# Expression of Stromal Cell-derived Factor 1 and CXCR4 Ligand Receptor System in Pancreatic Cancer: A Possible Role for Tumor Progression

**Takatomo Koshiba,[1] Ryo Hosotani, Yoshiharu Miyamoto, Jun Ida, Shoichiro Tsuji, Sanae Nakajima, Michiya Kawaguchi, Hiroyuki Kobayashi, Ryuichiro Doi, Toshiyuki Hori, Nobutaka Fujii, and Masayuki Imamura**

Departments of Surgery and Surgical Basic Science [T. K., R. H., Y. M., J. I., S. T., S. N., M. K., H. K., R. D., M. I.], Hematology and Oncology [T. H.], and Pharmaceutical Science [N. F.], Kyoto University, Kyoto 606-4897, Japan

## ABSTRACT

**To examine the expression of the stromal cell-derived factor 1 (SDF-1)/CXCR4 receptor ligand system in pancreatic cancer cells and endothelial cells, we performed immunohistochemical analysis for 52 pancreatic cancer tissue samples with anti-CXCR4 antibody and reverse transcription-PCR analysis for CXCR4 and SDF-1 in five pancreatic cancer cell lines (AsPC-1, BxPC-3, CFPAC-1, HPAC, and PANC-1), an endothelial cell line (HUVEC), and eight pancreatic cancer tissues. We then performed cell migration assay on AsPC-1 cells, HUVECs, and CFPAC-1 cells in the presence of SDF-1 or MRC-9 fibroblast cells. Immunoreactive CXCR4 was found mainly in pancreatic cancer cells and endothelial cells of relatively large vessels around a tumorous lesion. The immunopositive ratio in the pancreatic cancer was 71.2%. There was no statistically significant correlation with clinicopathological features. SDF-1 mRNA expressions were detected in all pancreatic cancer tissues but not in pancreatic cancer cell lines and HUVECs; meanwhile, CXCR4 mRNA was detected in all pancreatic cancer tissues, cancer cell lines, and HUVECs. The results indicate that the paracrine mechanism is involved in the SDF-1/CXCR4 receptor ligand system in pancreatic cancer. *In vitro* studies demonstrated that SDF-1 significantly increased the migration ability of AsPC-1 and HUVECs, and these effects were inhibited by CXCR4 antagonist T22, and that the coculture system with MRC-9 also increased the migration ability of CFPAC-1 cells, and this effect was significantly inhibited by T22. Our results suggested that the SDF-1/CXCR4 receptor ligand system may have a possible role in the pancreatic cancer progression through tumor cell migration and angiogenesis.**

## INTRODUCTION

Chemokines belong to the small molecule chemoattractive cytokine family and are grouped into CXC chemokines and CC chemokines, on the basis of the characteristic presence of four conserved cysteine residues (1–3). Chemokines mediate the chemical effect on target cells through G-protein-coupled receptors, which are characterized structurally by seven transmembrane spanning domains and are involved in the attraction and activation of mononuclear and polymorphonuclear leukocytes. The effects of CXC chemokines on cancer cells have been investigated in the case of IL[3]-8. Several studies have demonstrated the presence of IL-8 and its receptor in tumor tissues, which were involved in vascular endothelial cell proliferation and tumor neovascularization (4–7). It was also reported that IL-8 inhibited non-small cell lung cancer proliferation via the autocrine and paracrine pathway (8). IL-8 produced by malignant melanoma was found to induce cell proliferation via the autocrine pathway *in vitro* (9). These studies indicate that IL-8 is involved in the regulation of tumor progression through tumor angiogenesis and/or direct cancer cell growth.

SDF-1 was initially cloned by Tashiro *et al.* (10) and later identified as a growth factor for B cell progenitors, a chemotactic factor for T cells and monocytes, and in B-cell lymphopoiesis and bone marrow myelopoiesis (11–13). SDF-1 is a member of the CXC subfamily of chemokines, and its chemotactic effect is mediated by the chemokine receptor CXCR4 (12, 14). Most of the chemokine receptors interact with pleural ligands, and *vice versa*, but the SDF-1/CXCR4 receptor ligand system has been shown to involve a one-on-one interaction (15, 16). Furthermore, CXCR4 has been shown to function as a coreceptor for T lymphocytotrophic HIV-1 isolates (17). Recent studies have demonstrated that endothelial cells express CXCR4 and are strongly chemoattracted by SDF-1 (18–20). Tachibana *et al.* (15) reported that in the embryo of CXCR4 or SDF-1 knockout mice larger branches of the superior mesenteric artery were missing and that the resultant abnormal circulatory system led to gastrointestinal hemorrhage and intestinal obstruction. These findings suggest that SDF-1 and CXCR4 are involved in organ vascularization, as well as in the immune and hematopoietic system.

Recently, several studies have been conducted to detect the mRNA expression of CXCR4 and SDF-1 in solid tumors. The results are not uniform, and the relevance to cancer progression and tumor angiogenesis is not determined (21, 22). To clarify the role of the SDF-1/CXCR4 receptor ligand system in pancreatic cancer, we have investigated the expression of CXCR4 and SDF-1 with the aid of immunohistochemical analysis and RT-PCR in pancreatic cancer tissue and experimental chemotactic activity of SDF-1 in pancreatic cancer cells and vascular endothelial cells *in vitro*.

