The $G_{\alpha_{13}}$-Rho Signaling Axis Is Required for SDF-1-induced Migration through CXCR4*

Received for publication, September 25, 2006 Published, JBC Papers in Press, October 20, 2006, DOI 10.1074/jbc.M609062200

Wenfu Tan, Daniel Martin, and J. Silvio Gutkind

From the Oral and Pharyngeal Cancer Branch, NIDCR, National Institutes of Health, Department of Health and Human Services, Bethesda, Maryland 20892-4330

The CXC chemokine stromal-derived factor-1$\alpha$ (SDF-1) binds to CXCR4, a seven-transmembrane $G$ protein-coupled receptor that plays a critical role in many physiological processes that involve cell migration and cell fate decisions, ranging from stem cell homing, angiogenesis, and neuronal development to immune cell trafficking. CXCR4 is also implicated in various pathological conditions, including metastatic spread and human immunodeficiency virus infection. Although SDF-1-induced cell migration in CXCR4-expressing cells is sensitive to pertussis toxin treatment, hence involving heterotrimeric $G$ proteins of the $G_i$ family, whether other $G$ proteins participate in the chemotactic response to SDF-1 is still unknown. In this study, we took advantage of the potent chemotactic activity of SDF-1 in Jurkat T-cells to examine the nature of the heterotrimeric $G$ protein subunits contributing to CXCR4-mediated cell migration. We observed that whereas $G_i$ and $G_{\beta\gamma}$ subunits are involved in SDF-1-induced Rac activation and cell migration, CXCR4 can also stimulate Rho potently leading to the phosphorylation of myosin light chain through the Rho effector, Rho kinase, but independently of $G_i$. Furthermore, we found that $G_{\alpha_{13}}$ mediates the activation of Rho by CXCR4 and that the functional activity of both $G_{\alpha_{13}}$ and Rho is required for directional cell migration in response to SDF-1. Collectively, our data indicate that signaling by CXCR4 to Rho through $G_{\alpha_{13}}$ contributes to cell migration when stimulated by SDF-1, thus identifying the $G_{\alpha_{13}}$-Rho signaling axis as a potential pharmacological target in many human diseases that involve the aberrant function of CXCR4.

Chemokines are a group of chemoattractant cytokines that are involved in key developmental and homeostatic events, including leukocyte trafficking, angiogenesis, hematopoiesis, inflammation, immune response, and organogenesis, as well as in tumor progression and metastasis (1). They are divided into four subfamilies, CXC, CC, C, and CX3C, based on their structural properties and primary amino acid sequence (1, 2). The stromal-derived factor-1$\alpha$ (SDF-1),$^2$ also named CXCL12, belongs to the CXC subfamily and was first cloned from bone marrow stromal cells and shown to induce proliferation and differentiation of B cell progenitors (3). SDF-1 is expressed in a wide range of normal tissues such as bone marrow, lymph nodes, lung, liver, and brain (4). SDF-1 exerts functions on binding to CXCR4, a seven-transmembrane $G$ protein-coupled receptor (GPCR) (4–6) that is highly conserved across species and is expressed on a wide variety of cell types, including hematopoietic cells, vascular endothelial cells, neurons, microglia, and astrocytes (7). The SDF-1-CXCR4 signaling system is now known to be critical for the regulation of the migration, proliferation, differentiation, and survival of lymphocyte, as reflected by its key role in lymphocyte trafficking and overall immune surveillance (8). CXCR4 is also an obligatory co-receptor for the infection of T-cell tropic human immunodeficiency virus (HIV) strains (9, 10).

The function of SDF-1 and CXCR4 is not restricted to the immune system. Indeed, mouse embryos lacking either SDF-1 or CXCR4 display many lethal defects, including impaired hematopoiesis, malformations of the intestinal vasculature, cardiac ventricular septal defects, and abnormal migration of cerebellar neurons (11–13). In addition, SDF-1 and CXCR4 participate in a number of pathologic conditions that involve aberrant cell motility, such as the metastatic spread of cancerous cells (14). For example, CXCR4 is largely overexpressed in many frequently malignant tumors, including breast, lung, and prostate cancer, whereas the expression of SDF-1 is high in the organs in which these tumors metastasize, such as lymph nodes and lung (15). Furthermore, SDF-1 is a potent chemoattractant for breast cancer cells in vitro, and neutralizing CXCR4 antibodies inhibit the metastasis of these cells to organs with high expression of SDF-1 in vivo (16).

The signaling mechanisms by which CXCR4 promotes cell migration are not fully elucidated. It is well known that CXCR4 is coupled to pertussis toxin (PTX)-sensitive heterotrimeric $G$ proteins of the $G_i$ family, which promote the activation of phosphatidylinositol 3-kinase and the accumulation of D-3-phosphoinositide lipids (17, 18). Phosphatidylinositol 3-kinase signaling can in turn promote cell migration in response to SDF-1 by activating ITK, a Tec family kinase (19). However, whether...

