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Loss of C-terminal α-helix decreased SDF-1α-mediated signaling and chemotaxis without influencing CXCR4 internalization[1]

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KEY WORDS stromal cell-derived factor-1 alpha; CXC chemokine receptor 4; chemotaxis

ABSTRACT

AIM: To investigate the possibility that a novel α-helix-defective mutant of stromal cell-derived factor-1α (SDF-1α) (SDF-1/54R) acts as an antagonist of CXC chemokine receptor 4 (CXCR4). **METHODS:** According to the genetic sequence of natural SDF-1α, a recombinant α-helix-defective mutant of SDF-1α was designed and some biologic characteristics of this mutant were demonstrated. The migration of Jurkat cells was assessed with chemotactic assay. ERK phosphorylation was analyzed by Western blot with a specific anti-phospho-ERK1/2 antibody. Intracellular calcium influx was examined by flow cytometer with a calcium indicator dye Fluo-3AM. The CXCR4 on the cell surface was detected by flow cytometer with a PE conjoined anti-human CXCR4 antibody. **RESULTS:** Compared with native SDF-1α, SDF-1/54R displayed apparent decrease in chemotactic ability, ERK1/2 activation, and intracellular calcium influx in Jurkat cells. However, the binding to CXCR4 and inducing CXCR4 internalization of SDF-1/54R did not change outstandingly. Moreover, a competitive inhibitory effect of SDF-1/54R on the migration of Jurkat cells induced by native SDF-1α was confirmed. **CONCLUSION:** α-helix-defective mutant of SDF-1α, SDF-1/54R that remained both the N-terminus and the central β-sheet region, decreased SDF-1α-mediated signaling and chemotaxis but did not influence CXCR4 internalization, which suggested that SDF-1/54R might be developed as an anti-CHIV inhibitor with high biological potency and low side-effect.

INTRODUCTION

Stromal cell-derived factor-1α (SDF-1α, CXCL12), an only known biological natural ligand for chemokine CXC receptor 4 (CXCR4), regulates leukocyte and hematopoietic precursor migration, pre-B cell proliferation, and cerebellar development, as described by studies with SDF-1α and CXCR4-deficient mice[1,2]. CXCR4 is a member of the G-protein-coupled receptor family that expressed in a wide range of tissues and cell lines. Recently, an unexpected encounter between hu-

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man immunodeficiency virus (HIV) and chemokine system has led to a dramatic acceleration to understand the molecular mechanisms of HIV infection and open new perspectives for the development of effective therapeutics[3-6]. Since CXCR4 has been demonstrated as a co-receptor for the entry of HIV to host cells, a number of CXCR4 antagonists have been developed as potential therapeutic agents of HIV-infected individuals. As a special natural ligand of CXCR4, SDF-1α is certainly believed as a potent natural inhibitor of HIV. However, because of the multifunction of SDF-1α, the risk for SDF-1α to induce inflammatory side effects or to interfere with the physiology of the homeostatic chemokine system potentially limits its therapeutic uses. Therefore, according to the knowledge of structure-activity relationship of SDF-1α, modifying the original structure of SDF-1α should be a useful strategy to develop the antagonists of CXCR4 with high affinity for CXCR4 but without internal activity such as triggering cellular signal transduction.

Native SDF-1 mainly consists of three distinct structural regions: a N-terminus, a central core region of three antiparallel α-sheets, and a C-terminal amphiphilic α-helix[7]. Studies of synthetic peptides derived from SDF-1 N-terminus and SDF-1 mutants demonstrate that SDF-1 N-terminal residues play a key role as the major site for direct interaction with CXCR4 and signaling transduction[8]. And a cluster of positive charge residues on the central β-sheet region is important for SDF-1 to bind to CXCR4. Although the biological function of C-terminus of SDF-1 is still less clear, some studies suggest C-terminal fragment itself has no activity but serves to promote the activity of N-terminus[7].

In this study, a novel recombinant peptide structure of SDF-1, which remained both the N-terminus and the central β-sheet region but not the C-terminal α-helix, was designed according to natural SDF-1 genetic sequence. It was expected that this new α-helix-detective mutant of SDF-1α would act as an antagonist that could bind to CXCR4 but not trigger CXCR4 activation to induce related cellular signal transduction.