Received 5/20/99; revised 3/1/00; accepted 6/6/00.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] Supported in part by a grant from the Japanese Ministry of Education.

[2] To whom requests for reprints should be addressed, at Department of Surgery, Kishiwada City Hospital, 2-Gakuhara-cho, Kishiwada-shi, Osaka 596-8501, Japan. Phone: 81-75-751-3650; Fax: 81-75-751-3219.

[3] The abbreviations used are: IL, interleukin; SDF-1, stromal cell-derived factor 1; RT-PCR, reverse transcription-PCR; FBS, fetal bovine serum; HUVEC, human umbilical vein endothelial cell.

*Table 1* Comparison between expression of CXCR4 proteins and clinicopathological features in pancreatic ductal adenocarcinomas

| | No. of patients | Negative staining for CXCR4 | Positive staining for CXCR4 | $p$ ($\chi^2$ test) |
|---|---|---|---|---|
| Total | 52 | 15 | 37 (71.2%) | |
| Primary tumor | | | | |
| Limited to the pancreas (pT,2[a]) | 21 | 5 | 16 (76.2%) | |
| Extend out of the pancreas (pT3,4[a]) | 31 | 10 | 21 (70.0%) | 0.506 |
| Lymph node metastasis | | | | |
| Negative | 18 | 7 | 11 (61.1%) | |
| Positive | 34 | 8 | 26 (76.5%) | 0.250 |
| Metastasis to liver | | | | |
| Negative | 48 | 15 | 33 (68.9%) | |
| Positive | 4 | 0 | 4 (100%) | 0.091 |
| Stage (UICC[a]) | | | | |
| I | 8 | 3 | 5 (62.5%) | |
| II | 5 | 2 | 3 (60.0%) | |
| III | 20 | 6 | 14 (70.0%) | |
| IVa, b | 19 | 4 | 15 (78.9%) | 0.759 |

[a] UICC TNM Classification Ed. 5. Berlin: Springer-Verlag, 1977.

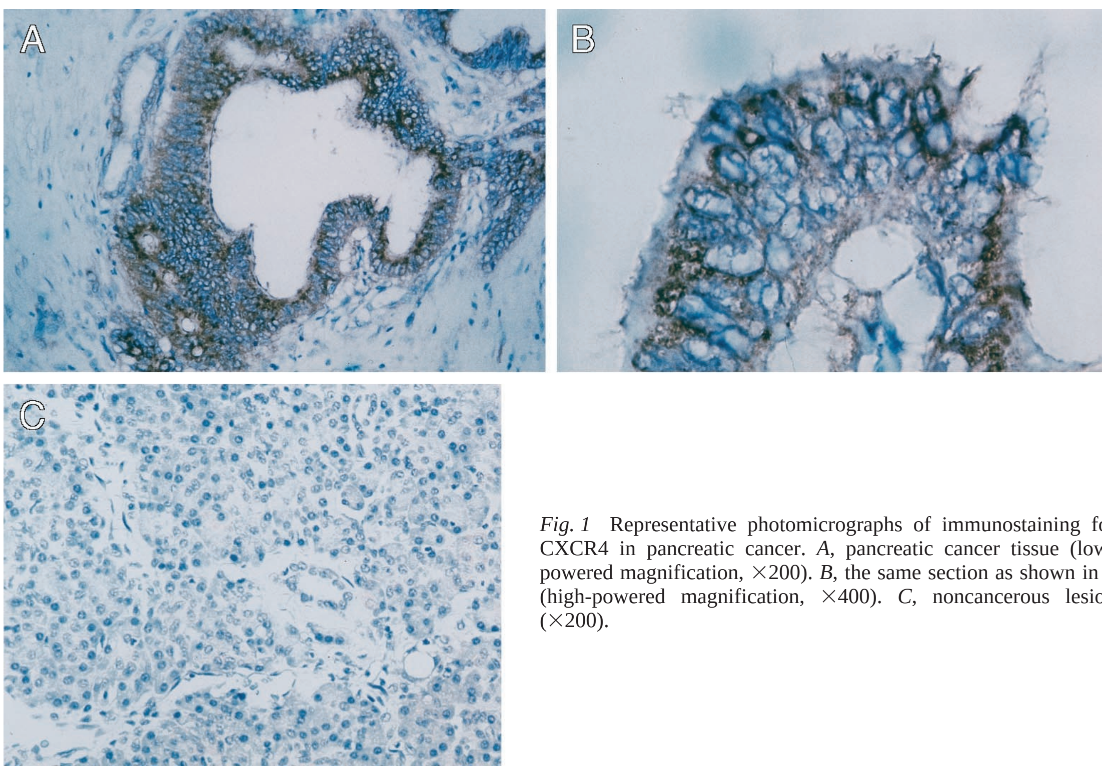


*Fig. 1* Representative photomicrographs of immunostaining for CXCR4 in pancreatic cancer. *A*, pancreatic cancer tissue (low-powered magnification, ×200). *B*, the same section as shown in *a* (high-powered magnification, ×400). *C*, noncancerous lesion (×200).