---

$^*\text{This work was supported by the Intramural Research Program of the National Institutes of Health, NIDCR. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked }^{\text{advertisement}}\text{ in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.}$

$^1\text{To whom correspondence should be addressed: Oral and Pharyngeal Cancer Branch, NIDCR, National Institutes of Health, 30 Convent Dr., Bldg. 30, Rm. 211, Bethesda, MD 20892-4340. Tel.: 301-496-6259; Fax: 301-402-0823; E-mail: sg39v@nih.gov.}$

$^2\text{The abbreviations used are: SDF, stromal cell-derived factor-1$\alpha$; GPCR, } G\text{ protein-coupled receptor; HIV, human immunodeficiency virus; PTX, pertussis toxin; ERK, extracellular signal-regulated kinase; MLC, myosin light chain; GFP, green fluorescent protein; PMA, phorbol-12-myristate-13-acetate; shRNA, short hairpin RNA; PAK, p21-activated kinase; GEF, guanine nucleotide exchange factor.}$
other heterotrimeric G proteins are also required for cell migration and, if so, the nature of their downstream targets is not fully understood. Here, we took advantage of the fact that Jurkat T-cells express CXCR4 receptors endogenously, and thus migrate readily in response to SDF-1, to begin dissecting the signaling network by which CXCR4 controls directional cell motility. We found that whereas Gᵢ regulates cell migration by activating Rac through Gᵢᵧ heterotrimeric G protein subunits, CXCR4 utilizes the α subunit of G₁₃, to activate Rho and that the functional activity of Gᵢ and G₁₃, hence the coordinated activation of Rac and Rho, are both required for CXCR4-induced cell migration in response to SDF-1.

EXPERIMENTAL PROCEDURES

Materials—Recombinant human stromal cell-derived factor 1 alpha and biotin-conjugated mouse monoclonal anti-CXCR4 antibody and mouse IgG₁₃ isotype antibody were purchased from R&D Systems (Minneapolis, MN). Rabbit monoclonal phospho-ERK1/2 (p-ERK1/2) and phospho-myosin light chain (p-MLC) and MLC antibodies were purchased from Cell Signaling Technology (Beverley, MA). Rabbit polyclonal antisera against ERK1/2, Rho, G₁₃, G₁₃, and α-tubulin were obtained from Santa Cruz Biotechnology (Santa Cruz, CA); mouse monoclonal antibody against Rac and rod α transducin were obtained from BD Transduction Laboratories. Monoclonal antibody against AU-1, AU-5, and GFP epitope were purchased from Covance (Burlingame, CA). AMD3100 octahydrochloride was purchased from Sigma. PTX and phorbol-12-myristate-13-acetate (PMA) were obtained from Calbiochem. Dual luciferase reporter system was purchased from Promega (Madison, WI).

Constructs—Expression vectors for Gα transducin (Gᵢ₃), G₁₃, G₁₃, G₁₃, G₁₃, PK-A, PAK-NL2, RhoN19, GFP fused to the RGS domain of PDZ-RhoGEF (GFP-RGS), and C3 toxin have been previously described (20–23).

Cell Lines and Transfection—Jurkat cells were cultured in RPMI 1640 (Sigma) supplemented with 10% fetal calf serum (BioWhittaker, Walkersville, MD). Human epithelial kidney 293-T cells were grown and maintained in Dulbecco’s modified Eagle’s medium (Sigma) containing 10% fetal calf serum. Cells were transfected by Lipofectamine PLUS (Invitrogen) following the manufacturer’s directions.

CXCR4 shRNA and Gα₁₃ shRNA—To knock down the expression of CXCR4 and Gα₁₃, CXCR4 shRNA, GCGCGGC-CAAGTTCTTATGTCATTAGTGAGCCACAGCATGT- AAGACGAACTAAGAATCTTCGCCATGCTCAGCTCGGGA, which targets CXCR4 mRNA sequence (GenBank accession number AY728138) at nucleotide 5457–5478, and Gα₁₃ shRNA, TGGCTTTGTAGTCATTAGTGAGCCACAGCATGT- AAGACGAACTAAGAATCTTCGCCATGCTCAGCTCGGGA, which targets Gα₁₃ mRNA sequence (GenBank accession number NM_006572) at nucleotide 412–438, were subcloned into the pENTR-shRNA vector between the Xhol and EcoRI sites. LR reaction (the Gateway attL × attR reaction) was performed according to the manufacturer’s instructions to transfer the CXCR4 shRNA or Gα₁₃ shRNA insert from pENTR-shRNA into PWPI-GW, a Gateway-compatible lentiviral destination vector (24).

Lentivirus Infection—Lentiviral stocks were prepared and titrated as previously reported using 293T cells as the packaging cells (25). Jurkat cells were incubated with viral supernatants for 12 h. After that, the cells were washed twice with phosphate-buffered saline and returned to normal growth medium.

Flow Cytometric Analysis—Cells were harvested and washed three times with phosphate-buffered saline. Following incubation with biotin-conjugated anti-CXCR4 antibody or isotype control antibody for 60 min at room temperature, the cells were treated with streptavidin-phycocerythrin-conjugated IgG (Vector Laboratories, Burlingame, CA) for 30 min at room temperature and analyzed with a BD Biosciences flow cytometer.

