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Stromal cell-derived factor-1 (CXCL12) activates integrins by direct binding to an allosteric ligand-binding site (site 2) of integrins without CXCR4.

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Abstract

Leukocyte arrest on the endothelial cell surface during leukocyte extravasation is induced by rapid integrin activation by chemokines. We recently reported that fractalkine induces integrin activation without its receptor CX3CR1 through binding to the allosteric site (site 2) of integrins. Peptides from site 2 bound to fractalkine and suppressed integrin activation by fractalkine. We hypothesized that this is not limited to membrane-bound fractalkine. We studied if stromal cell-derived factor 1 (SDF1), another chemokine that plays a critical role in leukocyte arrest, activates integrins through binding to site 2. We describe here that (1) SDF1 activated soluble integrin $\alpha v\beta 3$ in cell-free conditions, suggesting that SDF1 can activate $\alpha v\beta 3$ without CXCR4; (2) site 2 peptide bound to SDF1, suggesting that SDF1 binds to site 2; (3) SDF1 activated integrins $\alpha v\beta 3$, $\alpha 4\beta 1$, and $\alpha 5\beta 1$ on CHO cells (CXCR4-negative) and site 2 peptide suppressed the activation; (4) A CXCR4 antagonist AMD3100 did not affect the site 2-mediated integrin activation by SDF1; (5) Cell-surface integrins were fully activated in 1 min (much faster than activation of soluble $\alpha v\beta 3$) and the activation lasted at least for 1 h. We propose that the binding of SDF1 to cell-surface proteoglycan facilitates the allosteric activation process; (6) Mutations in the predicted site 2-binding site in SDF1 suppressed integrin activation. These results suggest that SDF1 (e.g., presented on proteoglycans) can rapidly activate integrins in an allosteric manner by binding to site 2 in the absence of CXCR4. The allosteric integrin activation by SDF1 is a novel target for drug discovery.

Keywords

Integrin activation; Stromal cell-derived factor-1; allosteric ligand-binding site; chemokine

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Integrins are a family of cell adhesion receptors that recognize ECM ligands, cell surface ligands, and soluble ligands (1). Integrins are transmembrane heterodimers, and at least 18 α and 8 β subunits are known (2). Leukocyte arrest on specific target vascular cells involves adhesive cascades mediated by sequential events, initiated by selectin-mediated adhesions (leukocyte rolling), followed by firm integrin-mediated arrest on immunoglobulin superfamily integrin ligands such as ICAM-1, VCAM-1 and MadCAM-1 (3). The leukocyte arrests involve an abrupt activation of leukocyte integrins by specialized chemokines displayed on the endothelial surface (arrest chemokines) (4–6). Leukocyte rolling is a critical step for leukocytes to encounter chemokines on the endothelial surface. Chemokines like CXCL12 (stromal cell-derived factor 1, SDF1), CCL21, CXCL1, CCL2 and CCL25 are the most potent physiological inducers of integrin-dependent leukocyte arrest, and subsequent leukocyte crawling over and diapedesis through vascular barriers. The mechanism of the abrupt activation of integrins by arrest chemokines, however, has not been established.

We previously reported that the chemokine domain of transmembrane chemokine fractalkine (FKN-CD) is an integrin ligand that binds to the classical ligand (e.g., RGD)-binding site of integrins (site 1) and induces integrin-FKN-CX3CR1 ternary complex (7). Also, the integrin-binding defective FKN mutant is an antagonist of FKN/CX3CR1 signaling, suggesting that integrins play a role in CX3CR1-dependent FKN signaling (7). It has been believed that integrin activation by chemokine is mediated by chemokine receptors and subsequently through signaling from inside the cells (inside-out signaling). Unexpectedly, we discovered that FKN-CD activates integrins in the absence of CX3CR1 by binding to another ligand-binding site (site 2) of integrins (8). Site 2 is located on the opposite side of site 1 in the integrin headpiece (8). Peptides from site 2 (e.g., residues 267–286 of β 3) directly bound to FKN-CD and suppressed FKN-CD-induced integrin activation (8). Thus FKN-CD binding to site 2 induces activation of site 1 through conformational changes (in an allosteric mechanism). We also identified the secreted phospholipase A2-type IIA (sPLA2-IIA) as another integrin ligand that binds to site 2 (in addition to site 1) and activates integrins (9). FKN is a transmembrane-type chemokine, and thus it is possible that the direct integrin binding and the site-2-mediated integrin activation are unique to FKN. It is unclear if soluble chemokines can activate integrins through binding to site 2. One of the soluble chemokine SDF1 is a potent chemoattractant for leukocytes and is believed to regulate signaling events through two different receptors, CXCR4 and CXCR7 in leukocytes (10–13). Binding of CXCL12 to CXCR4 induces trimeric G protein signaling leading to activation of the Src, phosphoinositide-3 kinase (PI3K)/AKT, ERK, and JNK pathways, contributing to pro-inflammatory function such as protease production and cellular migration (14,15). Dysregulated expression of SDF1/CXCR4 were reported in rheumatoid arthritis, systemic lupus erythematosus, multiple sclerosis and inflammatory bowel disease (16–19). Indeed, both small molecule CXCR4 antagonists and CXCR4 knock-out mice exhibited reduced joint inflammation in both humans and a mouse model of arthritis, suggesting that SDF1/CXCR4 play a role in the recruitment of inflammatory cells to the joint (20–23). However, it is unclear that SDF1 acts through only CXCR4 and CXCR7. SDF1 may activate integrins in a receptor-independent manner as in the case of FKN or sPLA2-IIA.