## MATERIALS AND METHODS

**Reagents and Antibodies.** SDF-1 and T22 (CXCR4 antagonist) were synthesized by N. F. (23, 24). The anti-CXCR4 mouse monoclonal antibody (clone IVR7) was developed by T. H. (25) and used for both immunohistochemical analysis and neutralization of cell migration. The anti-CD34 mouse monoclonal antibody QB-END/10 was obtained from Novocastra Laboratories (Newcastle, United Kingdom).

**Pancreatic Cancer Tissues.** Samples of 52 invasive ductal adenocarcinomas were obtained from patients with primary pancreatic cancers who underwent resection surgery at the Department of Surgery and Surgical Basic Science, Kyoto University, between 1991 and 1996. Samples were fixed in 4% paraformaldehyde or 10% formalin and embedded in paraffin, after which 4-μm sections were cut and placed on silane-coated slides for immunohistochemical studies. Part of the specimens was stained with H&E and microscopically examined to confirm the diagnosis. The clinicopathological characteristics of the 52 patients with ductal adenocarcinomas of the pancreas who were the subjects of this study are summarized in Table 1. There were 25 males and 27 females, with an age range of 41–79 years (median age, 63.1). Approximately 3-mm sections from each tumorous tissue and dissected lymph node were examined histologically to confirm the diagnosis and identify tumor extension, lymph node metastasis, liver metas-

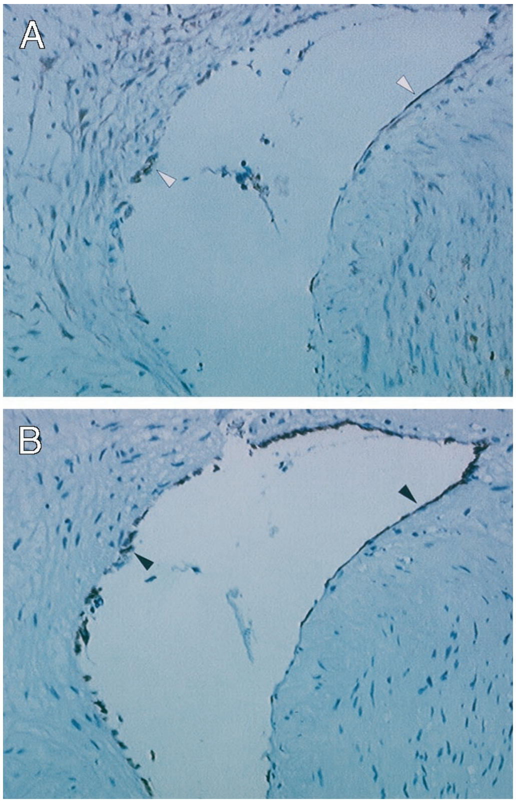


*Fig. 2* Representative photomicrographs of immunostaining for CXCR4 and CD34 in the relatively large vessel in pancreatic cancer tissue (×200). *A*, CXCR4 staining is detected in endothelial cells of large tube formation (*open arrowhead*). *B*, serial section of *A*; CD34 staining is detected in endothelial cells of large tube formation (*closed arrowhead*).

tasis, and stage of Union International Contre Cancer; the cancer was then staged accordingly.

For RT-PCR analysis, eight pancreatic cancer tissues, diagnosed as invasive ductal adenocarcinomas, were obtained at the time of surgery and immediately frozen with liquid nitrogen and stored at −80°C.

**Immunohistochemical Analysis.** The paraffin sections were dewaxed and pretreated in 0.01 M sodium citrate buffer (pH 6.0) for 20 min at 95°C to unmask tissue antigen. These sections were then incubated with 1% hydrogen peroxide in methanol for 15 min at room temperature to block endogenous peroxidase and then with PBS containing 5% normal goat serum for 30 min at room temperature to block any nonspecific reaction. Immunostaining was performed with anti-CXCR4 antibody (100 ng/ml) at 4°C overnight. The sections were incubated with goat antimouse IgG biotinylated second antibodies (Dakopatts, Glostrup, Denmark) diluted 1:300 for 1 h at room temperature and thereafter incubated in streptavidin-peroxidase complex for 30 min. The sections were then developed with diaminobenzidine-tetra-hydrochloride (0.03%) as the substrate for 3 min, counterstained with Mayers hematoxylin, and mounted. The evaluation of CXCR4 expression was simultaneously performed by two investigators (T. K. and Y. M.) without knowledge of the patients' clinicopathological features. We decided that the specimen should be regarded as positive when the intensity of staining was moderate or strong and negative when the intensity of staining was negative or weak. Several serial specimens of samples that were CXCR4 immunopositive were stained with anti-CD34 antibody to identify vascular endothelial cells. Furthermore, we exposed nonspecific mouse IgG as the primary antibody to several tissue specimens of pancreatic cancer to confirm the specificity of the results, and none of them showed any immunoreaction.