Chemotaxis Assay—Chemotaxis assay was determined with a 48-well Boyden chamber (NeuroProbe, Gaithersburg, MD) using a polystyrene pyrrolidone-free polycarbonate filter with a 5-μm pore size (Nucleopore; Corning Costar, Acton, MA). Filters were not coated with extracellular matrix molecules to prevent the influence of cell adhesion to the chemotactic response to SDF-1. Briefly, 50 μl of Jurkat cells transfected by Renilla luciferase with or without other constructs were added to the upper chamber, and a similar volume of cell suspension was kept as transfection efficiency control. The chemoattractant was added to the lower chamber. Cells migrated into the lower chamber were collected after 4 h of incubation, and Renilla luciferase activities present in cellular lysates were assayed using the dual luciferase reporter system. In each case, migration was calculated as the ratio between the luciferase activity in migrating cells (lower chamber) and the total luciferase activity in the 50-μl cell suspension.

Rho GTPase Pulldown Assay—In vivo Rho and Rac activity was assessed by a modified method described elsewhere (21). Briefly, after serum starvation overnight, cells were treated as indicated and lysed at 4 °C in a buffer containing 20 mM Hepes, pH 7.4, 0.1 M NaCl, 1% Triton X-100, 10 mM EGTA, 40 mM β-glycerophosphate, 20 mM MgCl₂, 1 mM Na₃VO₄, 1 mM dithiothreitol, 10 μg/ml aprotinin, 10 μg/ml leupeptin, and 1 mM phenylmethylsulfonyl fluoride. Lysates were incubated with glutathione S-transferase-aktivin-Rho binding domain previously bound to glutathione-Sepharose beads for Rho activity assay or with a purified, bacterially expressed glutathione S-transferase fusion protein containing the cdc42/Rac-interacting binding (CRIB) domain of PAK1 previously bound to glutathione-Sepharose beads for Rac activity. Associated GTP-bound forms of Rho or Rac were released with protein loading buffer and analyzed by Western blot analysis using a monoclonal antibody against Rho or Rac.

Immunoblot Analysis—Cells were lysed in lysis buffer (50 mM Tris-HCl, 150 mM NaCl, 1% Nonidet P-40) supplemented with protease inhibitors (0.5 mM phenylmethylsulfonyl fluoride, 1 μl/ml aprotinin and leupeptin) for 15 min at 4 °C. Equal amounts of protein were subjected to SDS-polyacrylamide gel electrophoresis and transferred onto a polyvinylidene difluoride membrane (Immobilon P; Millipore). The membranes were then incubated with the appropriate antibodies.

RESULTS

SDF-1 Induces Jurkat Cell Migration through CXCR4—To investigate the mechanism underlying the ability of SDF-1 to
promote cell migration, we took advantage of the Jurkat T cell line that expresses CXCR4 endogenously (26). We first confirmed CXCR4 expression on Jurkat cells by fluorescence-activated cell sorter analysis. Saos-2 cells, which do not express of CXCR4, were used as a negative control. As shown in Fig. 1A, we observed remarkable expression of CXCR4 on Jurkat cells, whereas no expression of CXCR4 was detected on Saos-2 cells (not shown). We then determined the functional activity of CXCR4 in Jurkat cells by analyzing the activation of ERK, a typical response elicited by GPCRs (20) by SDF-1. Exposure of Jurkat cells to SDF-1 resulted in the fast accumulation of the phosphorylated form of ERK (Fig. 1B), thus together demonstrating that Jurkat cells express functional CXCR4 receptors. We next evaluated whether SDF-1 could induce the migration of Jurkat cells using a modified Boyden Chamber system in which cell migration can be easily quantified using a luciferase-based assay. Using this system, we observed that Jurkat cells migrate efficiently toward wells containing various concentrations of SDF-1, reaching a maximum already at a concentration of 10 ng/ml of this chemotactant (Fig. 1C). The treatment with AMD3100 prevented the SDF-1-induced cell migration in a dose-dependent manner (Fig. 1D). These data indicated that SDF-1 can induce a remarkable migratory response in Jurkat cells through CXCR4.

Gi, and Gβγ Are Involved in the Migration of Jurkat Cells Induced by SDF—PTX, which ADP ribosylates Gαi, thereby uncoupling it from receptor activation (28), was used to investigate the role of Gi in the process of cell migration stimulated by SDF-1. The effect of PTX on the phosphorylation of ERK was used as positive control. Treatment of Jurkat cells with PTX resulted in a decrease of phosphorylation of ERK and cell migration in a dose-dependent fashion (Fig. 2, A and B). This confirmed that Gi participates in the migratory response initiated by CXCR4. As receptor stimulation of Gi induces the dissociation of Gβγ subunits from GTP-bound Gαi (29), we next evaluated the contribution of Gβγ to SDF-1-induced cell migration by the overexpression of Gα transducin, which blocks Gβγ function by quenching these G protein subunits upon their release from Gα (20). Indeed, expression of Gα transducin in Jurkat cells (Fig. 2C) prevented the phosphorylation of ERK induced by SDF-1 (Fig. 2D). In contrast, the phosphoryl-
induced by SDF-1 (Fig. 3). Treatment of Jurkat cells with PTX attenuated the Rac activity bound form of Cdc42, a Rac-related GTPase (not shown). Pre-treatment of Jurkat cells with PTX attenuated the activation of Rac bound to GTP as above. 