In the present study, we determined if SDF1 also activates integrins in the absence of its receptors. We found that SDF1 activated integrins in a site-2 specific and CXCR4-independent manner. We propose that activation of integrins by binding to site 2 of integrins is a common mechanism of rapid integrin activation by arrest chemokines.

Materials and Methods

Materials-

GST-fusion proteins of fibronectin type III domains 8–11 (FN8–11) (7), and fibronectin H120 fragment (FN-H120) were described (24). Fibrinogen γ -chain C-terminal domain that lacks residues 400–411 (γ C399tr) was synthesized as described (25).

Synthesis of SDF1-

The cDNA fragment of SDF1 was amplified using primers 5'-CGGGATCCAAGCCCGTCAGCCTGAGC-3' and 5'-CGGAATTCTCACATCTTGAACCTCTTGTTTAAAGC-3' with human SDF1 cDNA (Open Biosystems) as a template, and subcloned into the BamHI/EcoRI site of PET28a expression vector. The protein was synthesized in BL21 induced by isopropyl β -D-thiogalactoside as an insoluble protein. The protein was solubilized in 8 M urea, purified by Ni-NTA affinity chromatography, and refolded as previously described (26). The refolded protein was >90% homogeneous upon SDS-PAGE.

Activation of soluble α v β 3 by SDF1-

ELISA-type binding assays were performed as described previously (7). Briefly, wells of 96-well Immulon 2 microtiter plates (Dynatech Laboratories, Chantilly, VA) were coated with 100 μ l 0.1 M PBS containing γ C399tr for 2 h at 37°C. Remaining protein binding sites were blocked by incubating with PBS/0.1% BSA for 30 min at room temperature. After washing with PBS, soluble recombinant α v β 3 (5 μ g/ml) in the presence or absence of SDF1 was added to the wells and incubated in Hepes-Tyroses buffer (10 mM HEPES, 150 mM NaCl, 12 mM NaHCO₃, 0.4 mM NaH₂PO₄, 2.5 mM KCl, 0.1% glucose, 0.1% BSA) with 1 mM CaCl₂ for 1 h at room temperature. After unbound α v β 3 was removed by rinsing the wells with binding buffer, bound α v β 3 was measured using anti-integrin β 3 mAb (AV-10) followed by HRP-conjugated goat anti-mouse IgG and peroxidase substrates. For the time-course experiments, WT SDF1 and soluble α v β 3 were incubated for 1–60 min instead of 1 h.

Activation of integrins on the cell surface by SDF1-

Cells were cultured to nearly confluent in RPMI1640/10% FCS (K562 and U937 cells) or DMEM/10% FCS (CHO cells). Cells were resuspended with RPMI1640/0.02% BSA or DMEM/0.02% BSA and incubated for 30 min at room temperature to block protein binding sites. Cells were then incubated with WT SDF1 or mutants for 5 min at room temperature and then incubated with FITC-labeled integrin ligands (γ C399tr, FN-H120, or FN8–11) for 15 min at room temperature. Cells were washed with PBS/0.02% BSA and analyzed by FACSCalibur (Becton Dickinson, Mountain View, CA). For blocking experiments, SDF1 was preincubated with Fc- β 3 peptide for 30 min at room temperature. For blocking with

AMD3100, cells were preincubated with AMD3100 (10 μ M) for 30 min at room temperature. For time-course experiments, cells were incubated with WT SDF1 for 1–60 min at room temperature and then incubated with FITC-labeled integrin ligands for 5 min.

Fc site 2 peptide-

The Fc fragment of human IgG1 of the pFUSE-hIgG1-Fc1 vector was amplified and subcloned into the NdeI/NheI site of pET28a (designated pET28a-FcN). Site 2 peptide (QPNDGQSHVGSNDNHYSASTTM, residues 267–287 of β 3, C273 is changed to S) and a scrambled site 2 peptide (VHDSHYSGQGAMSDNTNSPQT) were synthesized as described (8) except that the BamHI/EcoRI fragment was subcloned into the BamHI/EcoRI site of pET28a-FcN. Proteins were synthesized in *E. coli* as insoluble proteins, purified by Ni-NTA affinity chromatography and refolded as described (7). The inserts were identified by DNA sequencing.

Binding of S2 peptide to SDF1-

ELISA-type binding assays were performed as described previously (7). Briefly, wells of 96-well Immulon 2 microtiter plates (Dynatech Laboratories, Chantilly, VA) were coated with Fc- β 3 peptide in 100 μ l 0.1 M PBS for 2 h at 37°C. Remaining protein binding sites were blocked by incubating with PBS/0.1% BSA for 30 min at room temperature. After washing with PBS, SDF1 was added to the wells and incubated in PBS for 2 h at room temperature. After unbound SDF1 was removed by rinsing the wells with PBS, bound SDF1 was measured using anti-SDF1 antibody (R&D systems MAB350, mouse monoclonal antibody to Lys22-Lys89 of SDF1) and HRP-conjugated anti-mouse IgG.