**Cell Lines and Culture Conditions.** Five human pancreatic cancer cell lines (CFPAC-1, BxPC-3, HPAC, AsPC-1, and PANC-1) and a human fetal lung fibroblast cell line (MRC-9) were purchased from the American Type Culture Collection. HUVECs were from Kurabo Industries (Osaka, Japan). The cell lines were maintained in the following media at 37°C in a humid atmosphere of 5% $CO_2$/95% air: CFPAC-1 cells in Iscove's modified Dulbecco's medium with 10% FBS, BxPC-3 cells in RPMI 1640 with 10% FBS, PANC-1 cells in DMEM, AsPC-1 cells in RPMI 1640 with 10% FBS, HPAC cells in DMEM/F-12 with 10% FBS, and HUVECs in S200 medium. Each medium contained 100 units/ml penicillin and 100 μg/ml streptomycin.

**RT-PCR.** Total RNA extraction from homogenized pieces of fresh frozen tissues of eight pancreatic cancer and cultured cells was performed with Trizol (Life Technologies, Inc., Eggenstein, Germany), according to the acid guanidium thiocyanate-phenol-chloroform method.

cDNA was synthesized with random priming from 1 μg of total RNA with the aid of a First-Strand cDNA Synthesis kit (Pharmacia Biotech, Uppsala, Sweden), according to the manufacturer's instructions. For the PCR reaction, 2 μl of cDNA solution were mixed with 2 μl of a specific primer (20 pM each), 5 μl of 10× reaction buffer, 10 μl of 1 mM dNTP mix, 0.5 μl of Taq DNA polymerase, and 28.5 μl of double distilled water for a total volume of 50 μl. The PCR reaction was performed in a Perkin-Elmer thermalcycler (Norwalk, CT) with the primers used for the amplification of SDF-1 and CXCR4 and specified below (26, 27). The amplification consisted of denaturation at 94°C for 30 s, annealing at 56°C for 30 s, and extension at 72°C for 30 s (33 cycles). A total of 10 μl of PCR products were separated onto 2.5% w/v agarose gels and stained with ethidium bromide.

Sense primers:

CXCR4, 5′-AGCTGTTGGTGAAAAGGTGGTCTATG-3′; and

SDF-1, 5′-CCGCGCTCTGCCTCAGCGACGGGAAG-3′.

Antisense primers:

CXCR4, 5′-GCGCTTCTGGTGGCCCTTGGAGTGTG-3′; and

SDF-1, 5′-CTTGTTTAAAGCTTTCTCCAGGTACT-3′.

**Migration Assay for Pancreatic Cancer Cells and HUVECs.** Cell migration assays were performed in triplicate by using 6.5-mm diameter chambers with 8-μm pore filters (Transwell, 24-well cell cultures; Costar, Boston, MA). Fifty microliters of fibronectin (100 μg/ml) were coated on the lower surfaces of filters. The filters were subsequently dried with air blown into a clean ventilator. AsPC-1 and CFPAC-1 cells and HUVECs were suspended at $2 \times 10^5$ cells/ml in serum-free media (RPMI 1640 containing 1% BSA), and then 200 μl of the cell suspension were added to the upper chamber. For AsPC-1

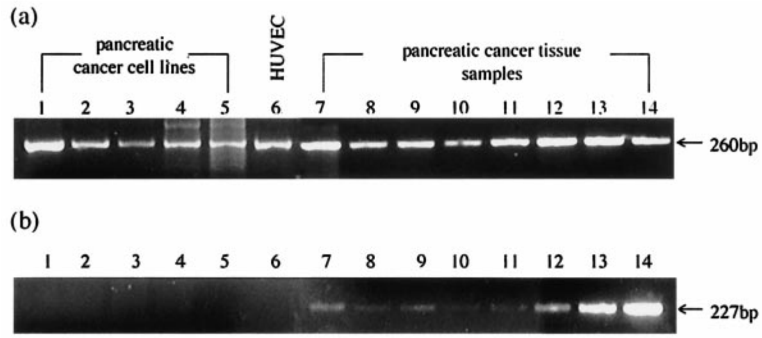


*Fig. 3* CXCR4 and SDF-1mRNA expressions. *a*, CXCR4 mRNA expression. *b*, SDF-1 mRNA expression. *Lane 1*, AsPC-1; *Lane 2*, BxPC-1; *Lane 3*, CFPAC-1; *Lane 4*, HPAC; *Lane 5*, PANC-1; *Lane 6*, HUVEC; *Lanes 7–14*, pancreatic cancer tissues.

cells and HUVECs, 100 ng/ml SDF-1, 100 ng/ml SDF-1 plus 10 μg/ml IVR7, or 100 ng/ml SDF-1 plus 1 μM T22 dissolved in 600 μl of serum-free media was placed in the lower well. For CFPAC-1 cells, subconfluent MRC-9 cells, or subconfluent MRC-9 cells plus 1 μM T22 were placed in the lower well. The chambers were then incubated for 12 h (AsPC-1 cells and HUVECs) or 8 h (CFPAC-1 cells) at 37°C in a humid atmosphere of 5% $CO_2$/95% air. After incubation, the filters were fixed in 10% acetic acid/90% methanol and stained with H&E. The upper surfaces of the filters were scraped twice with cotton swabs to remove nonmigrating cells. The experiments were conducted in triplicate wells, and the number of migrating cells in five high-power fields per filter were counted microscopically at ×400 magnification. Because the background migration without chemokines or fibroblasts varied among experiments, data were normalized as the migration index: the number of migrating cells in an experimental group/the number of migrating cells in control groups without chemokines or fibroblasts.