FIGURE 3. SDF-1 induces cell migration and Rac activation through G\textsubscript{13}. A, time course of the activation of Rac stimulated by SDF-1. Serum-starved Jurkat cells were stimulated with SDF-1 (10 ng/ml) for different times, and the GTP-bound form of Rac (GTP-Rac) was assessed in cellular lysates by pulldown assays. Western blot analysis of total Rac in each cell lysate was used as a control. B, PTX inhibits the activation of Rac induced by SDF-1. Jurkat cells were cultured overnight in serum-free growth medium with or without different concentrations of PTX and stimulated with SDF-1 (10 ng/ml) for 5 min. Cell lysates were used for pulldown analysis of GTP-bound Rac as above. C, expression of the AU1-tagged forms of PAK-N (PAKN) and PAK-NL2 (PAKL2) in Jurkat cells. Jurkat cells transfected with PAK-N and PAK-NL2 expression vectors were lysed for Western blot analysis with antibodies to the AU1 epitope or \(\alpha\)-tubulin as a control. D, inhibition of Rac leads to a decrease in Jurkat cell migration induced by SDF-1. Jurkat cells were transfected by PAK-N and the control construct PAK-NL2, and migration assays toward SDF-1 were performed as described above.

Signaling through G\textsubscript{13} Promotes the Migration of Jurkat Cells by Stimulating Rac—Because the small GTP-binding protein Rac plays a central role in the regulation of the actin-based cytoskeleton and cell movement (31), we then investigated whether this Rho-related GTPase is involved in the G\textsubscript{13}-mediated migration of Jurkat cells. As shown in Fig. 3A, by a pulldown assay we observed that stimulation of Jurkat cells with SDF-1 provoked the rapid accumulation of GTP-bound Rac, which reached a maximum at \(~5\) min. In parallel experiments, however, we did not detect the accumulation of the GTP-bound form of Cdc42, a Rac-related GTPase (not shown). Pre-treatment of Jurkat cells with PTX attenuated the Rac activity induced by SDF-1 (Fig. 3B). These data indicate that SDF-1 induces Rac activation through PTX-sensitive G proteins of the G\textsubscript{13} class. We next set out to explore whether Rac is necessary for the migratory response to SDF-1 by expressing in Jurkat cells the N-terminal region of PAK (p21-activated kinase), PAK-N, which includes its Cdc42/Rac-interactive binding motif, and PAK-NL2, which lacks two residues required for Rac binding and therefore serves as a specificity control (21). Fig. 3C shows the expression of the AU1-tagged forms of both PAK-N and PAK-NL2 after transfection of their expression vectors into Jurkat cells. Interestingly, as shown in Fig. 3D, expression of PAK-N dramatically reduced the migration of Jurkat cells induced by SDF-1, whereas PAK-NL2 did not exert any demonstrable effect (Fig. 3D). Collectively, our results suggest that G\textsubscript{13} participates in the SDF-1-induced migration of Jurkat cells by stimulating the activity of Rac, likely through G\(\beta\)\(\gamma\) subunits.

Rho Is Involved in Cell Migration Induced by SDF-1 through CXCR4—As cell migration often requires the coordinated activation of the small GTPases Rac and Rho, we next sought to explore whether Rho plays a role in the chemotactic response to SDF-1. We first examined whether SDF-1 promotes the activation of Rho. As shown in Fig. 4A, SDF-1 stimulation provoked a profound activation of Rho, as judged by the use of pulldown assays. To address whether Rho is involved in cell migration stimulated by SDF-1, we used both treatment with C3 toxin, by which ADP ribosylates and inhibits Rho (32), and the expression of an AU5-tagged negative dominant mutant of Rho, Rho N19. Treatment with C3 toxin from 0.1–5 ng/ml inhibited the migration of Jurkat cells toward wells containing SDF-1 (Fig. 4B). Similarly, the expression of the AU5-tagged form of Rho N19 in Jurkat cells (Fig. 4C) dramatically reduced the migration of these cells when induced by SDF-1 (Fig. 4D), while in control experiments the dominant negative mutant of Rac, but not of Cdc42, also prevented Jurkat cell migration (not shown). These data demonstrate that SDF-1 can activate Rho and that in turn Rho is involved in cell migration induced by this potent chemokine. To further investigate whether Rho activation in response to SDF-1 is indeed mediated through CXCR4, we limited the expression of this GPCR by RNA-interfering approaches (Fig. 4E). Our data showed that the knock down of the expression of CXCR4 in Jurkat cells resulted in a reduction in the ability of SDF-1 to promote both Rho activation and migration (Fig. 4, F and G).

Rho stimulation leads to the activation of Rho kinase, which promotes the accumulation of phospho-MLC by phosphorylating MLC directly or by inhibiting MLC phosphatase, thereby contributing to cell migration by regulating acto-myosin contraction (33). To explore whether Rho kinase is an important downstream effector of Rho activation in response to SDF-1, we treated Jurkat cells with the Rho kinase inhibitor Y27632 (34) and observed that SDF-1 stimulates the phosphorylation of MLC potently (Fig. 4H), which was abolished by the treatment with Y27632. Furthermore, Y27632 prevented the migration of Jurkat cells toward SDF-1 (Fig. 4I). These findings suggest that the stimulation of Rho by SDF-1 through CXCR4 results in Rho kinase activation and consequent MLC phosphorylation and that these downstream events initiated by Rho participate in cell migration toward SDF-1.