MATERIALS AND METHODS

Chemicals and reagents SuperScript Preamplification System for First Strand cDNA Synthesis Kit, ProBond Resin, TRIZOL Reagent for RNA extraction and RNA purification Reagent were from Life Technologies (Grand Island, NY). IPTG (isopropyl β-*D*-thiogalactoside) was purchased from Sigma Chemical Co (St Louis, MO). Recombinant SDF-1α protein was from R&D System (Minneapolis, MN). Plasmid extraction and purification reagent was from QIAGEN Genomics Inc (Hilden, Germany). Rabbit anti-phospho-ERK antibody was from New England Biolabs Inc (Beverly, MA). Rabbit anti-ERK antibody was from Santa Cruz Biotechnology (Santa Cruz, CA). PE conjugated anti-human CXCR4 antibody was from PharMingen (San Diego, CA). Western Blot Plus Chemiluminescence Reagent was from Life Science Inc (Boston, MA). Fluo-3AM was from Molecular Probes (Eugene, OR).

Plasmid and bacteria Bacterial expression plasmid: pET101/D-TOPO, one Shot TOP10 chemically competent cells, and BL21 Star (DE3) were purchased from Invitrogen.

Primers design T7 for PCR checking and DNA sequencing was produced by Invitrogen, and its sequence is as 5'-TAATACGACTCACTATAGGG -3'; The primers for cloning mice wild type of SDF-1α and mutant of SDF-1α were respectively synthesized (TAKARA) according to motif of natural SDF-1α. mSDF-1F: 5'-CACCATGAAACCAGTCAGCCTGAGCT-3' (wile type of SDF-1α, sense), mSDF-1R: 5'-GGACC-TCTTTCGAAATTTGTTC-3' (wile type of SDF-1α, antisense); mSDF-1-54F: 5'-CACCATGAAACCAGTC-AGCCTGAGCT-3' (α-helix-defective mutant of SDF-1α, sense), mSDF-1-54R: 5'-CGGGTCAATGCACAC-TTGTCTGT-3' (α-helix-defective mutant of SDF-1α, antisense).

Chemotactic chamber Transwell trays with 6-mm-diameter chambers and membrane pore size of 5 μm were produced by KURABO (Osaka, Japan).

Cloning wild type and α-helix-defective mutant of SDF-1α Total mRNA from mouse bone marrow was extracted with TRIZOL Reagent, and the first strand of cDNA was generated from 2 μg total RNA using oligo-dT primer and a SuperScript II Reverse Transcriptase (GIBCO BRL). PCR amplification for SDF-1α cDNA (wild type and α-helix-defective mutant) was performed initially by 94 ºC denaturation (3 min), followed by 30 cycles of three PCR steps of 60 s at 94 ºC, 45 s at 55 ºC, and 60 s at 72 ºC, and terminated with an extension prolongation for 5 min at 72 ºC. After amplification reaction, the PCR products were fractioned by 1.2 % agarose gel electrophoresis.

Construction of expression vector The PCR products were inserted into a bacterial expression

plasmid: pET101/D-TOPO to construct pET101/D-TOPO/SDF-1α and pET101/D-TOPO/SDF-1α-54R respectively. The recombinant expressive vectors transformed the competent bacteria TOP10 and positive clones were selected by 100 mg/L of ampicillin. The recombinant expression victors were extracted and purified by Qiagen Mini Plasmid Extraction and Purification Reagent and sequenced by using a 310 Genetic Analyzer (ABI) with the primers of T7/mSDF-1R.

Expression and purification of wild type and α-helix-defective mutant of SDF-1α The positive clones of BL21 bacteria transformed by pET101/D-TOPO/SDF-1α were inductively amplified in LB medium with IPTG. Then the steps below should be followed to purify the target proteins: (i) Harvest cells by centrifugation (3000×*g* for 5 min); (ii) Resuspend the cell pellet in 4 mL of Guanidinium Lysis Buffer (6 mol/L Guanidine HCl, 500 mmol/L NaCl, Phosphate 20 mmol/L, pH 7.8) and incubate with shaking at 60 ºC for 30 min; (iii) Centrifuge the lysate at 1500×*g* for 30 min to pellet the cellular debris. Transfer the supernatant to a fresh tube; (iv) Add 2 mL of Ni-Resin to the supernatant and bind for 15-30 min at room temperature using gentle agitation to keep the resin suspended in the lysate solution. Settle the resin by low speed centrifugation (800×*g*) and carefully aspirate the supernatant; (v) Wash the column with 4 mL of Denaturing Binding Buffer (Urea 8 mol/L, NaCl 500 mmol/L, Phosphate 20 mmol/L pH 7.8) and repeat this step one more time; (vi) Wash the column with 4 mL of Denaturing Wash Buffer (Urea 8 mol/L, NaCl 500 mmol/L, phosphate 20 mmol/L, pH 6.0) and repeat this step one more time; (vii) Wash the column with 8 mL of Native Wash Buffer (imidazole 20 mmol/L, NaCl 500 mmol/L, phosphate 20 mmol/L, pH 8.0) and repeat this step 3 more times for a total of 4 native washes; (viii) Elute the protein with 8 mL Native Elution Buffer (imidazole 500 mmol/L, NaCl 500 mmol/L, phosphate 20 mmol/L, pH 8.0). The amount of target protein was examined by means of SDS-PAGE electrophoresis.

