CXCR4 antagonist inhibits stromal cell-derived factor 1-induced migration and invasion of human pancreatic cancer

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Abstract
The stromal cell-derived factor-1 (SDF-1)/CXCR4 system is implicated in various instances of cell migration in mammals, including the migration of lymphocytes and the formation of metastases. We have recently synthesized a potent novel CXCR4 antagonist, TN14003. The purpose of this study was to investigate the role of SDF-1/CXCR4 axis in the pancreatic cancer metastasis via cell migration and invasion, and the inhibitory effect of TN14003 on pancreatic cancer cell metastasis. The expression of CXCR4 was detected in six pancreatic cancer cell lines by Western blotting and immunocytochemistry. In migration and invasion assays, SDF-1 stimulated both migration and invasion of cancer cells in a dose-dependent manner. The maximal effect of SDF-1 was observed at 100 ng/ml. SDF-1-induced migration and invasion of cancer cells were completely blocked by 100 nm TN14003. The stimulatory effect of SDF-1 on cancer migration and the inhibitory effect of TN14003 were mediated via the alteration in phosphorylation of mitogen-activated protein kinases. Treatment of cancer cells with 100 ng/ml SDF-1 resulted in a significant increase of actin polymerization, which was completely blocked by 100 nm TN14003. Taken together, SDF-1/CXCR4 axis is involved in pancreatic cancer metastasis through migration and invasion. The small molecule antagonists against CXCR4 such as TN14003 might be an effective anti-metastatic agent for pancreatic cancer. [Mol Cancer Ther. 2004;3(1):29–37]

Introduction
The chemokines are a family of low molecular weight cytokines that mediate the chemical effect on target cells through seven-transmembrane G-protein-coupled receptors (1–4). Stromal cell-derived factor-1 (SDF-1), which was initially cloned by Tashiro et al. (5), is a member of the CXC subfamily of chemokines and interacts with the seven-transmembrane G-protein-coupled receptor CXCR4, an exclusive receptor for SDF-1 (6, 7). Later, SDF-1 was identified as a growth factor for B-cell progenitors and also a chemotactic factor for T cells and monocytes relevant to B-cell lymphopoiesis and bone marrow myelopoiesis (6, 8, 9). Furthermore, SDF-1 was found to play a critical role in directed cell migration (6, 10) and embryonic development (8, 11, 12). Most importantly, the function of a subset of chemokine receptors as co-receptors for the entry of HIV-1 was clarified. CXCR4 and a CC-chemokine receptor, CCR5, represent major co-receptors for the entry of T-cell line-tropic HIV-1 (X4-HIV-1) and macrophage-tropic HIV-1 (R5-HIV-1), respectively (13, 14).

Recently, several studies have been conducted to detect the expression of CXCR4 and SDF-1 in solid tumors. The results are not uniform, and the relevance to cancer progression and tumor angiogenesis is unclear (15–17). In breast cancer, the SDF-1/CXCR4 system has been implicated in the formation of metastasis (18). Before this, we found that SDF-1 mRNA expression is detected in pancreatic cancer tissues, but is not detected in pancreatic cancer cell lines, whereas CXCR4 mRNA expression is detected in both pancreatic cancer tissues and cancer cell lines (19). To date, however, the role of interaction between SDF-1 and CXCR4 in pancreatic cancer progression has not been defined.

The specific ligands for these receptors are able to inhibit HIV infection (20–23). As for the malignant solid tumors, neutralizing the interactions of SDF-1/CXCR4 by administration of an antibody to CXCR4 significantly impairs metastasis of breast cancer cells to regional lymph node and lung in the animal model. For this reason, efforts have focused on developing a specific antagonist for chemokine receptors.

A CXCR4 antagonist, T22, was previously discovered as an anti-HIV peptide based on chemical conversions of horseshoe crab self-defense peptides, tachyplesins and polyphemusins (24). On the basis of T22 structure, we reported the synthesis of a novel CXCR4 inhibitor, T140 (25). T140 strongly antagonizes CXCR4 function, though it is not stable in serum due to the cleavage of COOH-terminal Arg14. Consequently, we reported that the COOH-terminal amidation and the double-L-citrulline (Cit)-scanning of T140 led to development of a novel effective CXCR4 inhibitor, TN14003, which possesses high selectivity index and complete stability in serum, without significant change in the secondary structure (25). In this
study, we investigated the role of SDF-1/CXCR4 axis in human pancreatic cancer cells, and tested the inhibitory effect of the novel CXCR4 inhibitor, TN14003, on in vitro cell functions that are relevant to metastasis.