Ga\textsubscript{12/13} Is Involved in SDF-1-induced Cell Migration, and This Effect Is Mediated by Rho—As both G\textsubscript{13} and Rho activity were required for SDF-1-induced cell migration, we asked whether Rho functions downstream of G\textsubscript{13}. However, as shown in Fig. 5A, treatment of Jurkat cells with PTX failed to inhibit Rho activation in response to SDF-1 and instead caused a slight enhancement of Rho activity (Fig. 5A). As Ga\textsubscript{12} and Ga\textsubscript{13} can promote the activation of Rho elicited by GPCRs through directly binding to the RGS domain of RGS-containing Rho guanine nucleotide exchange factors (GEFs) (22, 35), we then examined whether the Ga\textsubscript{12/13}-Rho signaling axis is required...
CXCR4 Signals to Rho through \( \alpha_{13} \)

**FIGURE 4.** Rho is involved in SDF-1-stimulated cell migration. **A**, time course of Rho activation induced by SDF-1. Jurkat cells were serum starved overnight and stimulated with SDF-1 (10 ng/ml) for the indicated times. Rho activity was assessed in cell lysates by pulldown analysis of GTPbound Rho. Total Rho in each cell lysate was determined as a control. **B**, treatment with C3 toxin inhibits the migration of Jurkat cells. Jurkat cells were transfected with various concentrations of a C3 toxin expression vector, and their migration to wells containing SDF-1 (10 ng/ml) was assayed as described above. **C**, expression of RhoN19 in Jurkat cells. Jurkat cells were transfected with AU5-tagged RhoN19, and Western blot analysis was performed to show expression of the AU5 epitope on RhoN19. **D**, inhibition of Rho prevents Jurkat cell migration induced by SDF-1. Jurkat cells were transfected with RhoN19 and used for migration assays toward SDF-1 (10 ng/ml) as above. **E**, knock down decreased Jurkat cell migration induced by SDF-1. Pretreatment of Jurkat cells with the indicated concentrations of the Rho kinase inhibitor Y27632 suppressed the phosphorylation of the MLC (H) and the migration of the Jurkat cells in response to SDF-1 (I).

**FIGURE 5.** \( \alpha_{12/13} \) is involved in the migration of Jurkat cells induced by SDF-1. A role for \( \alpha_{12/13} \) in Rho activation. **A**, inhibition of the activity of \( \alpha_{12/13} \) by PTX fails to prevent Rho activation. Jurkat cells treated with different concentrations of PTX overnight were stimulated with SDF-1 (10 ng/ml; 5 min), and Rho activity in cell lysates was assayed as described above. **B**, expression of the GFP-tagged RGS domain of PDZ-RhoGEF in Jurkat cells. Western blot analysis with antibodies to GFP or \( \alpha \)-tubulin was performed in lysates from Jurkat cells transfected with the GFP-tagged RGS domain of PDZ-RhoGEF (GFP-RGS) or vector control. **C**, inhibition of \( \alpha_{12/13} \) suppresses the activation of Rho by SDF-1. Jurkat cells transfected with GFP-RGS were stimulated with SDF-1 (10 ng/ml) for 5 min and Rho activity measured in cell lysates by pull-down analysis. **D**, inhibition of \( \alpha_{12/13} \) diminishes SDF-1-induced cell migration. Jurkat cells transfected with different amounts of GFP-RGS were used for migration assays toward SDF-1 (10 ng/ml) as above. **E and F**, reconstitution of the CXCR4-\( \alpha_{13} \)-signaling axis in 293T cells. E, time course of ERK phosphorylation in response to SDF-1 in 293T cells transfected with expression vectors for GFP or CXCR4. Cells treated with 10 ng/ml SDF-1 were collected at the indicated time for immunoblot analysis. **F**, CXCR4 is involved in Rho activation in response to SDF-1 in CXCR4-transfected cells. 293T cells transfected with GFP or CXCR4 with or without GFP RGS were lysed after treatment with SDF-1 and used for pulldown assay.
Knock Down of $\alpha_{13}$ Prevents the Activation of Rho and Cell Migration in Response to SDF-1 in Jurkat Cells.