Docking simulation-

Docking simulation of interaction between SDF1 and integrin α v β 3 (closed headpiece form, PDB code 1JV2) was performed using AutoDock3 as described (27). We used the headpiece (residues 1–438 of α v and residues 55–432 of β 3) of α v β 3 (closed form, 1JV2.pdb). Cations were not present in integrins during docking simulation, as in the previous studies using α v β 3 (open form, 1L5G.pdb) (27,28). The ligand is presently compiled to a maximum size of 1024 atoms. Atomic solvation parameters and fractional volumes were assigned to the protein atoms by using the AddSol utility, and grid maps were calculated by using AutoGrid utility in AutoDock 3.05. A grid map with 127 \times 127 \times 127 points and a grid point spacing of 0.603 Å included the headpiece of α v β 3 (residue 1–438 of α v and residues 55–432 of β 3). Kollman “united-atom” charges were used. AutoDock 3.05 uses a Lamarckian genetic algorithm (LGA) that couples a typical Darwinian genetic algorithm for global searching with the Solis and Wets algorithm for local searching. The LGA parameters were defined as follows: the initial population of random individuals had a size of 50 individuals; each docking was terminated with a maximum number of 1 \times 10⁶ energy evaluations or a maximum number of 27,000 generations, whichever came first; mutation and crossover rates were set at 0.02 and 0.80, respectively. An elitism value of 1 was applied, which ensured that the top ranked individual in the population always survived into the next generation. A maximum of 300 iterations per local search was used. The probability of performing a local search on an individual was 0.06, whereas the maximum number of consecutive successes or failures before doubling or halving the search step size was 4.

Other methods-

Treatment differences were tested using ANOVA and a Tukey multiple comparison test to control the global type I error using Prism 7 (Graphpad Software).

Results

SDF1 activates soluble integrin $\alpha v\beta 3$ in cell-free conditions without CXCR4-

We previously reported that transmembrane chemokine FKN activates integrins in a CX3CR1-independent and site 2-dependent manner (8). This may be a mechanism of rapid integrin activation by FKN that leads to strong adhesion of leukocytes to the endothelial surface during leukocyte extravasation (4–6). It is unclear if this is limited to the transmembrane chemokine FKN. To address this question, we performed docking simulation of interaction between SDF1 (PDB code 1VMC) and integrin $\alpha v\beta 3$ (PDB code 1JV2), which has been shown to have a closed headpiece (29). The simulation predicts that SDF1 binds to site 2 of $\alpha v\beta 3$ (docking energy 18.5 kcal/mol) (Fig. 1a). The amino acid residues involved in the predicted model are shown in Table 1. To determine if SDF1 binds to site 2, we studied if site 2 peptide of $\beta 3$ fused to Fc (Fc- $\beta 3$) bind to SDF1. When SDF1 (His-tagged) is immobilized directly to wells of 96-well microtiter plate it did not bind to Fc- $\beta 3$ (data not shown). So, we immobilized Fc- $\beta 3$, incubated with soluble SDF1, and detected SDF1 using anti-SDF1 antibody (Fig. 1b). We found that SDF1 bound to Fc- $\beta 3$ to the level much higher than that of parent Fc or scrambled $\beta 3$ peptide (Fc- $\beta 3$ scr), suggesting that SDF1 binds to site 2. We studied if soluble chemokine SDF1 enhances the binding of soluble $\alpha v\beta 3$ (extracellular domains) to its specific ligand $\gamma C399$ tr in cell-free conditions. To keep soluble $\alpha v\beta 3$ in an inactive state, 1 mM Ca^{2+} was included in the assay medium. We found that SDF1 markedly enhanced the binding of soluble $\alpha v\beta 3$ to $\gamma C399$ tr in a concentration- (Fig. 1c) and time-dependent manner (Fig. 1d). These findings suggest that SDF1 can activate soluble $\alpha v\beta 3$ in cell-free conditions without CXCR4, while SDF1 at $> 5 \mu g/ml$ was required to detect enhanced ligand binding to soluble $\alpha v\beta 3$. We describe that SDF1 activated integrins on the cell surface at much lower SDF1 concentrations (see below).

SDF1 activates cell-surface integrin $\alpha v\beta 3$ in a CXCR4-independent manner-

It has been reported that CHO cells do not normally express CXCR4 or respond to SDF1 (30). We studied if SDF1 activates cell-surface integrin $\alpha v\beta 3$ using CHO cells that express recombinant $\alpha v\beta 3$ ($\beta 3$ -CHO). We used DMEM that includes high $[Ca^{2+}]$ to keep integrins inactive for integrin activation assays. We previously reported that site 2 peptides did not bind to $\gamma C399$ tr (8). We incubated the cells with FITC-labeled $\gamma C399$ tr in the presence of SDF1 and measured the bound FITC by flow cytometry. We found that SDF1 markedly enhanced binding of $\gamma C399$ tr to $\alpha v\beta 3$ in a dose-dependent manner. One ng/ml (0.083 nM) SDF1 induced detectable $\alpha v\beta 3$ binding to $\gamma C399$ tr in $\beta 3$ -CHO cells (Fig. 2a). The enhanced binding was inhibited by Fc- $\beta 3$, but not by control Fc or Fc- $\beta 3$ scr (Fig. 2b). These findings suggest that SDF1 activates $\alpha v\beta 3$ by binding to site 2. AMD3100, an inhibitor of CXCR4, did not affect SDF1-induced activation of $\alpha v\beta 3$ (Fig. 2c). While CHO cells do not express CXCR4, these findings confirmed that CXCR4 is not involved in SDF1-induced activation of $\alpha v\beta 3$ on CHO cells. We substituted Lys residues in the predicted SDF1-integrin interface at positions 24, 27, and 43 of SDF1 to Glu in combination. The SDF1 mutants did not

