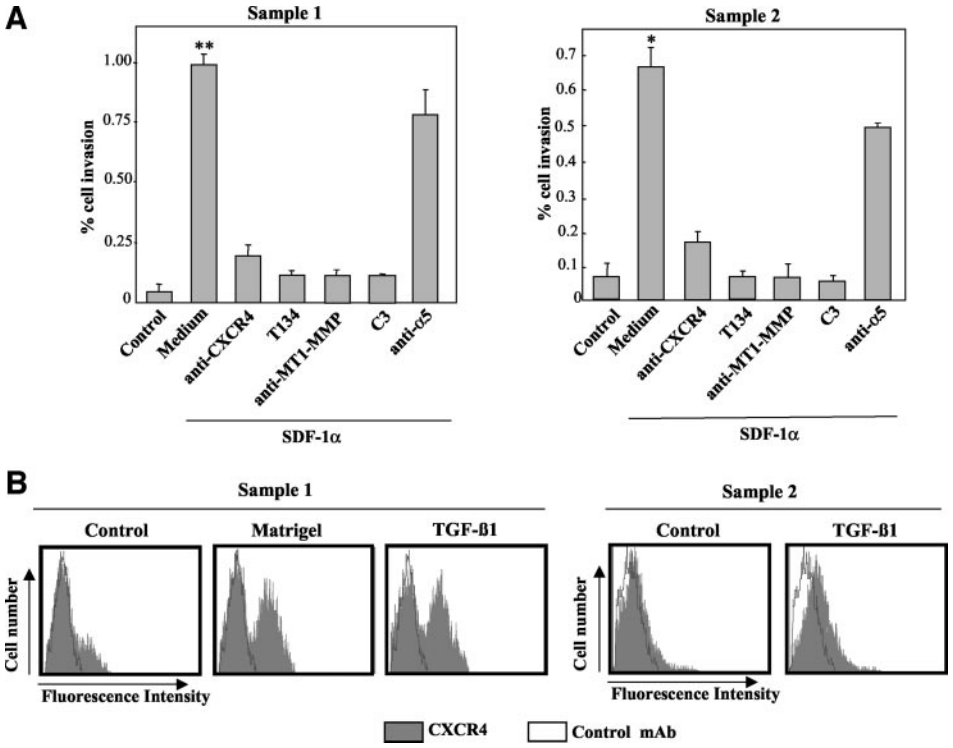


Fig. 9. Stromal cell-derived factor-1$\alpha$ (*SDF-1$\alpha$*) promotes invasion of metastatic lymph node melanoma cells across Matrigel reconstituted basement membranes. *A*, two different melanoma samples were resuspended in invasion medium and subjected to Matrigel invasion assays with SDF-1$\alpha$ in the presence of anti-CXCR4, anti-MT1-MMP, anti-$\alpha$5 monoclonal antibody or no antibodies (*Medium*) or in the presence of T134 or C3 transferase. *Control* denotes invasion performed in the absence of SDF-1$\alpha$. After 24 h, invaded cells were fixed, stained, and counted as in Fig. 1. Results of the Student's two-tailed *t* test for significant stimulation of invasion were as follows: **, $P < 0.01$; *, $P < 0.05$. *B*, melanoma cells were resuspended in serum-free DMEM and cultured for 20 h in this medium alone (*Control*), in plates coated with Matrigel (*Matrigel*), or in the presence of transforming growth factor-$\beta$1 (*TGF-$\beta$1*; 2.25 ng/ml). After incubation, cells were detached, washed, and incubated with anti-CXCR4 (*shaded curves*) or control P3X63 (*unshaded curves*) monoclonal antibody (*mAb*), followed by incubation with FITC-conjugated secondary antibody and analysis by flow cytometry. Shown are two results representative of four separate experiments.

MMP activity, activation of RhoA, and possibly an increase in CXCR4 expression are involved in this invasion.

## DISCUSSION

Basement membranes constitute biological barriers that must be traversed by tumor cells to invade surrounding tissues during metastasis. Chemokines are potential candidates to guide and activate tumor cells through basement membranes and tissues on binding to their membrane G-protein-coupled receptors. Melanoma cells express the chemokine receptor CXCR4, which interacts with its ligand SDF-1$\alpha$, triggering their adhesion as well as mitogen-activated protein kinase activation (5, 20). Reconstituted basement membranes such as Matrigel retain structural and functional characteristics resembling those of basement membranes *in vivo*, providing a useful system to test the invasive potential of tumor cells. In the present study we showed that SDF-1$\alpha$ promotes invasion of Matrigel basement membrane by the BLM, Mel 57, and MeWo melanoma cell lines, as well as invasion by melanoma cells isolated from lymph node metastases. In addition to Matrigel invasion, SDF-1$\alpha$ was also capable of triggering invasion of BLM cells across type I collagen, a constituent of dermal connective tissues and bones.