A. Jurkat cells express $\alpha_{13}$. The expression of $\alpha_{12}$ and $\alpha_{13}$ in Jurkat cells was analyzed by Western blot with heterotrimeric G protein $\alpha$ subunit-specific antibodies, using antibodies to $\alpha$-tubulin as a loading control. 293T cells transfected with empty vector (−) or $\alpha_{12}$ and $\alpha_{13}$ expression vectors were used as negative and positive controls, respectively. B. Knock down of $\alpha_{13}$ by lentiviruses carrying $\alpha_{13}$ shRNA. Jurkat cells infected with lentiviruses carrying GFP or $\alpha_{13}$ shRNA were lysed at the indicated times after infection and used for Western blot analysis of $\alpha_{13}$ expression. Western blot for $\alpha$-tubulin was used as a loading control. C. Knock down of $\alpha_{13}$ partially decreases the phosphorylation of ERK and nearly abolishes the activation of Rho induced by SDF-1. The accumulation of phosphorylated ERK and GDP-bound Rho in response to SDF-1 (10 ng/ml; 5 min) was examined as described above in Jurkat cells 10 days after infection with lentiviruses carrying GFP or $\alpha_{13}$ shRNA. D. Knock down of $\alpha_{13}$ inhibits the migration of Jurkat cells stimulated by SDF-1. The migration of Jurkat cells to wells containing SDF-1 (10 ng/ml) was examined as described above 10 days after infection with lentiviruses carrying GFP or $\alpha_{13}$ shRNA and normalized by their respective migration to serum-free growth medium.

The expression of $\alpha_{13}$ and expressed it stably in Jurkat cells by a recently developed lentiviral system (24). As shown in Fig. 6B, $\alpha_{13}$ expression was decreased 6 days after infection of Jurkat cells with a lentivirus carrying $\alpha_{13}$ shRNA and was almost eliminated 10 days after infection, whereas a control GFP-expressing lentivirus had no demonstrable effect. As shown in Fig. 6C, the activation of Rho in Jurkat cells mediated by CXCR4 was significantly blocked after $\alpha_{13}$ knock down. In addition, phosphorylation of ERK elicited by SDF-1 was slightly decreased, suggesting an unexpected role for $\alpha_{13}$ in ERK activation by SDF-1. Remarkably, we also found that knock down of the expression of $\alpha_{13}$ reduced the migration mediated by CXCR4, in alignment with the result obtained by the $\alpha_{12/13}$ dominant negative mutant (Fig. 6D). Taken together, our data suggest that CXCR4 is coupled to $\alpha_{13}$ in addition to $\alpha_1$, and that activation of Rho through the $\alpha$ subunit of $G_{13}$ is required to induce cell migration in response to SDF-1.

DISCUSSION

In the present study, we used Jurkat T-cells that express CXCR4 endogenously as an experimental model system to examine the contribution of heterotrimeric G proteins and their downstream targets in cell migration induced by SDF-1. Using a simple luciferase-based system to monitor cell migration, we first observed that SDF-1 elicits a robust chemotactic response through CXCR4 in Jurkat cells. This response was abolished by treatment with PTX, confirming the key role for G proteins of the Gi family in lymphocyte migration toward chemokine gradients (36). In this regard, we observed that G$\beta\gamma$ subunits, when released from Gi, are strictly required for CXCR4-mediated cell migration and that the chemotactic response to SDF-1 involves the coordinated activation of the small GTPases Rac and Rho by CXCR4. Of interest, whereas the activation of Rac was mediated by Gi, likely though its G$\beta\gamma$ subunits, the activation of Rho was insensitive to PTX treatment, thus suggesting that CXCR4 may utilize G proteins distinct from Gi, to stimulate Rho. Indeed, by a combination of dominant negative and RNA interference approaches, we now show that G proteins of the $G_{12/13}$ family are also required to induce cell migration in response to SDF-1 and that $G_{13}$ links CXCR4 to the activation of Rho by SDF-1 in Jurkat T cells.

Small GTP-binding proteins of the Rho family, including Rho, Rac, and Cdc42, play a central role in regulating the dynamic organization of the actin-based cytoskeleton in mammalian cells (37). During the process of cell migration, each member of this Rho GTPase family plays a distinct role. Rho is important for regulating the formation of contractile actin-myosin filaments, which form stress fibers, and for maintaining focal adhesions at the rear of the migrating cells, whereas Rac is involved in forming actin-rich membrane ruffles, referred to as lamellipodia, at the leading edge of the migrating cells and is recognized to be a driving force for cell migration (38). Cdc42 has been demonstrated to be critical in regulating cell polarity and filopodia formation, thereby controlling the direction of the cell migration (39). Hence, the coordinated activation of Rho GTPases represents a key regulatory event during the migration of cells toward a chemoattractant gradient. How this
CXCR4 Signals to Rho through Gα₁₃

coordination is achieved is under current intense investigation (37, 40, 41).

In Jurkat T cells, we observed that SDF-1 induces the rapid activation of both Rac and Rho, but not Cdc42, in line with prior studies using human peripheral blood lymphocytes (42, 43). Of interest, PTX treatment suppressed the activation of Rac while slightly enhancing Rho activity. This observation is consistent with a role for Gα₁ in signaling from chemokine receptors to Rac (42) and prior studies that demonstrated that the activity of Rho can be negatively regulated by Rac (44, 45). However, the nature of the molecular mechanism by which Gα₁ regulates Rac is at the present not fully understood. A recent study suggests that Vav1, a Rac GEF that is activated upon tyrosine phosphorylation (46), changes its localization and activity upon SDF-1 stimulation of lymphocytes (47). Furthermore, given the fact that CXCR4 activates a Rac GEF that is activated upon tyrosine phosphorylation (46), it remains not fully understood. A recent study suggests that Vav1, initiated by SDF-1 through CXCR4. Furthermore, the results of cell migration. For example, concertina, an ortholog of Rho in response to SDF-1 may provide novel therapeutic targets for pharmacological intervention in many pathological conditions, ranging from increasing the efficiency on bone marrow transplantation to treating cancer metastasis and HIV infection.