**Statistical Analysis.** The distribution of categorical data between CXCR4 immunostaining in pancreatic cancers and clinicopathological characteristics were assessed by $\chi^2$ test. Results of migration assays were assessed with Student's *t* test. The level of significance was defined as $P < 0.05$.

## RESULTS

**Immunohistochemical Analysis of CXCR4 in Pancreatic Cancer Tissues.** The distribution of CXCR4 protein expression in pancreatic cancer tissue was examined by means of immunohistochemical analysis of pancreatic cancer tissue samples obtained at surgical operation. Fig. 1 shows representative immunostainings of cancerous and noncancerous regions in pancreatic cancer tissues. Staining of the CXCR4 protein was identified in the cytoplasm and/or cell membrane of cancer cells, but was not detected in the normal acinar cells and ductal cells of noncancerous region in pancreatic cancer tissue. Negative or weak staining for the CXCR4 protein was observed in a majority of the infiltrating inflammatory cells in the specimens. The immunopositive ratio of cancer cells in the pancreatic cancer tissue specimens was 71.2% (37 of 52). Table 1 summarizes the relationship between CXCR4 expression and clinicopathological features of 52 pancreatic cancers. There was no significant correlation between the expression of CXCR4 protein and the clinicopathological variables examined (*i.e.*, tumor extension, lymph node metastasis, liver metastasis, and Union International Contre Cancer stage). CXCR4 immunoreactivities were observed in endothelial cells of relatively large vessels around the tumorous lesions, but were scarcely found in the endothelial cells of microvessels inside tumorous lesions (Fig. 2, *A* and *B*).

**CXCR4 and SDF-1 mRNA Expression.** We performed RT-PCR using specific primers, as described in "Materials and Methods," to confirm CXCR4 and SDF-1 mRNA expression in pancreatic cancer cells, endothelial cells (HUVECs), and pancreatic cancer tissues. CXCR4 mRNA expressions were clearly detected in five pancreatic cancer cell lines, HUVECs, and eight pancreatic cancer tissue samples (Fig. 3*a*). On the other hand, SDF-1 mRNA expression was not detected in five pancreatic cancer cell lines and HUVECs, but was identified in eight pancreatic cancer tissue samples (Fig. 3*b*).

**Effect of SDF-1 on Chemotaxis of Pancreatic Cancer Cells and HUVECs.** Transwell migration assays were performed to examine the effects of SDF-1 on motility of pancreatic cancer cells (AsPC-1) and endothelial cells (HUVEC). At a concentration of 100 ng/ml, SDF-1 induced chemotaxis of AsPC-1 cells, which was approximately double that of the control. One micromolar of T22 (CXCR4 antagonist) and 10 μg/ml of IVR7 (neutralizing CXCR4 antibody) completely blocked the chemotaxis of AsPC-1 induced by 100 ng/ml SDF-1 (Fig. 4*a*). At a concentration of 100 g/ml SDF-1 induced an approximately quadruple chemotaxis of HUVECs. One micromolar of T22 caused a 33% reduction of the chemotaxis of HUVECs in the presence of containing 100 ng/ml SDF-1 (Fig. 4*b*).

We also performed a migration assay for pancreatic cancer cells (CFPAC-1) cocultured with fibroblasts (MRC-9) to examine whether SDF-1 participates in the chemotaxis effect when it is cocultured condition. Coculturing with MRC-9 induced an ~9-fold chemotaxis of CFPAC-1 when compared with that of control. T22 significantly reduced the chemoattractive effect of MRC-9, but did not completely block this effect (Fig. 4*c*). RT-PCR generated SDF-1 mRNA expression in MRC-9 cells under these conditions (data not shown).

## DISCUSSION

SDF-1 belongs to the CXC chemokine family and is a ligand for CXCR4. The role of the SDF-1/CXCR4 receptor ligand system has been investigated mainly in the field of immunology, especially in the mechanism of infection of T lymphocytotrophic HIV-1 and for the prevention of HIV-1