**Materials and Methods**

**SDF-1 and TN14003**

We synthesized TN14003 and SDF-1 as described previously (25). TN14003 shows the highest level of anti-HIV activity and antagonism of target cell entry by X4-HIV-1 among all the CXCR4 antagonists that have been reported to date, and possesses complete stability in serum (25).

**Reagents and Antibodies**

The following reagents and reagents were purchased: An affinity-purified goat anti-CXCR4 polyclonal antibody (sc-6190, Santa Cruz Biotechnology, Santa Cruz, CA); a mouse anti-human CXCR4 monoclonal antibody (MAB172, R&D Systems, Minneapolis, MN); anti-phospho-p44/42 mitogen-activated protein (MAP) kinase antibody (9106, Cell Signaling Technology, Beverly, MA); anti-p44/42 MAP kinase antibody (#9102, Cell Signaling Technology); PD98059 (Calbiochem, San Diego, CA); Cy3-conjugated AffiniPure goat anti-mouse IgG (Jackson ImmunoResearch Laboratories, West Grove, PA); and rhodamine-phalloidin (R-415, Molecular Probes, Eugene, OR).

**Cell Line and Culture Conditions**

Human pancreatic cancer cell lines (CFPAC-1, Capan-2, AsPC-1, BxPC-3, and SUIT-2) were maintained in the following media at 37°C in a humid atmosphere of 5% CO₂/95% air. CFPAC-1 cells were cultured in Iscove's modified Dulbecco's medium with 10% fetal bovine serum (FBS). CFPAC-1 cells were cultured in DMEM with 10% FBS. BxPC-1 cells, Capan-2 cells, AsPC-1 cells, and SUIT-2 cells were cultured in RPMI 1640 with 10% FBS. Each medium contained 100 units/ml penicillin and 0.1 mg/ml streptomycin.

**Expression of CXCR4 in Pancreatic Cancer Cells**

Serum-starved subconfluent cells were harvested, washed in cold PBS, and lysed in ice-cold lysis buffer [10 mM PBS (pH 7.4), 1% NP40, 0.5% sodium deoxycholate, 0.1% SDS, 5 mM EDTA] supplemented with 1% phenylmethylsulfonyl fluoride and 0.02 mg/ml gabeptate mesilate, a synthetic protease inhibitor (FOY, Ono Pharmaceutical, Osaka, Japan) for 30 min at 4°C. The lysate was homogenized and centrifuged at 15,000 rpm for 30 min at 4°C to remove debris, and the protein concentration was measured using a colorimetric protein assay kit (Pierce, Rockford, IL). The extracted protein was subjected to Western blotting, as previously described (26). Equal amounts of protein were loaded onto 10% SDS-polyacrylamide gels and the proteins were transferred to polyvinylidene difluoride membrane (Bio-Rad, Richmond, CA). Blots were blocked at 4°C overnight with 5% (w/v) nonfat milk in TTBS buffer (10 mM Tris-HCl, 150 mM NaCl, 0.5% Tween 20). The blots were incubated for 1 h at room temperature with the primary antibody against CXCR4 (sc-6190, Santa Cruz Biotechnology) diluted at 1:100 with TTBS. The blots were incubated for 1 h with a second antibody (200 ng/ml alkaline phosphatase-conjugated anti-goat IgG, Pierce). Immunoreactive proteins were visualized using alkaline phosphatase solution supplemented with 100 mM Tris-HCl, 100 mM HCl, 5 mM MgCl₂, 0.03% nitroblue tetrazolium, and 0.017% 5-bromo-indolyphosphate P-toluidine salt.

**Immunocytochemistry**

Pancreatic cancer cells were seeded on coverslips and incubated for 24 h at 37°C in a humid atmosphere of 5% CO₂/95% air. The coverslips with cells were then fixed with 4% paraformaldehyde in PBS for 10 min, washed with PBS, permeabilized in 1% Triton X-100 in PBS for 15 min, washed, and blocked with TTBS with 1% BSA. For CXCR4 staining, fixed and permeabilized cells were incubated with mouse anti-human CXCR4 monoclonal antibody (MAB172, R&D Systems) diluted in 1% BSA-TTBS (0.01 mg/ml) for 2 h at 37°C, and rinsed 3 times with TTBS, and then incubated for 30 min with secondary antibody (Cy3-conjugated AffiniPure goat anti-mouse IgG, Jackson ImmunoResearch Laboratories, West Grove, PA) and rhodamine-phalloidin (R-415, Molecular Probes, Eugene, OR).