induce integrin activation (Fig. 2d), suggesting that SDF1 binding to site 2 is required for integrin activation. This also suggests that these Lys residues are involved in site 2 binding (Fig. 4b–d), in consistent with the docking model. It has been reported that arrest chemokines very quickly activate integrins (4–6). We measured the time-course of SDF1-induced integrin activation in β 3-CHO cells. We found that SDF1 were fully activated in 1 min (the shortest time point we can take in our assay) (Fig. 2e), which is much faster than SDF1-induced activation of soluble integrins in solution (Fig. 1a). We propose that SDF1 may be rapidly concentrated on the cell surface by binding to cell-surface proteoglycans and this process markedly facilitates the SDF1-induced integrin activation.

SDF1 activates α 4 β 1 and α 5 β 1 through site 2 in a CXCR4-independent manner-

α v β 3 is not a typical leukocyte integrin, but integrins α 4 β 1 and α 5 β 1 play a role in leukocyte functions (1,2). We studied if SDF1 activates α 4 β 1 and α 5 β 1 in CHO cells that express α 4 β 1 (α 4-CHO) or parent CHO cells (α 5 β 1+) in DMEM that includes high $[Ca^{2+}]$ to keep integrins inactive. We measured the levels of binding of FITC-labeled ECM ligands [H120, a fibronectin fragment specific to α 4 β 1 (24) and FN8–11, another fibronectin fragment that contains the cell-binding domain specific to α 5 β 1 (14)] to cell surface integrins, and bound FITC was measured by flow cytometry. We obtained very similar results in α 4 β 1 (Fig. 3) and α 5 β 1 (Fig. 4) to that for α v β 3: One ng/ml SDF1 clearly enhanced integrin α 4 β 1 binding to specific ligand H120, while 100 ng SDF1 was required to detect enhanced binding of integrin α 5 β 1 to FN8–11. SDF1 markedly enhanced binding of ECM ligands to integrins α 4 β 1 and α 5 β 1 and the enhanced binding was inhibited by Fc- β 3, but not by control Fc or Fc- β 3scr. Also, AMD3100 did not affect the SDF1-induced activation of integrins α 4 β 1 and α 5 β 1. Importantly, SDF1 activated integrins in 1 min by binding to site 2 and the activation lasted at least for 1h. These findings suggest that SDF1-induced activation of integrins α 4 β 1 and α 5 β 1 in a site 2-dependent manner is not limited to α v β 3.

Discussion

The present study establishes that a) soluble chemokine SDF1 directly binds to the allosteric binding site (site 2) in integrins. We further obtained evidence that b) SDF1 activated soluble α v β 3 in cell-free conditions, which does not involve signal transduction through CXCR4; c) Fc- β 3 peptide, not control Fc- β 3scr, bound to SDF1, suggesting that SDF1 binds to site 2; d) SDF1 activated integrins α v β 3, α 5 β 1, and α 4 β 1 on the cell surface and Fc- β 3 peptide, but not control Fc- β 3scr, suppressed SDF1-induced integrin activation; and e) CXCR4 antagonist AMD3100 did not suppress SDF1-mediated integrin activation on CHO cells, which suggests that SDF1 activates integrins by binding to site 2 in an allosteric manner, independent of CXCR4. f) Also, cell-surface integrins were fully activated within 1 min by SDF1, while it took over 1 h for soluble α v β 3 to be fully activated. We were not able to take time points shorter than 1 min. These findings suggest that cell-surface integrin activation by binding of SDF1 to site 2 is rapid and stable. Since SDF1 induced enhanced ligand binding to integrins occurs within or close to biological concentrations of SDF1 (1 or 100 ng/ml), suggesting that the SDF1-induced allosteric integrin activation is biologically relevant. It is unlikely that SDF1 at the biological concentrations (e.g., <10 ng/ml) detectably binds to

integrins that has relatively low affinity to ligands (KD 10^{-6} to 10^{-7} M). SDF1 is known to bind to heparin and proteoglycans and thus expected to be highly concentrated on the cell surface proteoglycans (33,34). Therefore, we expect that SDF1 that has been presented and concentrated on the surface binds to site 2 of integrins and rapidly activates integrins independent of CXCR4 in physiological and pathological conditions. It is still unclear, however, if SDF1 binds to site 1. SDF1 and FKN are part of “arrest chemokines”, which are involved in tight binding of leukocytes to endothelial surface through rapid integrin activation (sub second) and subsequent extravasation (4). We propose that integrin activation by SDF1 and FKN (and perhaps other arrest chemokines) in a cognate GPCR-independent manner plays a role in rapid integrin activation (e.g., during leukocyte extravasation). The binding of chemokines to site 2 is a novel target for drug discovery.