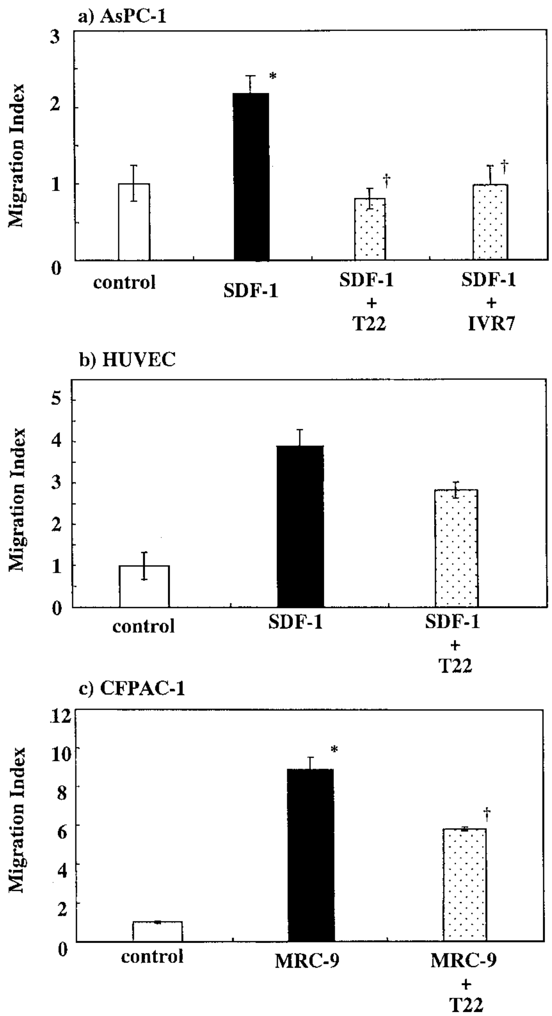


*Fig. 4* Effect of SDF-1 on chemotaxis of pancreatic cancer cells and HUVECs. *a*, AsPC-1. *b*, HUVEC. *c*, CFPAC-1. □, the condition containing no stimulatory factors (medium only in the lower well); ■, the condition containing a stimulatory factor (100 ng/ml SDF-1 or MRC-9 in the lower well); ▩, the condition containing both stimulatory and inhibitory factors 1 μM T22 or 10 μg/ml IVR7). Mean ± SEM ($n = 3$). *, significant increase *versus* control ($P < 0.05$, *t* test). †, significant inhibition by T22 or IVR7 ($P < 0.05$).

infection. Investigators have also paid attention to the role of the SDF-1/CXCR4 receptor ligand system in cancer tissues.

In this study, we first used immunohistochemical methods to examine CXCR4 expression in pancreatic cancer tissues. Immunoreactive CXCR4 was found in the cytoplasm and/or cell membrane of pancreatic cancer cells. Although CXCR4 staining in pancreatic cancer tissue was heterogeneous and showed differences between specimens, it was found mainly in cancer cells: the immunopositive ratio for the pancreatic cancer tissue specimens was 71.2% (37 of 52). There was a tendency for the immunopositive ratio of CXCR4 in tumors with lymph node metastasis or liver metastasis to be higher than in tumors without these features, but no statistically significant correlation with clinicopathological features were found. Several studies have demonstrated either overexpression or reduced expression of CXCR4 and SDF-1 mRNA in solid tumors. Sehgal *et al.* (21, 28) reported overexpression of CXCR4 mRNA in glioblastoma multiform tumor tissue and breast cancer tissue. They also found that CXCR4 expression and its ligand interaction were deeply involved in cell proliferation in glioblastoma cell lines (28). They concluded that CXCR4 plays an important role of proliferation and tumorigenic properties of human glioblastoma tumors (21, 28). Barnard and his colleagues (29, 30) showed the contrary results that CXCR4 mRNA expression was reduced in hepatocellular carcinoma tissue when compared with noncancerous tissue, but was not changed in colon, esophageal, and gastric cancer. They also found reduced mRNA expression of SDF-1 in these malignant tissues (22). Thus, there is a diversity of views on the role of the SDF-1/CXCR4 receptor ligand system in malignant tissues. In the current study, SDF-1 mRNA expressions were detected in all pancreatic cancer tissues (eight of eight) but were not detected in pancreatic cancer cell lines (zero of five), whereas CXCR4 mRNA was detected in both pancreatic cancer tissues (eight of eight) and cancer cell lines (five of five). The results indicate that the paracrine mechanism may be involved in the SDF-1/CXCR4 receptor ligand system in pancreatic cancer.

We have demonstrated that CXCR4 mRNA expression was present in HUVEC endothelial cell lines and that the migratory capability of HUVECs was increased by SDF-1 stimulation. Several other studies have demonstrated similar results. *In situ* hybridization and immunocytochemistry revealed both transcript and protein expression in cultured endothelial cells, as well as in the endothelium of normal aorta. SDF-1 stimulated mobilization of intracellular calcium at a moderate level, confirming the expression of a functional receptor on the endothelial surface (20). The mRNA expression level of CXCR4 in vascular endothelial cells is highest of several CC and CXC chemokine receptors, and SDF-1 induced pronounced chemotaxis of vascular endothelial cells, and this effect was stronger than that of other chemokines such as gIP10, IL-8, MIP-1a, MCP-1, eotaxin, and RANTES (18). Such evidence suggests that the SDF-1/CXCR4 receptor ligand system may be involved in angiogenesis.

Recently it has been demonstrated that SDF-1 plays an important role in organ vascularization. In CXCR4 knockout mice, the formation of the small vascular network that surrounds the stomach was well preserved, but the large vessels were missing, which led to hemorrhage and intestinal obstruction, and the mice lacking CXCR4 died *in utero*. As expected, SDF-1 knockout mice showed a similar phenotype (15, 16). In our immunohistochemical study of CXCR4 and CD34, CXCR4 protein expression was detected in the endothelial cells of relatively large vessels around tumorous lesions. We did not find the direct evidence of CXCR4 expression in microvessels inside the tumor; however, these findings suggest that SDF-1 may be involved in tumor growth by way of modeling relative large vessels in pancreatic cancer tissues.