Immunoblot analysis Immunoblot analysis was performed as described previously[9]. Briefly, cells were lysed in lysis buffer containing 1 % Nonidet P-40, pH 7.6 Tris-HCl 20 mmol/L, NaCl 0.15 mol/L, edetic acid 3 mol/L, egtazic acid 3 mmol/L, phenylmethylsulfonyl fluoride 1 mmol/L, sodium vanadate 2 mmol/L, aprotinin 20 g/L and leupeptin 5 g/L. Insoluble material was removed by centrifugation (10 000×*g* for 30 min, 4 ºC). For Immunoblotting, the cell lysates were separated on 10 % SDS-polyacrylamide gels, and blotted onto a nitrocellulose membrane. Nonspecific binding was blocked by incubating the membrane with 0.05 % Tween-20/PBS containing 5 % non-fat dry milk at room temperature for 1 h. Membranes were incubated with the primary antibody overnight at 4 ºC and then with a horseradish peroxidase-conjugated secondary antibody, and the specific immune complexes were detected using Western Blot Plus Chemiluminescence Reagent.

Flow cytometric analysis For analysis of amounts of CXCR4 on cell face, Jurkat cells were plated into each well of a 24-well tissue culture plate and treated with wild type or α-helix-defective mutant of SDF-1α for indicated time. The cells were harvested and stained with a PE conjugate anti-human CXCR4 antibody for 30 min on ice. After twice washes with PBS, the cells were analyzed using a FACScalibur flow cytometer (Becton Dickinson). The percent of receptor internalization was calculated from the mean channel fluorescence values of cells treated with ligand-free buffer.

Chemotaxic assay Migration of Jurkat cells was assessed in disposable Transwell trays with 6-mm-diameter chambers and membrane pore size of 5 µm. The wild-type of SDF-1α and α-helix-defective mutant of SDF-1α were diluted in RPMI-1640 containing 1 g/L of BSA. 600 µL of each diluted samples were added to the lower wells and incubated at 37 ºC for 30 min. To upper wells 200 µL of the suspension of Jurkat cells at 5×10^9/L were added. After 2 h at 37 ºC in a 5 % CO_2 incubator, cells that migrated to the lower wells were counted.

Intracellular calcium measurement Jurkat cells (5×10^9/L) were loaded with a calcium indicator dye Fluo-3AM (4 µmol/L; Molecular Probes, Eugene, OR) for 45 min in FCS-free RPMI1640 at room temperature in the dark. Cells were then washed 3 times with same medium and maintained in darkness until use. After addition of SDF-1/WT (4 nmol/L) or SDF-1/54R (10 nmol/L) for 15 min, the intercellular Fluo-3AM fluorescence signals were analyzed by flowcytometer with an excitation light of 488 nm and an emission light of 530 nm.

RESULTS

Design and construction of α-helix-defective mutant of SDF-1α In contrast to the functional importance of the N-terminus and the central β-sheet region of SDF-1α in interaction with CXCR4, the C-ter-

minal α-helix of SDF-1α is still poorly understood in terms of its relevance to the biological function of SDF-1α though a few studies have been done. To further clarify the functional role of the C-terminus of SDF-1α, a pair of special primers (mSDF-1/54F and mSDF-1/54R) were designed according to natural SDF-1α genetic sequence, by which, the residues from 55 to 67 in the C-terminus of SDF-1α were removed. After being cloned and expressed, a novel peptide structure of SDF-1α (named SDF-1/54R) was constructed. This peptide remained both the N-terminus of SDF-1α and the central β-sheet region of SDF-1α but lacked the C-terminal α-helix (Fig 1).