The finding that CXCR4 utilizes a Gα₁₂/₁₃-Rho signaling axis to promote cell migration may have broad implications as the SDF-1/CXCR4 signaling system plays a critical role in many physiological processes, such as axon guidance, stem cell homing, tissue damage repair, and hematopoietic cell trafficking (8, 13, 14, 17), in which the contribution of Gα₁₂/₁₃ and Rho can now be investigated. Similarly, although signaling through Gα₁ can now be investigated. Similarly, although signaling through Gα₁ can now be investigated. Similarly, although signaling through Gα₁ can now be investigated. Similarly, although signaling through Gα₁ can now be investigated. Similarly, although signaling through Gα₁ can now be investigated. Similarly, although signaling through Gα₁ can now be investigated. Similarly, although signaling through Gα₁ can now be investigated. Similarly, although signaling through Gα₁ can now be investigated. Similarly, although signaling through Gα₁ can now be investigated. Similarly, although signaling through Gα₁ can now be investigated. Similarly, although signaling through Gα₁ can now be investigated. Similarly, although signaling through Gα₁ can now be investigated. Similarly, although signaling through Gα₁ can now be investigated. Similarly, although signaling through Gα₁ can now be investigated. Similarly, although signaling through Gα₁ can now be investigated. Similarly, although signaling through Gα₁ can now be investigated. Similarly, although signaling through Gα₁ can now be investigated. Similarly, although signaling through Gα₁ can now be investigated. Similarly, although signaling through Gα₁ can now be investigated. Similarly, although signaling through Gα₁ can now be investigated. Similarly, although signaling through Gα₁ can now be investigated. Similarly, although signaling through Gα₁ can now be investigated. Similarly, although signaling through Gα₁ can now be investigated. Similarly, although signaling through Gα₁ can now be investigated. Similarly, although signaling through Gα₁ can now be investigated. Similarly, although signaling through Gα₁ can now be investigated. Similarly, although signaling through Gα₁ can now be investigated. Similarly, although signaling through Gα₁ can now be investigated. Similarly, although signaling through Gα₁ can now be investigated. Similarly, although signaling through Gα₁ can now be investigated. Similarly, although signaling through Gα₁ can now be investigated. Similarly, although signaling through Gα₁ can now be investigated. Similarly, although signaling through Gα₁ can now be investigated. Similarly, although signaling through Gα₁ can now be investigated. Similarly, although signaling through Gα₁ can now be investigated. Similarly, although signaling through Gα₁ can now be investigated. Similarly, although signaling through Gα₁ can now be investigated. Similarly, although signaling through Gα₁ can now be investigated. Similarly, although signaling through Gα₁ can now be investigated. Similarly, although signaling through Gα₁ can now be investigated. Similarly, although signaling through Gα₁ can now be investigated. Similarly, although signaling through Gα₁ can now be investigated. Similarly, although signaling through Gα₁ can now be investigated. Similarly, although signaling through Gα₁ can now be investigated. Similarly, although signaling through Gα₁ can now be investigated. Similarly, although signaling through Gα₁ can now be investigated. Similarly, although signaling through Gα₁ can now be investigated. Similarly, although signaling through Gα₁ can now be investigated. Similarly, although signaling through Gα₁ can now be investigated. Similarly, although signaling through Gα₁ can now be investigated. Similarly, although signaling through Gα₁ can now be investigated. Similarly, although signaling through Gα₁ can now be investigated. Similarly, although signaling through Gα₁ can now be investigated. Similarly, although signaling through Gα₁ can now be investigated. Similarly, although signaling through Gα₁ can now be investigated. Similarly, although signaling through Gα₁ can now be investigated. Similarly, although signaling through Gα₁ can now be investigated. Similarly, although signaling through Gα₁ can now be investigated. Similarly, although signaling through Gα₁ can now be investigated. Similarly, although signaling through Gα₁ can now be investigated. Similarly, although signaling through Gα₁ can now be investigated. Similarly, although signaling through Gα₁ can now be investigated. Similarly, although signaling through Gα₁ can now be investigated. Similarly, although signaling through Gα₁ can now be investigated. Similarly, although signaling through Gα₁ can now be investigated. Similarly, although signaling through Gα₁ can now be investigated. Similarly, although signaling through Gα₁ can now be investigated. Similarly, although signaling through Gα₁ can now be investigated. Similarly, although signaling through Gα₁ can now be investigated. Similarly, although signaling through Gα₁ can now be investigated. Similarly, although signaling through Gα₁ can now be investigated. Similarly, although signaling through Gα₁ can now be investigated. Similarly, although signaling through Gα₁ can now be investigated. Similarly, although signaling through Gα₁ can now be investigated. Similar.
CXCR4 Signals to Rho through Ga13