Activating signals originated on SDF-1$\alpha$/CXCR4 interaction therefore lead to the invasion of melanoma cells across Matrigel, indicating that matrix degradation and migration mechanisms had been stimulated in these cells. Expression of the membrane-type metalloproteinase MT1-MMP has been found in malignant melanoma (45, 46), as well as on melanoma BLM cells (60), raising the possibility that the proteolytic activity of this metalloproteinase could contribute to melanoma cell dissemination. We demonstrate here that MT1-MMP activity is necessary for SDF-1$\alpha$-promoted invasion of BLM and metastatic melanoma cells across Matrigel, as well as for BLM invasion through type I collagen matrices. Moreover, SDF-1$\alpha$ increased MT1-MMP expression on BLM cells, as well as its intrinsic fibrinogenolytic activity, and enhancement of MT1-MMP expression by transfection with MT1-MMP-GFP forms resulted in up-regulated BLM invasion across Matrigel in response to SDF-1$\alpha$. These data suggest that increased expression of MT1-MMP in response to SDF-1$\alpha$ could represent one of the mechanisms behind MT1-MMP proteolytic activity on Matrigel and type I collagen during SDF-1$\alpha$-promoted melanoma cell invasion. Whether increased pro-MMP-2 activation by MT1-MMP takes place during SDF-1$\alpha$-triggered BLM invasion requires further investigation.

Promotion by SDF-1$\alpha$ of melanoma cell invasion associated with the stimulation of matrix digestion should take place coordinately with activation of locomotion mechanisms to allow migration of these cells across basement membranes. Earlier reports revealed that ectopic expression of constitutively active mutant forms of RhoA and Rac1 in different tumor cell types stimulated tumor cell locomotion and invasion (53–55). Here we showed that SDF-1$\alpha$ activates RhoA, Rac1, and Cdc42 on BLM melanoma cells and that expression of dominant-negative forms of RhoA and Rac1, but not Cdc42, or blocking of RhoA activation by C3 transferase, resulted in a substantial reduction in SDF-1$\alpha$-dependent melanoma cell Matrigel potential invasiveness. On the other hand, BLM transfectants expressing active Rac1 displayed enhanced invasiveness in response to SDF-1$\alpha$ above the levels achieved by transfectants expressing wild-type forms of this GTPase, suggesting that activated Rac1 functionally synergizes with SDF-1$\alpha$-promoted Matrigel invasion. Together, these data strongly suggest that activation of RhoA and Rac1 functions on melanoma cells in response to SDF-1$\alpha$ constitutes a potential mechanism that could contribute to their invasion across basement membranes in response to this chemokine. It does not exclude, however, that although SDF-1$\alpha$ is the trigger of BLM cell motility, it might not be the only stimulus contributing to RhoA and Rac1 activation during invasion, because integrin-mediated cell interactions with ECM components of Matrigel or mitogens present in Matrigel might also activate these GTPases (64).

In conjunction with activation of matrix digestion and cell migration by SDF-1$\alpha$, we show here that incubation of BLM and metastatic melanoma cells with Matrigel or type I collagen, as well as with

TGF-$\beta$1, a major cytokine present in Matrigel, led to substantial enhancement in cell surface CXCR4 expression. Moreover, ectopic expression of CXCR4-GFP forms in BLM cells led to enhanced invasion in response to SDF-1$\alpha$, suggesting that an increase in CXCR4 receptors could be a mechanism involved in SDF-1$\alpha$-promoted invasion. Furthermore, anti-TGF-$\beta$ antibodies inhibited both increased CXCR4 expression and SDF-1$\alpha$-promoted BLM Matrigel invasion. Therefore, these data suggest that enhancement in CXCR4 expression on the surface of melanoma cells on contact with basement membrane components, including TGF-$\beta$1, and/or with type I collagen might lead to a more efficient cell response to SDF-1$\alpha$ and thus contribute to increased invasiveness. It is likely that melanoma cells become exposed to TGF-$\beta$ signaling because TGF-$\beta$ is present in the tumor microenvironment (65), and this could result in up-regulated CXCR4 expression, as reported here. The mechanisms underlying the modulation of CXCR4 expression by TGF-$\beta$1, as well as by type I collagen, remain to be determined. Previous work showed that expression of CXCR4 on T lymphocytes is also subject to modulation by TGF-$\beta$1 (66, 67).