Purification of SDF-1/WT and SDF-1/54R The SDF-1/WT (wide type of SDF-1α) and SDF-1/54R proteins were expressed in an *E coli* strain, BL21Star, and purified by Ni-Resin immobile affinity chromatograph. Since both recombinants SDF-1/WT and SDF-1/54R proteins were not contained enough in the solution portion of the homogenate, a hybrid condition's purification protocol was used, and the eluted proteins were refolded by gradually changing buffer to the nature condition. The purity of the recombinant proteins was detected by SDS-PAGE analysis with Bromophenol Blue staining. The result showed that the molecular weight sizes of both SDF-1/WT and SDF-1/54R were matched the expected sizes, and the purity of both SDF-1/WT and SDF-1/54R was approximately 95 % (Fig 2).

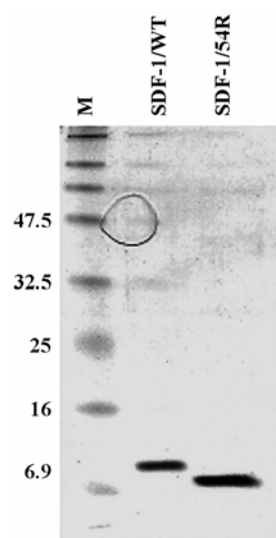


Fig 2. Purification of both SDF-1/WT and SDF-154R Recombinant wild type and mutant of SDF-1 proteins were purified form *E coli* and detected by SDS-PAGE with Bromophenol Blue staining.

The α-helix structure is important for chemotactic activity of SDF-1α Induction of T lymphocyte migration through the interaction with CXCR4 was demonstrated as one of the most important functions of natural SDF-1α. Since the cellular membrane of Jurkat cells, a T lymphocytes cell lines, constitutively expressed CXCR4, both SDF-1/WT and SDF-1/54R were tested for their chemotactic ability in Jurkat cells. Compared with the Jurkat cells without any treatment, SDF-1/WT induced dose-dependent chemotaxis of Jurkat cells and reached the peak at the concentration about 20 nmol/L. But α-helix-defective mutant, SDF-1/54R, did not show significantly chemotactic ability at the same concentration. And even though the concentration was increased to 100 nmol/L, its chemotactic ability still retained at lower level (Fig 3). The result suggest that the α-helix structure is important for chemotactic activity of SDF-1α.

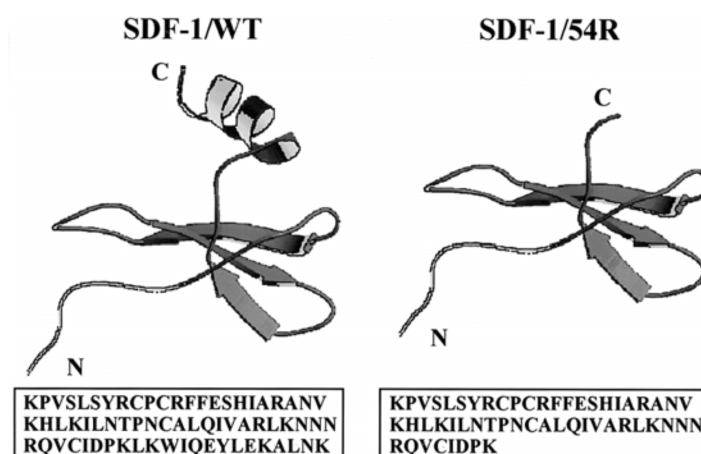


Fig 1. Design of α-helix-defective mutant of SDF-1α. SDF-1/54R was prepared by deleting 55-67 amino acids at C-terminus of SDF-1α. The amino acid sequence is shown.

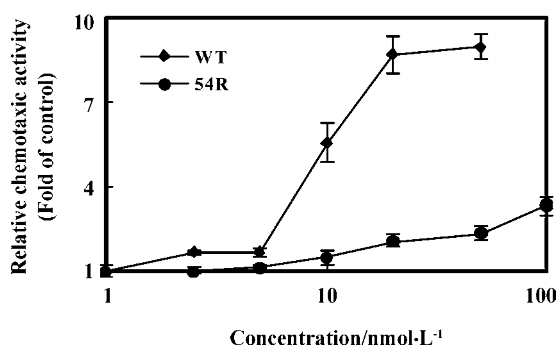


Fig 3. The α-helix structure is important for chemotactic activity of SDF-1. The chemotactic activity of both SDF-1/WT and SDF-1/54R in Jurkat cells were preformed with transwell trays with a membrane pore size of 5 μm. The background was the mean number of migrating cells without any treatment. The data shown was one of three different experiments.