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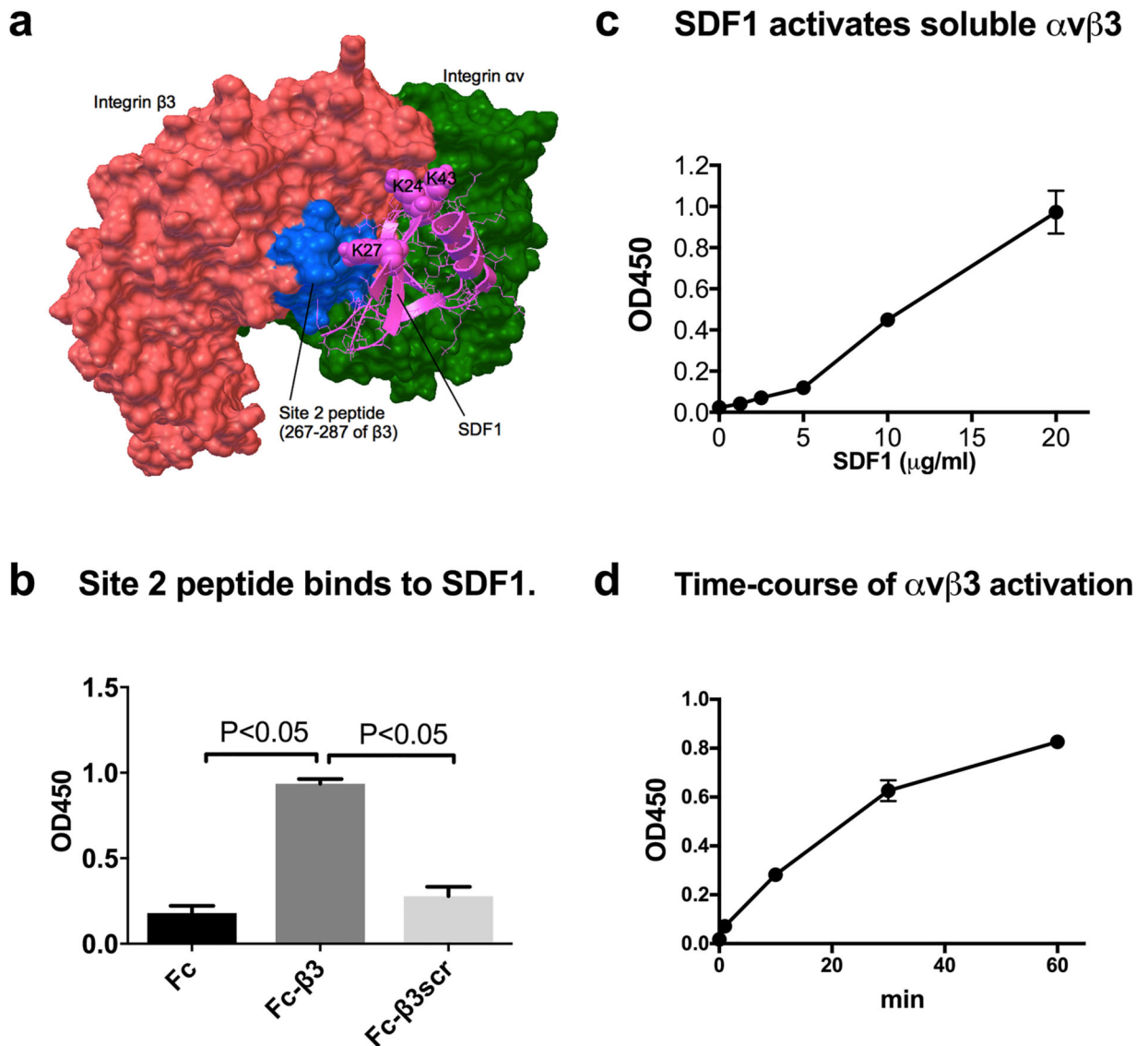


Fig. 1. SDF1 binds to and allosterically activates $\alpha v \beta 3$ without cognate receptor CXCR4.

(a) Docking model of SDF1- $\alpha v \beta 3$ (inactive) interaction. The position of site 2 peptide of $\beta 3$ is shown in blue. The simulation predicted amino acid residues involved in integrin binding (Table 1), including Lys24, Lys27, and Lys43 of SDF1. (b) Peptide derived from site 2 (Fc- $\beta 3$) binds to SDF1. SDF1 binding to immobilized Fc- $\beta 3$ peptide, scrambled peptide (Fc- $\beta 3$ scr), or Fc was measured by using anti-SDF1 antibody. Data are shown as means \pm SEM of triplicate experiments. (c) SDF1 activates soluble $\alpha v \beta 3$ in cell-free conditions. The binding of soluble $\alpha v \beta 3$ to immobilized $\gamma C399$ tr was measured as described in the methods section. Data are shown as means \pm SEM of triplicate experiments. (d) Time course of SDF1-induced activation of soluble $\alpha v \beta 3$. Data are shown as means \pm SEM of triplicate experiments.