**In Vitro Proliferation Assay**

Pancreatic cancer cells (AsPC-1, PANC-1, and SUIT-2) were seeded at a density of 5000 cells per well into 96-well plates in culture medium containing 10% FBS. After 24 h, the cultures were washed and refed with medium alone (control) or with medium containing SDF-1 or TN14003 at various concentrations. After 3 days, the number of viable cells was counted using Cell Counting Kit 8 (Dojindo Co., Kumamoto, Japan) according to the manufacturer's instructions. The assay reagent is a tetrazolium compound (WST-x8) that is reduced by live cells into a colored formazan product measured at 450 nm. The quantity of formazan product measured at 450 nm is directly proportional to the number of live cells in the culture. The experiments were repeated in triplicate wells.

**In Vitro Migration Assays**

Migration of cancer cells was assayed using 6.5-mm-diameter chambers with 8-μm pore filters (Transwell, 24-well cell culture, Coster, Boston, MA). Pancreatic cancer cells were suspended at 2 × 10⁶ cells/ml in serum-free media, and then 0.2 ml cell suspension was added to the upper chamber. Then, 0.5 ml serum-free media with various concentrations of SDF-1 was added to the lower chamber. In another set of experiments, 0.5 ml serum-free media with 10 ng/ml of SDF-1 (fixed concentration) plus various concentrations of TN14003 was added to the lower chamber. The number of migrating cells in five high-power fields per filter was counted microscopically at ×400 magnification. Because the background migration without SDF-1 varied among experiments, data were normalized as the migration
index: the number of migrating cells in an experimental chamber/the number of migrating cells in control chamber without SDF-1. In another experiment, cells were pretreated with 30 μM PD98059 (Calbiochem), a MEK1 inhibitor, before SDF-1 treatment.

**In Vitro Invasion Assays**

Invasion of cancer cells was assayed using a Biocoat Matrigel invasion chamber (Becton Dickinson, Bedford, MA), which consists of an 8-μm pore size polyethylene terephthalate (PET) membrane that has been overlaid with Matrigel (basement membrane matrix). PANC-1, SUIT-2, or AsPC-1 cells were seeded at 2 × 10^5 cells/ml in serum-free media, and then 0.2 ml cell suspension was added to the upper chamber. Next, 0.5 ml serum-free media with 100 ng/ml of SDF-1 (fixed concentration) plus various concentrations of TN14003 was added to the lower chamber. The chambers were incubated for 12 h at 37°C in a humid atmosphere of 5% CO₂/95% air. After incubation, the filters were fixed and stained with Diff-Quick reagent (Dade Behring). The upper surface of the filters was scraped twice with cotton swabs to remove non-invading cells. The experiments were repeated in triplicate wells, and the number of invading cells in five high-power fields per filter was counted microscopically at ×400 magnification. The data were processed by the method described in migration assay.

**Detection of p44/42 MAP Kinases in Pancreatic Cancer Cells**

Cells were put in DMEM containing low levels of BSA (0.5%) to render the cells quiescent. Then the cells were incubated with 100 ng/ml SDF-1 or 100 ng/ml SDF-1 plus 100 nm TN14003 for 5 min to 1 h at 37°C. In another setting, PANC-1 cells were preincubated for 1 h at 37°C with 10 μM PD98059 (Calbiochem), a MEK1 inhibitor, before SDF-1 treatment. After treatment with SDF-1, cells were lysed for 60 min in phosphorylation-inhibitory radioimmunoprecipitation assay (RIPA) buffer containing 50 mM HEPES (pH 7.0), 250 mM NaCl, 0.1% NP40, 1 mM phenylmethylsulfonyl fluoride, and 20 μg/ml gabexate mesilate, then the lysate was sonicated for 10 s. Total extracts were cleaned by centrifugation at 12,000 rpm for 10 min at 4°C, and the supernatants were collected. Protein concentrations were measured using a protein assay kit (Tonein-TP, Otsuka Pharmaceutical, Tokyo, Japan). The lysates were resuspended in 1 volume of the gel loading buffer that contained 50 mM Tris-HCl (pH 6.7), 4% SDS, 0.02% bromophenol blue, 20% glycerol, and 4% 2-mercaptoethanol, and then boiled at 95°C for 90 s. The extracted protein was subjected to Western blotting, as described in previous study, to detect p44/42 MAP kinase expression. After stripping and blocking, the same blots were re-probed with anti-p44/42 MAP kinase antibody (#9102, Cell Signaling Technology) to measure total MAP kinase.