T22 is a small synthesized peptide consisting of 18 amino acid residues, which is an analogue of polyphemusin II isolated from the hemocyte debris of American horseshoe crabs (23). T22 is a CXCR4 antagonist that inhibits $Ca^{2+}$ mobilization induced by SDF-1 stimulation through CXCR4, and IVR7 monoclonal antibody blocks HIV entry into T cells, as well as T22 (24, 25). In the current study, we first demonstrated that T22 significantly antagonized SDF-1-stimulated migration of AsPC-1 pancreatic cancer cells and HUVEC endothelial cells. IVR7, which was used in the immunohistochemical study, also significantly blocked chemoattractive action of SDF-1 in AsPC-1 cells. It is well known that the interaction between cancer cells and stromal cells is deeply involved in tumor invasion and metastasis. We were able to demonstrate that MRC-9 fibroblast cells significantly increased the migratory capability of CFPAC-1 cells and that T22 significantly reduced this capability when they are cocultured. *In vitro* findings indicate that SDF-1 acts as a chemoattractive factor for pancreatic cancer cells and endothelial cells and is, at least in part, involved in the mechanism of cancer cell migration resulting from fibroblast coculture. The mode of action of chemokines depends heavily on the local environment. Secreted SDF-1 is through to act by creating a gradient for CXCR4-bearing cells. The secreted protein may be localized by binding to extracellular matrix. In this situation, *in vitro* migration assays may not predict *in vivo* function. *In vitro* findings, however, indicate that SDF-1 acts as a chemoattractive factor for pancreatic cancer cells and endothelial cells and is, at least in part, involved in the mechanism of cancer cell migration resulting from fibroblast coculture.

In conclusion, our results suggest that the SDF-1/CXCR4 receptor ligand system may have a possible role in the pancreatic cancer progression through tumor cell migration and angiogenesis. Because T22 suppressed the migration of both pancreatic cancer cells and endothelial cells *in vitro*, additional *in vivo* studies are warranted to examine whether T22 suppresses the tumor spread and tumor angiogenesis to clarify the role of the SDF-1/CXCR4 receptor ligand system in pancreatic cancer.