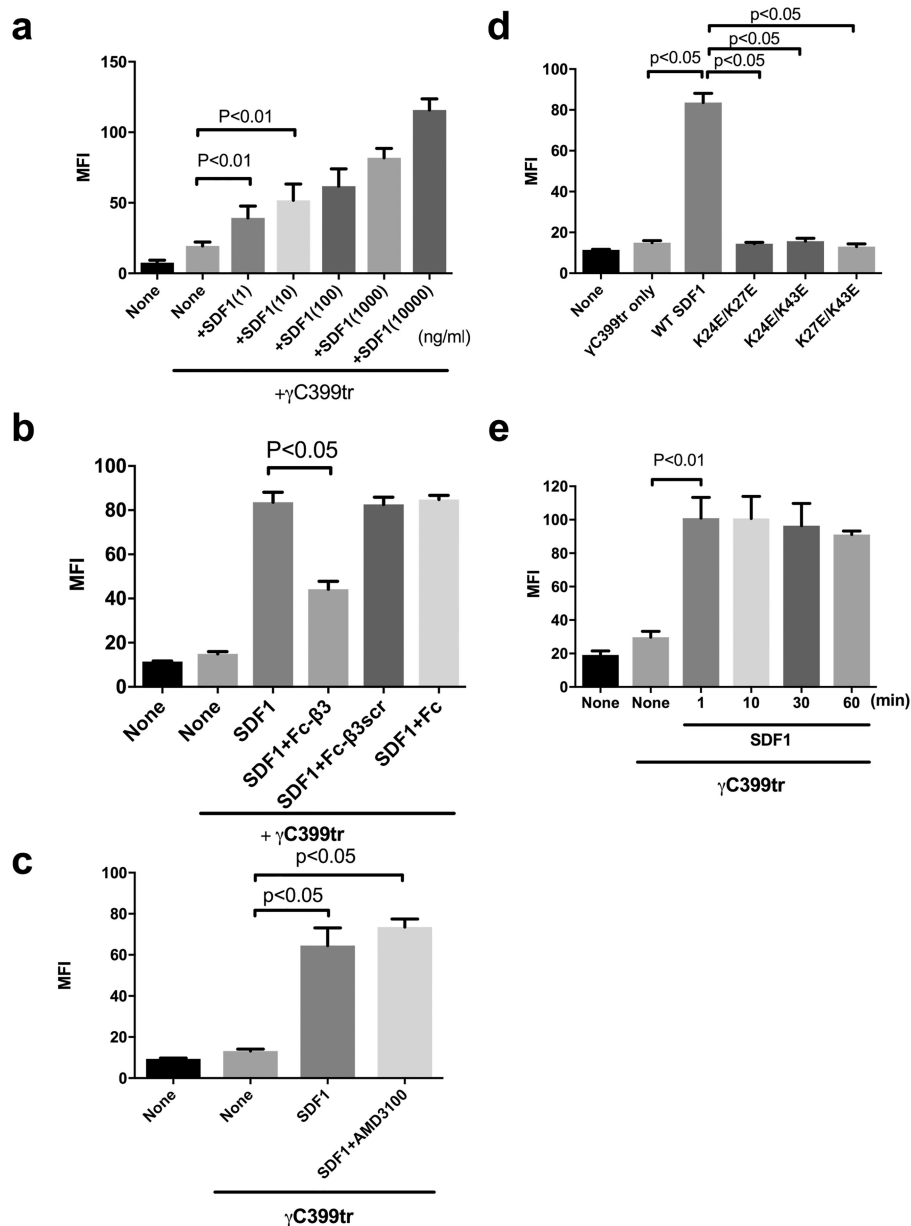


Fig. 2. SDF1 activates integrin $\alpha v \beta 3$ on the cell surface by binding to site 2 in a CXCR4-independent manner.

The binding of FITC-labeled $\gamma C399tr$ to the cell-surface $\alpha v \beta 3$ on $\beta 3$ -CHO cells in the presence of SDF and Fc- $\beta 3$ peptide was measured using flow cytometry as described in the methods section. MFI= median fluorescent intensity. Data are shown as means \pm SEM of triplicate experiments. (a) SDF1 activates integrin $\alpha v \beta 3$ on $\beta 3$ -CHO cells in a dose-dependent manner ($n=4$). (b) Fc- $\beta 3$ peptide, not Fc- $\beta 3scr$ or Fc, suppresses SDF1-mediated activation of integrin $\alpha v \beta 3$. (c) AMD3100, a CXCR4 inhibitor, does not affect SDF1-mediated $\alpha v \beta 3$ activation in an allosteric manner. (d) SDF1 mutations in the predicted site 2-binding interface of SDF1 are defective in $\alpha v \beta 3$ activation. (e) SDF1 fully activates cell-surface $\alpha v \beta 3$ in 1 min and the activation lasts for 1 h.