**Actin Cytoskeleton**

Pancreatic cancer cells were seeded on coverslips and incubated for 24 h at 37°C in a humid atmosphere of 5% CO₂/95% air. Then the cells were incubated in serum-free medium containing 0.1% BSA with 100 ng/ml SDF-1 or with 100 ng/ml SDF-1 plus 100 nm TN14003 for 1 h at 37°C. The coverslips with cells were then fixed with 4% paraformaldehyde in PBS for 10 min, washed with PBS, permeabilized in 1% Triton X-100 in PBS for 15 min, washed, and blocked with TTBS with 1% BSA. For visualization of filamentous actin, the cells were exposed to rhodamine-phalloidin for 30 min at 37°C and washed with TTBS. After final wash, coverslips were mounted on the slide-glass using 50% solution of glycerol in PBS. The cells were examined under a fluorescence microscope (Olympus).

**Adhesion Assay**

The adhesion of pancreatic cancer cells to the elements of extracellular matrix (ECM) was evaluated. Pancreatic cancer cells were grown to subconfluent state and then harvested by 0.25% trypsin/EDTA (Invitrogen Corp., Carlsbad, CA) in 1 min. Cells were preincubated with SDF-1 or with SDF-1 plus TN14003. The same volume of vehicle was added to cells as a control. Preincubated cells were plated onto a 96-well microplate which was precoated with various kinds of ECM elements, that is, 0.01 mg/ml plasma fibronectin, 0.01 mg/ml vitronectin or Matrigel, or 96-well Microtest Plate (BioCoat, Becton-Dickinson Japan, Tokyo, Japan) precoated with collagen I, collagen IV, or laminin. Then the cells were incubated for 3 h at 37°C in 5% CO₂/95% air to allow cell attachment. Cells were washed gently with PBS 3 times to remove detached cells. The number of adherent cells was measured by WST method using Cell Counting Kit 8 (Dojindo). Experiments were repeated 3 times in triplicate wells.

**Statistical Analysis**

Statistical comparisons were performed by a two-way ANOVA for repeated measures, followed by a post hoc Turkey test, or Student’s two-tailed t test. P < 0.05 was considered to be significant.

**Results**

**Expression of CXCR4 in Pancreatic Cancer Cells**

The expression of CXCR4 in six pancreatic cancer cell lines (CFFAC-1, Capan-2, AsPC-1, PANC-1, BxPC-3, and SUIT-2) was examined. Western blot analysis demonstrated definite expression of immunoreactive CXCR4 protein in...
all pancreatic cancer cell lines. The strength of CXCR4 expression was similar among the six cell lines (Fig. 1A). Immunocytochemistry demonstrated the expression of CXCR4 in all pancreatic cancer cells (Fig. 1B).

Effect of SDF-1 on Pancreatic Cancer Cell Proliferation

The effect of SDF-1 on cell proliferation was examined in pancreatic cancer cell lines (AsPC-1, PANC-1, and SUIT-2). After incubation for 72 h, the cell proliferation was not significantly changed by SDF-1 at concentrations from 50 to 200 ng/ml (Fig. 2A).

Effect of TN14003 on Pancreatic Cancer Cell Proliferation

We found that the 50% effective concentration (EC50) of TN14003 against HIV-induced cytopathogenicity in MT-4 cells was 0.6 nM, and the 50% cytotoxic concentration (CC50) of TN14003 against human peripheral blood mononuclear cells (PBMCs) was 410 \( \pm \) 10 nM (25). Then, the selectivity index (SI = [CC50/EC50]) of TN14003 in this system was 680,000, and the complete inhibitory concentration of TN14003 was conjectured between 20 and 80 nM from these data. Thus, the effect of TN14003 at concentrations up to 10 \( \mu \)M was tested in terms of proliferation of pancreatic cancer cells (AsPC-1, PANC-1, and SUIT-2). TN14003 showed no inhibitory effect on cell proliferation at concentrations up to 100 nM (Fig. 2B). The cell proliferation was slightly suppressed at 1 and 10 \( \mu \)M, but the suppression was not statistically significant for PANC-1 and SUIT-2. TN14003 at 10 \( \mu \)M suppressed the proliferation of AsPC-1 (Fig. 2B). Therefore, the following experiments were performed by using TN14003 at concentrations less than 10 \( \mu \)M.