Type I collagen is a main constituent of interstitial stroma, which must be traversed by tumor cells in addition to basement membranes during invasion (26). MT1-MMP digestion of type I collagen was shown previously to confer cell invasiveness (68). The present results show that MT1-MMP activity is needed during SDF-1$\alpha$-promoted BLM cell invasion of type I collagen matrices, which might involve increased MT1-MMP expression in response to SDF-1$\alpha$, as well as by type I collagen (43). Furthermore, up-regulated CXCR4 expression in response to type I collagen could represent an additional factor contributing to the increased invasiveness in response to SDF-1$\alpha$. These data raise the possibility that SDF-1$\alpha$/CXCR4 interactions might provide melanoma cells with additional means to invade across stroma rich in type I collagen.

Collectively, the results from the present work indicate that SDF-1$\alpha$ confers on melanoma cells a remarkable potential to invade across reconstituted basement membranes and type I collagen matrices, involving SDF-1$\alpha$-promoted stimulation of MT1-MMP activity and RhoA- and Rac1-dependent cell motility, which could be contributed by increased CXCR4 expression during invasion. Because MT1-MMP function and activation of Rho GTPases have been shown to play important roles during tumor cell invasion (28, 29, 53–55), the identification of SDF-1$\alpha$ as a potential stimulatory molecule for both types of molecules during melanoma cell invasion provides important additional knowledge of the mechanisms stimulating melanoma cell dissemination. SDF-1$\alpha$ present in the tumor environment might work together with other cytokines and cell adhesion events, playing important roles in the activation of MT1-MMP and Rho GTPases in melanoma cells and contributing to the acquisition of an invasive phenotype. It is likely that cell locomotion and ECM degradation are functionally interdependent for melanoma cells to invade in response to SDF-1$\alpha$ stimuli. In this regard, it has been reported that MMP levels can be modulated by RhoA and Rac1 (69–71). We show here that blocking of SDF-1$\alpha$-promoted RhoA and Rac1 activation on BLM cells inhibits up-regulation of MT1-MMP mRNA and protein expression by this chemokine, which is compatible with regulation of gene expression or post-transcriptional changes. These data suggest that SDF-1$\alpha$-triggered, Rho GTPase-dependent signaling leading to the control of MT1-MMP expression could represent a coordination mechanism between cell motility and cell surface proteolysis that possibly influences invasiveness in response to SDF-1$\alpha$.

Recent *in vivo* data have shown that chemokines can contribute to the homing of tumor cells to distinct organs in a manner similar to that for differential leukocyte tissue distribution, which depends on the chemokine expression pattern. For example, CXCR4 expression conveyed metastasis of human breast cancer cells into regional lymph nodes and lung (5). In line with these findings, down-regulation of CXCR4 expression by RNA interference resulted in inhibition of breast cancer cell invasiveness *in vitro* (72). In melanoma, the expression of CXCR4 and CCR7 on B16 murine cells enhanced their pulmonary and lymph node metastatic potential, respectively (22, 24, 25). Together with the *in vitro* melanoma cell invasion results presented here, these data indicate that SDF-1$\alpha$ could play important roles during melanoma cell metastasis, providing both invasion and migratory directions for basement membrane and interstitial stroma infiltration in the skin (9), as well as representing an important stimulus for homing of these cells to additional sites where this chemokine is expressed, such as lymph nodes, lung, and liver (5). The finding that CXCR4 expression could be modulated during melanoma cell invasion provides an attractive potential mechanism for controlling their dissemination. In support of this hypothesis, the involvement of the von Hippel-Lindau tumor suppressor pVHL in the modulation of CXCR4 expression has been reported recently, suggesting that it could control tumor cell dissemination (73).

In conclusion, interaction of SDF-1$\alpha$ with CXCR4 on melanoma cells conveys invasive, migratory, and activating signals to these cells, similar to the signals for motile cells such as leukocytes. Characterization of the stimuli modulating CXCR4 expression and intracellular signaling triggered by SDF-1$\alpha$ could contribute to a better knowledge of the scenario of tumor cell metastasis and could lead to potential findings to improve therapeutic approaches.

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