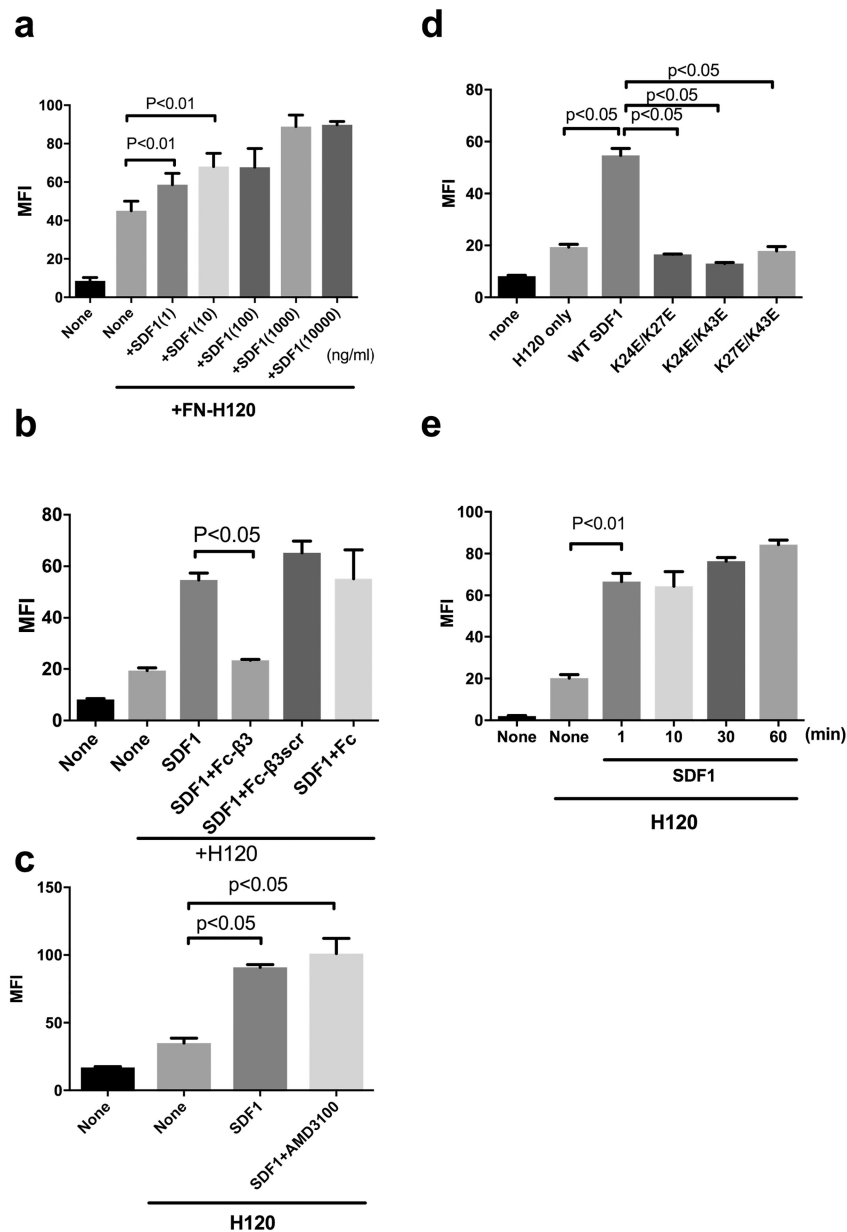


Fig. 3. SDF1 activates integrin $\alpha 4\beta 1$ on the cell surface by binding to site 2 in a CXCR4-independent manner. $\alpha 4$ -CHO cells were incubated with FITC-labeled ECM ligands (the fibronectin fragment H120 specific to $\alpha 4\beta 1$) in the presence of SDF1 (20 $\mu\text{g}/\text{ml}$) and/or Fc- $\beta 3$ peptide (200 $\mu\text{g}/\text{ml}$) and the binding of the ligands was measured in flow cytometry. MFI= median fluorescent intensity. Data are shown as means \pm SEM of triplicate experiments. (a) SDF1 activates integrin $\alpha 4\beta 1$ on $\alpha 4$ -CHO cells in a dose-dependent manner ($n=4$). (b) Fc- $\beta 3$ peptide, not Fc- $\beta 3$ scr or Fc, suppresses SDF1-mediated activation of integrin $\alpha 4\beta 1$. (c) AMD3100, a CXCR4 inhibitor, does not affect SDF1-mediated integrin $\alpha 4\beta 1$ activation in an allosteric manner. (d) SDF1 mutations in the predicted site 2-binding interface of SDF1 are defective in $\alpha 4\beta 1$ activation. (e) SDF1 fully activates cell-surface $\alpha 4\beta 1$ in 1 min and the activation lasts for 1 h.