DECEMBER 22, 2006 • VOLUME 281 • NUMBER 51

J. Biol. Chem. 279, 29816–29820
20. Crespo, P., Xu, N., Simonds, W. F., and Gutkind, J. S. (1994) Nature 369, 418–420
21. Montaner, S., Sodhi, A., Servitja, J. M., Ramsdell, A. K., Barac, A., Sawai, E. T., and Gutkind, J. S. (2004) Blood 104, 2903–2911
22. Fukuhara, S., Chikumi, H., and Gutkind, J. S. (2000) FEBS Lett. 485, 183–188
23. Marinissen, M. J., Chiariello, M., Tanos, T., Bernard, O., Narumiya, S., and Gutkind, J. S. (2004) Mol. Cell 14, 29–41
24. Wiznerowicz, M., and Trono, D. (2003) J. Virol. 77, 8957–8961
25. Basile, J. R., Barac, A., Zhu, T., Guan, K. L., and Gutkind, J. S. (2004) Cancer Res. 64, 5212–5224
26. Peacock, J. W., and Jirik, F. R. (1999) J. Immunol. 162, 215–223
27. Donzella, G. A., Schols, D., Lin, S. W., Este, J. A., Nagashima, K. A., Madden, P. J., Allaway, G. P., Sakmar, T. P., Henson, G., De Clercq, E., and Moore, J. P. (1998) Nat. Med. 4, 72–77
28. Ui, M., and Katada, T. (1990) Adv. Second Messenger Phosphoprotein Res. 24, 63–69
29. Hamm, H. E. (1998) J. Biol. Chem. 273, 669–672
30. Ueda, Y., Hirai, S., Osada, S., Suzuki, A., Mizuno, K., and Ohno, S. (1996) J. Biol. Chem. 271, 23512–23519
31. Bar-Sagi, D., and Hall, A. (2000) J. Biol. Chem. 271, 23512–23519
32. Kikuchi, A., Yamamoto, K., Fujita, T., and Takai, Y. (1988) J. Biol. Chem. 263, 16303–16308
33. Burridge, K., and Wennerberg, K. (2004) Cell 116, 167–179
34. Narumiya, S., Ishizaki, T., and Uehata, M. (2000) Methods Enzymol. 325, 273–284
35. Fukuhara, S., Murga, C., Zohar, M., Igishi, T., and Gutkind, J. S. (1999) J. Biol. Chem. 274, 5868–5879
36. Murphy, P. M. (2001) N. Engl. J. Med. 345, 833–835
37. Etienne-Manneville, S., and Hall, A. (2002) Nature 420, 629–635
38. Ridley, A. J., Schwartz, M. A., Burridge, K., Firtel, R. A., Ginsberg, M. H., Borisy, G., Parsons, J. T., and Horwitz, A. R. (2003) Science 302, 1704–1709
39. Nobes, C. D., and Hall, A. (1995) Biochem. Soc. Trans. 23, 456–459
40. Xu, J., Wang, F., Van Keymeulen, A., Herzmark, P., Straight, A., Kelly, K., Takuwa, Y., Sugimoto, N., Mitchison, T., and Bourne, H. R. (2003) Cell 114, 201–214
41. Barac, A., Basile, J., Vazquez-Prado, J., Gao, Y., Zheng, Y., and Gutkind, J. S. (2004) J. Biol. Chem. 279, 6182–6189
42. Nishita, M., Aizawa, H., and Mizuno, K. (2002) Mol. Cell Biol. 22, 774–783
43. Vicente-Manzanares, M., Cabrero, J. R., Rey, M., Perez-Martinez, M., Ursa, A., Itoh, K., and Sanchez-Madrid, F. (2002) J. Immunol. 168, 400–410
44. Sander, E. E., ten Klooster, J. P., van Delft, S., van der Kammen, R. A., and Collard, J. G. (1999) J. Cell Biol. 147, 1009–1022
45. Zondag, G. C., Evers, E. E., ten Klooster, J. P., Janssen, L., van der Kammen, R. A., and Collard, J. G. (2000) J. Cell Biol. 149, 775–782
46. Crespo, P., Schuebel, K. E., Ostrom, A. A., Gutkind, J. S., and Bustelo, X. R. (1997) Nature 385, 169–172
47. Vicente-Manzanares, M., Cruz-Adalia, A., Martin-Cofreces, N. B., Cabrero, J. R., Dosil, M., Alvarado-Sanchez, B., Bustelo, X. R., and Sanchez-Madrid, F. (2005) Blood 105, 3026–3034
48. Van Leeuwen, F. N., Olivo, C., Giepmans, B. N., Collard, J. G., and Moolenaar, W. H. (2003) J. Biol. Chem. 278, 400–406
49. Rosenfeldt, H., Vazquez-Prado, J., and Gutkind, J. S. (2004) FEBS Lett. 572, 167–171
50. Radhika, V., and Dhanasekaran, N. (2001) Oncogene 20, 1607–1614
51. Xu, N., Voyno-Yasenetskaya, T., and Gutkind, J. S. (1994) Biochem. Biophys. Res. Commun. 201, 603–609
52. Parks, S., and Wieschaus, E. (1991) Cell 64, 447–458
53. Offermanns, S., Mancino, V., Revel, J. P., and Simon, M. I. (1997) Science 275, 533–536
54. Stantchev, T. S., and Broder, C. C. (2001) Cytokine Growth Factor Rev. 12, 219–243