Effect of TN14003 on SDF-1-Induced Migration and Invasion of Pancreatic Cancer Cells

SDF-1 stimulated the migration of pancreatic cancer cells (Fig. 3). Maximal effect was observed at 100 ng/ml of SDF-1 in all pancreatic cancer cell lines, and maximal migration indices were 214 \( \pm \) 27% in PANC-1 cells, 191 \( \pm \) 35% in AsPC-1 cells, and 159 \( \pm \) 17% in SUIT-2 cells, respectively. Supramaximal suppression was observed at concentrations over 100 ng/ml in this system. Next, the inhibitory effect of TN14003 on SDF-1-induced migration was tested. The migration induced by SDF-1 at 100 ng/ml was inhibited by TN14003 in PANC-1, AsPC-1, and SUIT-2 cells, and was completely blocked by TN14003 at 100 nM (Fig. 4).

The effects of SDF-1 and TN14003 on cancer cell invasion were tested. SDF-1 at 100 ng/ml induced maximal effect and the maximal invasion indices were 150 \( \pm \) 14% in PANC-1 cells, 180 \( \pm \) 15% in AsPC-1 cells, and 168 \( \pm \) 8% in SUIT-2 cells. The invasion induced by SDF-1 at 100 ng/ml was inhibited by TN14003 in all cancer cells, and was completely blocked by TN14003 at 100 nM (Fig. 5). Meanwhile, T22, a lead compound for TN14003 (27), did not completely block the migration and invasion at the same concentration.

Effect of SDF-1 and TN14003 on Actin Cytoskeleton of PANC-1 Cells

Serum-starved cells displayed low levels of F-actin as judged by rhodamine staining (Fig. 6A). After treatment with SDF-1 at 100 ng/ml for 1 h, PANC-1 cells showed intense F-actin staining in the periphery of the cells, distinct pseudopodia formation, and stress fibers (actin polymerization, Fig. 6B). Addition of TN14003 at 100 nM eradicated the SDF-1-induced actin polymerization (Fig. 6C). The cells that express actin polymerization were counted microscopically in five high-power fields at \( \times 400 \) magnification. The ratio of cells with actin polymerization was 41 \( \pm \) 8% (\( n = 3 \)) in control medium, 75 \( \pm \) 7% (\( n = 3 \)) in cells with SDF-1, and 47 \( \pm \) 7% (\( n = 3 \)) in cells with SDF-1 plus TN14003.

Involvement of p44/42 MAP Kinases in Pancreatic Cancer Cell Mobility

To explain the mechanism by which TN14003 inhibited the migration and invasion of pancreatic cancer cells, we next investigated the involvement of p44/42 MAP kinases. Pretreatment of PANC-1 cells with 100 ng/ml SDF-1, the phosphorylated p44/42 MAP kinase (Erk1/2) was transiently increased peaking at 10 min following addition of SDF-1 and dropping back to baseline thereafter (Fig. 7A). An addition of TN14003 abolished the SDF-1-induced phosphorylation of p44/42 MAP kinase.

Therefore, we next investigated whether the phospho-p44/42 MAP kinase is involved in the cancer cell mobility. Pretreatment of PANC-1 cells with PD98059 resulted in a suppression of SDF-1-induced phosphorylation of p44/42 MAP kinase (Fig. 7B). At the same time, pretreatment of PANC-1 cells with PD98059 abolished the increased migration induced by SDF-1 (Fig. 7C).