weakly induced the phosphorylation in ERK1/2 (Fig 4). In addition, it has been known that the increase of in-

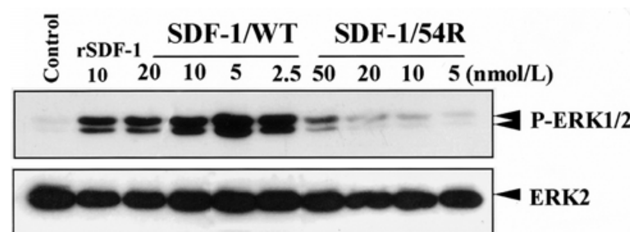


Fig 4. The α-helix of SDF-1α is important for activation of MEK/ERK pathway. The Jurkat cells were stimulated with or without SDF-1/WT and SDF-1/54R for 2 min. The cells were subjected to immunoblotting analysis with a specific anti-phospho-ERK1/2 body (upper panel). The membrane was stripped of antibody and immunoblotted with anti-ERK2 antibody (lower panel).

The α-helix of SDF-1α is important for activation of MEK/ERK pathway The activation of MEK/ERK cascade is known as a main mechanism for the intracellular pathway associated with SDF-1α activating CXCR4, which is the critical molecular base of the biological function of SDF-1α[10]. To further investigate why α-helix-defective mutant of SDF-1α failed to chemoattract Jurkat cells, the effect of SDF-1/54R on activating ERK1/2 (extracellular signal-regulated kinase) was tested by western blotting with a specific anti-phospho-ERK1/2 antibody. Upon treatment with SDF-1/ WT, a dramatic increase in the phosphorylation of ERK1/2 was observed, and the highest level of phosphorylation of the kinase was obtained by treatment with 5 nmol/L of SDF-1/WT, whereas treatment with 5 nmol/L of SDF-1/54R failed to induce ERK1/2 activation. Increasing the dose of SDF- 1/54R just tracellular free Ca^{2+}, a key event triggered by SDF-1α stimulation, is dependent on the activation of CXCR4 and intercellular signal transduction[11]. To further characterize the property of α-helix-defective SDF-1 in inducing intercellular signal transduction, we measured the effect of SDF-1/54R on triggering intracellular Ca^{2+} influx in Jurkat cells. In this study, SDF-1/54R was tested in comparison to SDF-1 for its ability to induce intracellular calcium transients. A rapid and transient increase in the intracellular calcium concentration was observed followed by the addition of SDF-1/WT for a few minutes. On the contrary, addition of SDF-1/54R induced a weak change in the intracellular calcium flux (Fig 5). These data were consistent with those from chemotaxis assay. Taken together, the findings suggested that α-helix in the structure of SDF-1α played a key role in activating CXCR4 and triggering signaling

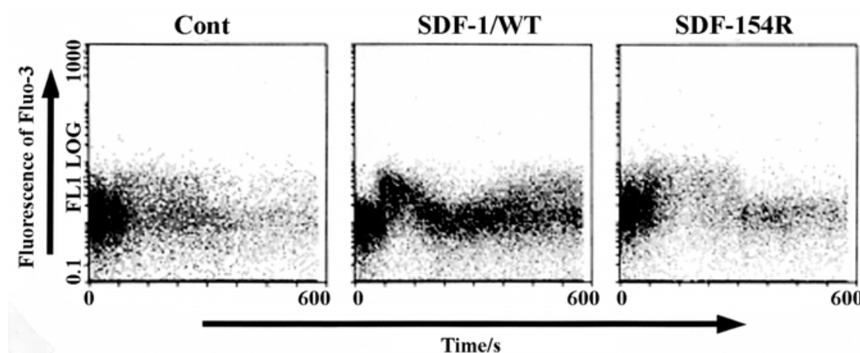


Fig 5. The α-helix-defective mutant of SDF-1α induced a distinct intracellular Ca^{2+} pattern from that SDF-1/WT-induced. The effects of both SDF-1/WT (4 nmol/L) and SDF-1/54R (10 nmol/L) on intracellular Ca^{2+} influx in Jurkat cells were measured by flow cytometric analysis with Fluo-3AM staining.

cascade in cell migration or other biological function.