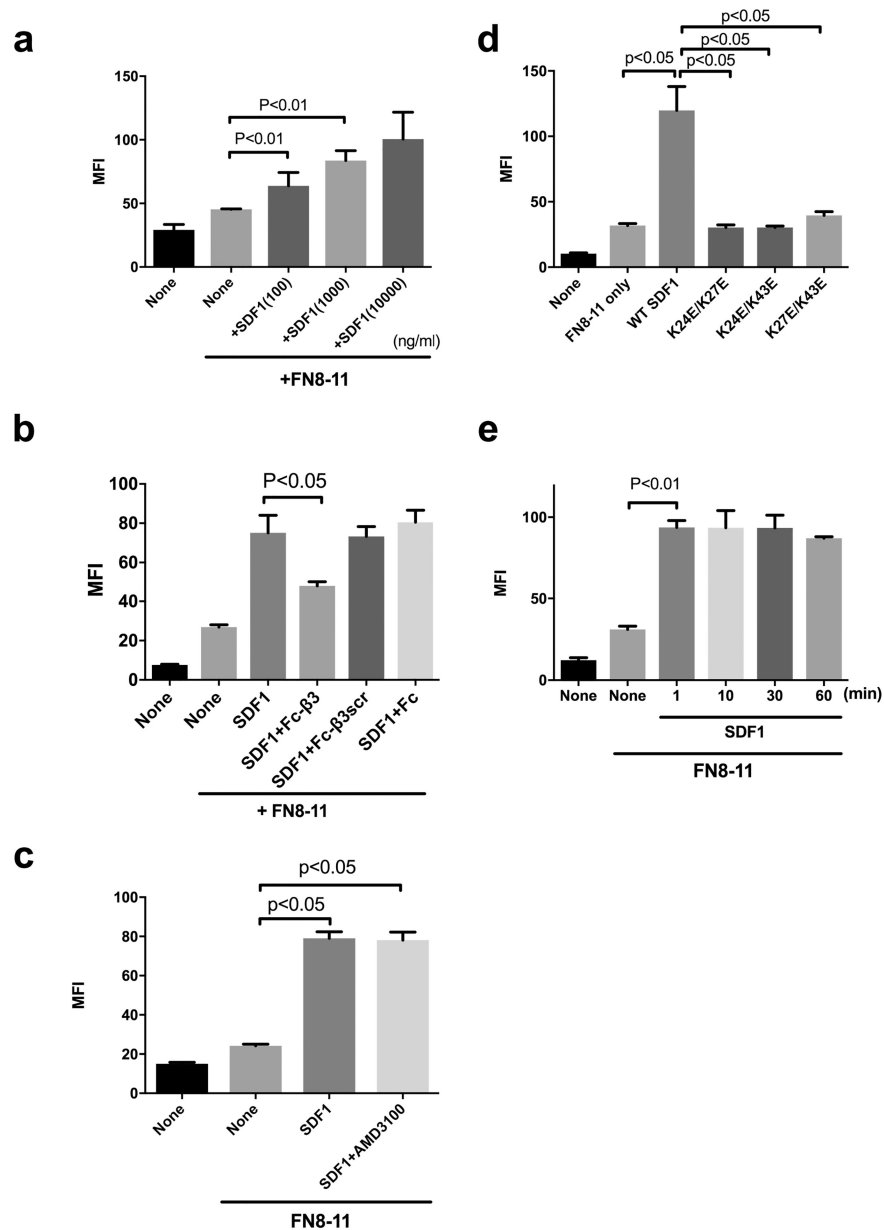


Fig. 4. SDF1 activates integrins $\alpha 5\beta 1$ on the cell surface by binding to site 2 in a CXCR4-independent manner.

Parent CHO cells ($\alpha 5\beta 1+$) were incubated with FITC-labeled ECM ligands (the fibronectin fragment FN8–11 specific to $\alpha 5\beta 1$) in the presence of SDF1 (20 $\mu\text{g}/\text{ml}$) and/or Fc- $\beta 3$ peptide (200 $\mu\text{g}/\text{ml}$) and the binding of the ligands was measured in flow cytometry. MFI= median fluorescent intensity. Data are shown as means \pm SEM of triplicate experiments. (a) SDF1 activates integrin $\alpha 5\beta 1$ on CHO cells in a dose-dependent manner ($n=4$). (b) Fc- $\beta 3$ peptide, not Fc- $\beta 3\text{scr}$ or Fc, suppresses SDF1-mediated activation of integrin $\alpha 5\beta 1$. (c) AMD3100, a CXCR4 inhibitor, does not affect SDF1-mediated integrin $\alpha 5\beta 1$ activation in an allosteric manner. (d) SDF1 mutations in the predicted site 2-binding interface of SDF1

are defective in $\alpha_5\beta_1$ activation. (e) SDF1 fully activates cell-surface $\alpha_5\beta_1$ in 1 min and the activation lasts for 1 h.

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Table 1.

Amino acid residues involved in the interaction between SDF1 and integrin $\alpha v\beta 3$ (site 2).

SDF1	αv	$\beta 3$
Arg8, Arg12, Phe13, Glu15, His17, Val18, Ala19, asn22, Lys24, Lys27 , Leu29, Arg41, Leu42, Lys43 , Asn44, Asn45, Asn46, Arg47, Gln48	Tyr18, Lys42, Asn44, Val51, Glu52, Phe88, Ser90, His91, Gln92, Trp93, Leu111, His113, Arg122	Pro160, Val161, Ser162, Pro163, Met165, Tyr166, Ile167, Ser168, Glu171, Glu174, Asn175, Met187, Ala263, Gly264, Ile265, Gln267, Asp270, Gln272, Cys273, His274, Val275, Ser277, Asp278, His280, Tyr281, Ser282, Ala283, Ser284, Thr285, Thr286, Met287

Amino acid residues within 0.6 nm between SDF1 and $\alpha v\beta 3$ were selected using pdb viewer (version 4.1).

Amino acid residues in $\beta 3$ site 2 peptide (S2- $\beta 3$) are shown in bold.