Cell Adhesion Assay

The effects of SDF-1 on pancreatic cancer cell adhesion to the elements of ECM were tested. The number of adherent...
cells to fibronectin, vitronectin, and collagen I was not changed by SDF-1 (Fig. 8, A–C). In contrast, the number of adherent cells to Matrigel was increased by SDF-1 (Fig. 8D). Matrigel is a solubilized basement membrane matrix extracted from the Engelbreth-Holm-Swarm mouse tumor, and is composed of collagen IV, laminin, and other elements. Therefore, we tested the effects of SDF-1 on cell adhesion to collagen IV and laminin-coated plate. SDF-1 induced increase of pancreatic cancer cell adhesion to laminin but not to collagen IV (Fig. 8, E and F). The increasing effect of SDF-1 on pancreatic cancer cell adhesion to laminin was not reversed by an addition of TN14003 (data not shown).

Discussion
In this report, we investigated the expression of CXCR4 in pancreatic cancer cell lines and determined the effects of its ligand, SDF-1. We showed that all the six human pancreatic cancer cell lines express the CXCR4 receptors by Western blotting and immunocytochemistry. Recent reports have demonstrated that CXCR4 receptors are expressed in most small cell lung cancer cells (16), ovarian cancer cells (28), and melanoma cells (29). In contrast, CXCR4 mRNA expression in colon, esophageal, or gastric cancers were not different from that of non-cancerous tissues, and CXCR4 mRNA expression in hepatocellular carcinoma were reduced when compared to non-cancerous liver tissue (17, 30). We found that CXCR4 mRNA was detected in both pancreatic cancer tissues and cancer cell lines, whereas SDF-1 mRNA expression was detected in all pancreatic cancer tissues but was not detected in pancreatic cancer cell lines (19). These findings indicate that the paracrine mechanism may be involved in the SDF-1/CXCR4 ligand receptor system in those solid tumors that express CXCR4 receptors. The transcription
factors that regulate CXCR4 expression are currently unknown. Recently, it has been reported that NF-κB regulates the motility of cancer cells by directly up-regulating the expression of CXCR4 (31).

In pancreatic cancer cell lines, we showed that SDF-1 had no direct effect on cell proliferation. Our result is in accordance with the recent study which showed that SDF-1 had no proliferative effects on rhabdomyosarcoma (32). It also agrees with a report demonstrating lack of a proliferative effect by SDF-1 on colony-forming units-megakaryocyte (CFU-MK) (33) or lymphohematopoietic cells (34). In contrast, recent reports demonstrated that SDF-1 stimulates proliferation of small cell lung cancer cells (NCI-H69) in the presence of serum (16), that SDF-1 acts together with thrombopoietin to enhance the development of CFU-MK in a murine model (35) and that SDF-1 at low doses enhances the proliferation of peripheral blood CD34+ cells (36). In addition, antisense CXCR4 overexpression in glioblastoma cells caused inhibition of cell proliferation, suggesting that SDF-1/CXCR4 system is involved in cell proliferation in glioblastoma cell lines as well (15, 37). We suggest that these differences may be due to the different culture system or to the different target cells.

Although we did not find any effect of SDF-1 on proliferation of pancreatic cancer cells, we found that SDF-1 stimulates cell metastasis and invasive behavior. In migration assay by Transwell chamber, SDF-1 significantly increased migration of pancreatic cancer cells. The cancer cells responded to SDF-1 in a similar manner, and maximal effect by SDF-1 was observed at 100 ng/ml. Invasion assay by Matrigel-coated invasion chamber also showed that SDF-1 significantly stimulated invasion of pancreatic cancer cell. Previous reports similarly showed that invasion through Matrigel was stimulated by SDF-1 in CXCR4-expressing ovarian cancer cells (38), prostate cancer cells (39), and myeloma cells (40). Video microscopic examination revealed that SDF-1 stimulated the motility of small cell lung cancer cells (16).

It has recently been reported that CXCR4 was highly expressed in malignant but not normal breast tissue, and that its ligand, SDF-1, is expressed in those organs where breast cancer metastasis is frequently found (bone marrow, lymph node, lung, and liver) (18). Furthermore, neutralizing the interactions of SDF-1/CXCR4 by administration of an antibody to CXCR4 significantly impairs metastasis of breast cancer cells to regional lymph node and lung in their breast cancer metastasis model. These reports, taken together with the current results, indicate that SDF-1/CXCR4 interaction may be generally important for the metastasis of solid tumors that express CXCR4.