SDF-1/54R inhibited migration of Jurkat cells induced by native SDF-1α An important question that needs to be answered is why the defection of α-helix compromised the effects of native α-helix on the migration and relevant cellular signal transduction of T cells? A possible explanation was that α-helix-defective mutant of SDF-1α lost its capacity in CXCR4 binding. Thus we examined whether SDF-1/54R has an inhibitory effect on native SDF-1α-mediated chemotaxis? Jurkat cells were pre-incubated with a series of concentration of SDF-1/54R for 2 h and then the cells were subjected to chemotaxis assay with 20 nmol/L of SDF-1/WT. The results showed that the cells migration induced by SDF-1/WT was abolished by pre-incubation with SDF-1/54R in a dose-dependent manner (Fig 6). At the concentration higher than 0.1 μmol/L, the native SDF-1α-mediated cell migration was significantly inhibited compared to the cells treated with SDF-1/WT alone, and the IC_{50} was approximately 38 nmol/L of SDF-1/54R. It suggested that the binding ability of SDF-1/54R with CXCR4 was not altered due to deleting α-helix though some of its intrinsic biologic functions such as inducing the migration of T cells, triggering CXCR4 mediated intercellular signaling transduction were lost.

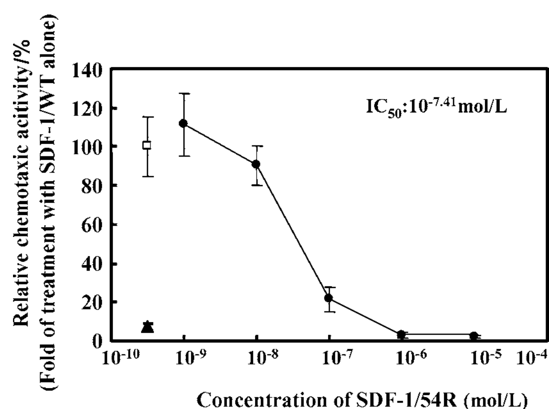
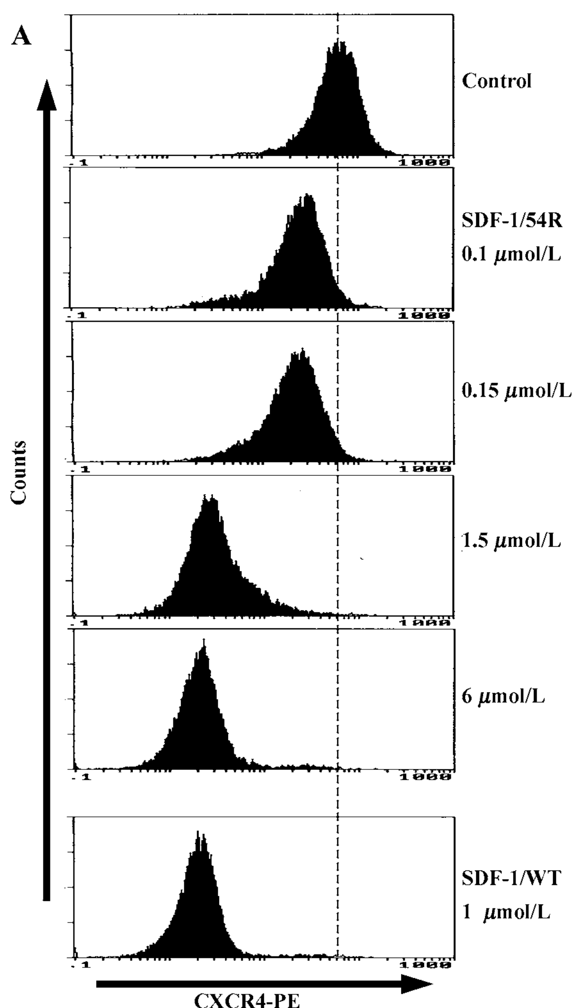


Fig 6. Pre-treatment with SDF-1/54R inhibited chemotaxic activity of wild type of SDF-1α. Jurkat cells were pre-incubated with or without indicated concentration of SDF-1/54R at 37 ºC for 2 h. The chemotactic activity of wild type of SDF-1 (20 nmol/L) in the cells was detected, and IC_{50} of SDF-1/54R was calculated. □: treatment with wild-type of SDF-1 alone; ▲: non-treatment control.

The α-helix-defective mutant of SDF-1α induced CXCR4 internalization The competitive inhibitory effect of SDF-1/54R on native SDF-1α-induced migration of Jurkat cells provided us essential evidence that SDF-1/54R probably reserved the binding ability to CXCR4. Considering CXCR4 internalization is dependent on its ligand binding, the receptor internalization was investigated to determine whether SDF-1/54R was able to bind to CXCR4 on Jurkat cells. The cells were treated with SDF-1/WT or SDF-1/54R, and the amount of CXCR4 on cell surface was measured by flow cytometer analysis with special PE-conjoined anti-CXCR4 antibody. After the exposure to SDF-1/54R, the CXCR4 internalization was rapidly induced in a dose-dependent manner. This effect of SDF-1/54R on down-regulation of the amount of CXCR4 on the cell surface was clearly observed even at a low dose, and the maximal effect appeared at 1.5 μmol/L of SDF-1/54R (Fig 7). The complete disappearance of CXCR4 from cell surface was also determined by a longer time-course test. Treatment with 1 μmol/L of SDF-1/54R resulted in a continued down-regulation of the amount of CXCR4 as longer as 72 h (Fig 8). These results further confirmed that the binding ability of SDF-1/54R to CXCR4 was not affected due to the loss of α-helix structure. In addition, ligand-mediated internalization of the receptor CXCR4 has been verified as the main mechanisms of chemokine-mediated inhibition of HIV-1 entry host cells[12]. Therefore, the effect of SDF-1/54R on inducing CXCR4 internalization should be helpful to its therapeutic uses in anti-AIDS.

DISCUSSION

Presently, although many anti-HIV agents are available, further development of new drugs is desired for multiple combine therapies that affect different stages of HIV-replication[13]. The unexpected encounter between the fields of chemokines and HIV has surely opened new perspectives for understanding the mechanisms of AIDS pathogenesis, as well as for developing effective therapies and vaccines. Increasing evidences support the fact that SDF-1α acts as a potential inhibitor of HIV due to its binding to the major HIV co-receptor CXCR4[3,4]. However, one of the major hurdles for developing safe and effective co-receptor inhibitors from native SDF-1α is the risk of interfering with multiple physiologic function of SDF-1α, thereby causing potentially harmful side-effects. Therefore, designing the special antagonists for CXCR4 through modifying the native structure of SDF-1α may be a useful way for the development of anti-HIV agents. In this paper,



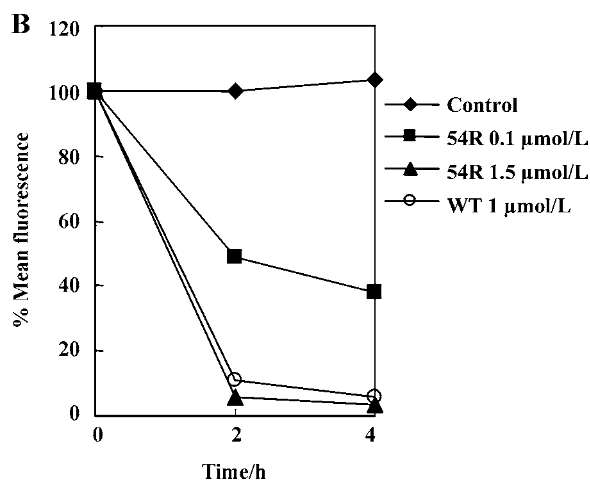


Fig 7. The α-helix-defective form of SDF-1 induced CXCR4 internalization. A: Jurkat cells were treated with or without SDF-1/54R or SDF-1/WT for 2 h. B: Jurkat cells were treated with or without SDF-1/54R or SDF-1/WT for 2 and 4 h. The CXCR4 on the cells surface was stained with a PE conjoined anti-human CXCR4 antibody and detected by flow cytometer.

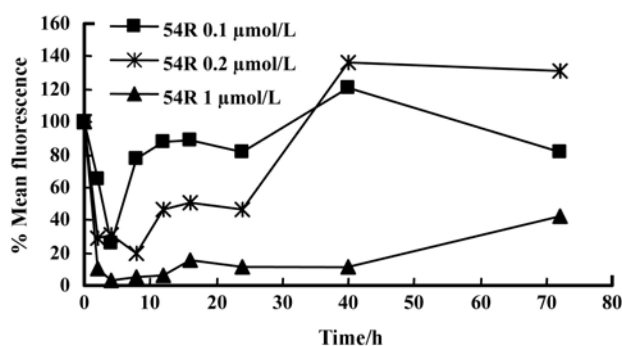


Fig 8. The time course of CXCR4 internalization induced by α-helix-defective form of SDF-1α. Jurkat cells were treated with indicated concentration of SDF-1 54R at 37 °C for 2-72 h. The CXCR4 on the cells surface was stained with a PE conjoined anti-human CXCR4 antibody and detected by flow cytometer. The level of CXCR4 on the surface of the cells was shown with the relative percentage of mean fluorescence of the cells without treatment.