In tumor cells, high levels of actin polymerization are required for the formation of stress fiber and pseudopodia, which in turn are implicated in the enhancement of cell migration and invasion. We showed that the treatment of pancreatic cancer cells with SDF-1 resulted in a dramatic increase in actin polymerization, which is needed for the invasion of malignant cells into tissues and for efficient metastasis. These findings suggest that SDF-1/CXCR4 ligand receptor system plays an important role in invasion as well as metastasis in pancreatic cancer.

Since CXCR4 was identified as a co-receptor for the entry of T-cell line-tropic (T-tropic) HIV-1 (13, 14), development
for CXCR4 antagonists seemed an ideal approach to discover an effective anti-HIV agent. Several specific antagonists for CXCR4 have been developed to date; however, there was no effective agent that satisfied both pharmacological and clinical requirements. The CXCR4 antagonist T22, which was derived from chemical conversions of horseshoe crab self-defense peptides, tachypleins, and polyphemusins, had previously been discovered as an anti-HIV peptide (24).

On the basis of the structure-activity relationship study (SAR) of T22, we previously synthesized a more effective analogue, T140 (27). This agent showed the highest level of anti-HIV activity and antagonism of target cell entry by X4-HIV-1 among all the CXCR4 antagonists that have been developed.

Figure 7. Effect of TN14003 on SDF-1-induced phosphorylation of p44/42 MAP kinase (Erk 1 and 2) in pancreatic cancer cells. A, PANC-1 cells were treated with SDF-1 alone or SDF-1 plus TN14003. Cells were harvested at 5, 10, 30, and 60 min, and phospho-p44/42 MAP kinase and total MAP kinase were detected as described. SDF-1 induced the maximal level of phosphorylation of p44/42 MAP kinase at 10 min. The addition of TN14003 eradicated the SDF-1-induced phospho-p44/42 MAP kinase. B, PANC-1 cells were treated with SDF-1 alone or SDF-1 plus PD98059. Cells were harvested at 10 min and phospho-p44/42 MAP kinase and total MAP kinase were detected as described. Pretreatment of PANC-1 cells with PD98059 eradicated the SDF-1-induced phospho-p44/42 MAP kinase. C, pancreatic cancer cells were stimulated by SDF-1 at 100 ng/ml with or without pretreatment of PD98059. The SDF-1-stimulated migration was inhibited by the pretreatment of PD98059. Columns, mean of three separate experiments in triplicate wells; bars, SE. * P < 0.05 compared with control. # P < 0.05 compared with SDF-1.

Figure 8. The effects of SDF-1 on pancreatic cancer cell adhesion to the elements of extracellular matrix. The number of adherent cells to fibronectin (A), vitronectin (B), and collagen I (C) was not changed by SDF-1. The number of adherent cells to Matrigel (D) was increased by SDF-1. The number of adherent cells to collagen IV was not changed (E), but that to laminin (a component of Matrigel) was increased by SDF-1 (F). Columns, mean of three separate experiments; bars, SE. * P < 0.05 compared with control.
Role of SDF-CXCR4 Axis in Pancreatic Cancer Metastasis

In this study, we found that human pancreatic cancer cells express the chemokine receptor CXCR4, which mediate ligand-dependent cell migration and invasion in vitro, and that migration and invasion of pancreatic cancer cells induced by SDF-1 were completely blocked by 100 nM TN14003. The IC_{50} of TN14003 on cancer cell migration and invasion was almost one-tenth that of T22. We demonstrated that TN14003 inhibited SDF-1-induced actin polymerization of pancreatic cancer cells. In contrast, SDF-1 stimulated adhesion of pancreatic cancer cells to laminin, one of the ECM components, but the addition of TN14003 did not reverse the effect of SDF-1. Therefore, TN14003 seems to mainly antagonize the stimulatory effect of SDF-1 on the mobility of pancreatic cancer cells. The C-it-substitution for a reduction of total cationic charges in the molecule is thought to be useful for developing effective anti-metastatic peptides, which could be a new type of anti-cancer agents against CXCR4.

In conclusion, the results in this study indicate that the SDF-1-CXCR4 ligand receptor axis may play an important role in metastasis of pancreatic cancer, and that CXCR4 receptor antagonists could inhibit metastatic behavior of pancreatic cancer cells. Therefore, it is strongly suggested that TN14003 could be an effective anti-metastatic agent. Small-molecule antagonists of chemokine receptors like TN14003 may be useful in treating cancer patients.

References


Molecular Cancer Therapeutics

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