it was confirmed that SDF-1/54R, a recombinant mutant, deleted the C-terminal α-helix of SDF-1α, showed not only low abilities to induce the chemotaxis and signal transduction in Jurkat cells but also a clear inhibitory effect on SDF-1α-mediated migration of these cells, whereas it did not lose its binding affinity with CXCR4 and function to induce CXCR4 internalization.

Previous studies on the structure-activity relationship have established that in 3 distinct structure regions, the N-terminus played a major role in the direct interaction of SDF-1α with the receptor[7]. The residues 12-17 of the loop region, which was termed as RFFESH motif and well defined in the SDF-1α structure, formed a receptor-binding site, which was proposed to be an important initial docking site of SDF-1α with the N-terminal residues of CXCR4. In addition, four cysteines that take shape two disulfide bonds (one is between cysteine 11 and 36 and the other between cysteine 13 and 52) have been believed to be essential for stabilizing the 3 dimensional structure of SDF-1α due to they act as a basic motif to form three-sheets with short loops in a Greek key formation[14]. Just as SDF-1/54R contains intact N-terminal structure and the four cysteines, it is foresighted that it remains the binding affinity to CXCR4. Consistently, the results indicated that exposure of Jurkat cells that constitutively express CXCR4 to SDF-1/54R induced rapidly CXCR4 internalization, which has been well known as a sensitive indicator of ligand binding with G-protein coupled receptor(s). It suggests that deletion of C-terminal α-helix dose not lose the binding activity of SDF-1α with

its receptor CXCR4.

Although the 8 N-terminal residues of SDF-1 made up an essential region for activation of receptor, it is also possible that multiple mechanism(s) may be involved in the activation of CXCR4 and signal transduction process. For example: normally binding with cell surface glycosaminoglycans (GAGs) including heparin is required for chemokines to stabilize receptor binding as well as to exert full biological activity[15]. The C-terminal helices have been verified to be the GAG binding site in CXC chemokines such as IL-8[16] and PF-4[17]. And the positive charge residues of chemokine have been believed to be required for GAG binding and the C-terminus of SDF-1α possesses many positive charge residues[18]. Being consistent with these findings, our data revealed that the functions of SDF-1/54R in activating CXCR4 and inducing cellular signal transduction dramatically decreased, whereas its binding affinity for CXCR4 did not change. It seems that the interaction of SDF-1/54R with CXCR4 may be transient, which is sufficient for CXCR4 internalization, but not adequate to stimulate CXCR4-dependent activation of hetrotrimeric guanine nucleotide-binding proteins (G-proteins). Therefore, it is not an accident that SDF-1/54R loses its other biological functions, including inducing signal transduction and chemotaxis.

Chemokine-mediated inhibition of HIV-1 entry appears to result from the combination of three mechanisms: (i) steric blocking of the interaction between gp120 and the co-receptor[19]; (ii) ligand-mediated internalization of the receptor, which reduces its availability for use by gp120[12]; and (iii) interference with receptor recycling[20]. To date, several findings have indicated that chemokine receptor internalization is a major mechanism of chemokines preventing HIV infection from host cells[21]. The results here shown that the CXCR4 internalization on Jurkat cells was rapidly induced by SDF-1/54R in a dose-dependent manner, which suggested that this new mutant of SDF-1 should be useful for inhibition of HIV-1 entry to target cells.

It is important to regard the clinical applicability of any agonists of SDF-1α, because drug-induced chemokine receptor activation could produce some side effects that may preclude its use in patients. Because of the character of SDF-1/54R, which can trigger CXCR4 receptor internalization without effects of intracellular calcium mobilization and cell migration, this peptic compound derived from native SDF-1 may be a valuable drug candidate for preventing from HIV infection and/or treatment of other pathological processes driven by the SDF-1/CXCR4 interplay, such as rheumatoid arthritis[22], breast cancer metastasis[23], arteriosclerosis[24], and asthma[25].

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