## REFERENCES


1. Baggiolini, M., Dewald, B., and Moser, B. Human chemokines: an update. Annu. Rev. Immunol., *15*: 675–705, 1997.
2. Rollins, B. J. Chemokines. Blood, *90*: 909–928, 1997.
3. Kelner, G. S., Kennedy, J., Bacon, K. B., Kleyensteuber, S., Largaespada, D. A., Jenkins, N. A., Copeland, N. G., Bazan, J. F., Moore, K. W., Schall, T. J., *et al.* Lymphotactin: a cytokine that represents a new class of chemokine. Science (Washington DC), *266*: 1395–1399, 1994.
4. Smith, D. R., Polverini, P. J., Kunkel, S. L., Orringer, M. B., Whyte, R. I., Burdick, M. D., Wilke, C. A., and Strieter, R. M. Inhibition of interleukin 8 attenuates angiogenesis in bronchogenic carcinoma. J. Exp. Med., *179*: 1409–1415, 1994.
5. Miller, L. J., Kurtzman, S. H., Wangss, Y., Anderson, K. H., Lindquist, R. R., and Kreutzer, D. L. Expression of interleukin-8 receptors on tumor cells and vascular endothelial cells in human breast cancer tissue. Anticancer Res., *18*: 77–81, 1998.
6. Arenberg, D. A., Kunkel, S. L., Polverini, P. J., Glass, M., Burdick, M. D., and Strieter, R. M. Inhibition of interleukin-8 reduces tumorigenesis of human non-small cell lung cancer in SCID mice. J. Clin. Invest., *97*: 2792–2802, 1996.
7. Strieter, R. M., Polverini, P. J., Arenberg, D. A., Walz, A., Opdenakker, G., Van Damme, J., and Kunkel, S. L. Role of C-X-C chemokines as regulators of angiogenesis in lung cancer. J. Leukoc. Biol., *57*: 752–762, 1995.
8. Wang, J., Huang, M., Lee, P., Komanduri, K., Sharma, S., Chen, G., and Dubinett, S. M. Interleukin-8 inhibits non-small cell lung cancer proliferation: a possible role for regulation of tumor growth by autocrine and paracrine pathways. J. Interferon Cytokine Res., *16*: 53–60, 1996.
9. Schadendorf, D., Moller, A., Algermissen, B., Worm, M., Sticherling, M., and Czarnetzki, B. M. IL-8 produced by human malignant melanoma cells *in vitro* is an essential autocrine growth factor. J. Immunol., *151*: 2667–2675, 1993.
10. Tashiro, K., Tada, H., Heilker, R., Shirozu, M., Nakano, T., and Honjo, T. Signal sequence trap: a cloning strategy for secreted proteins and type I membrane proteins. Science (Washington DC), *261*: 600–603, 1993.
11. Nagasawa, T., Kikutani, H., and Kishimoto, T. Molecular cloning and structure of a pre-B-cell growth-stimulating factor. Proc. Natl. Acad. Sci. USA, *91*: 2305–2309, 1994.
12. Bleul, C. C., Fuhlbrigge, R. C., Casasnovas, J. M., Aiuti, A., and Springer, T. A. A highly efficacious lymphocyte chemoattractant, stromal cell-derived factor1 (SDF-1). J. Exp. Med., *184*: 1101–1109, 1996.
13. Nagasawa, T., Hirota, S., Tachibana, K., Takakura, N., Nishikawa, S., Kitamura, Y., Yoshida, N., Kikutani, H., and Kishimoto, T. Defects of B-cell lymphopoiesis and bone-marrow myelopoiesis in mice lacking the CXC chemokine PBSF/SDF-1. Nature (Lond.), *382*: 635–638, 1996.
14. Wells, T. N., Power, C. A., Lusti Narasimhan, M., Hoogewerf, A. J., Cooke, R. M., Chung, C. W., Peitsch, M. C., and Proudfoot, A. E. Selectivity and antagonism of chemokine receptors. J. Leukoc. Biol., *59*: 53–60, 1996.
15. Tachibana, K., Hirota, S., Iizasa, H., Yoshida, H., Kawabata, K., Kataoka, Y., Kitamura, Y., Matsushima, K., Yoshida, N., Nishikawa, S., Kishimoto, T., and Nagasawa, T. The chemokine receptor CXCR4 is essential for vascularization of the gastrointestinal tract. Nature (Lond.), 393: 591–594, 1998.
16. Zou, Y. R., Kottmann, A. H., Kuroda, M., Taniuchi, I., and Littman, D. R. Function of the chemokine receptor CXCR4 in haematopoiesis and in cerebellar development. Nature (Lond.), *393*: 595–599, 1998.
17. Feng, Y., Broder, C. C., Kennedy, P. E., and Berger, E. A. HIV-1 entry cofactor: functional cDNA cloning of a seven-transmembrane, G protein-coupled receptor. Science (Lond.), *272*: 872–877, 1996.
18. Gupta, S. K., Lysko, P. G., Pillarisetti, K., Ohlstein, E., and Stadel, J. M. Chemokine receptors in human endothelial cells. Functional expression of CXCR4 and its transcriptional regulation by inflammatory cytokines. J. Biol. Chem., *273*: 4282–4287, 1998.
19. Feil, C., and Augustin, H. G. Endothelial cells differentially express functional CXC-chemokine receptor-4 (CXCR-4/fusin) under the control of autocrine activity and exogenous cytokines. Biochem. Biophys. Res. Commun., *247*: 38–45, 1998.
20. Volin, M. V., Joseph, L., Shockley, M. S., and Davies, P. F. Chemokine receptor CXCR4 expression in endothelium. Biochem. Biophys. Res. Commun., *242*: 46–53, 1998.
21. Sehgal, A., Keener, C., Boynton, A. L., Warrick, J., and Murphy, G. P. CXCR-4, a chemokine receptor, is overexpressed in and required for proliferation of glioblastoma tumor cells. J. Surg. Oncol., *69*: 99–104, 1998.
22. Shibuta, K., Begum, N. A., Mori, M., Shimoda, K., Akiyoshi, T., and Barnard, G. F. Reduced expression of the CXC chemokine hIRH/SDF-1α mRNA in hepatoma and digestive tract cancer. Int. J. Cancer, *73*: 656–662, 1997.
23. Masuda, M., Nakashima, H., Ueda, T., Naba, H., Ikoma, R., Otaka, A., Terakawa, Y., Tamamura, H., Ibuka, T., Murakami, T., *et al.* A novel anti-HIV synthetic peptide, T-22 ([Tyr5,12, Lys7]-polyphemusin II). Biochem. Biophys. Res. Commun., *189*: 845–850, 1992.
24. Murakami, T., Nakajima, T., Koyanagi, Y., Tachibana, K., Fujii, N., Tamamura, H., Yoshida, N., Waki, M., Matsumoto, A., Yoshie, O., Kishimoto, T., Yamamoto, N., and Nagasawa, T. A small molecule CXCR4 inhibitor that blocks T cell line-tropic HIV-1 infection. J. Exp. Med., *186*: 1389–1393, 1997.
25. Hori, T., Sakaida, H., Sato, A., Nakajima, T., Shida, H., Yoshie, O., and Uchiyama, T. Detection and delineation of CXCR-4 (fusin) as an entry and fusion cofactor for T cell-tropic HIV-1 by three different monoclonal antibodies. J. Immunol., *160*: 180–188, 1998.
26. Wang, J. F., Liu, Z. Y., and Groopman, J. E. The α-chemokine receptor CXCR4 is expressed on the megakaryocytic lineage from progenitor to platelets and modulates migration and adhesion. Blood, *92*: 756–764, 1998.
27. Ohashi, T., Arai, M., Kato, H., Kubo, M., Fujii, M., Yamamoto, N., Iwamoto, A., and Kannagi, M. High SDF-1 expression in HIV-1 carriers does not correlate with CD8+ T-cell-mediated suppression of viral replication. Virology, *244*: 467–472, 1998.
28. Sehgal, A., Ricks, S., Boynton, A. L., Warrick, J., and Murphy, G. P. Molecular characterization of CXCR-4: a potential brain tumor-associated gene. J. Surg. Oncol., *69*: 239–248, 1998.
29. Begum, N. A., Shibuta, K., Mori, M., and Barnard, G. F. Reduced expression of the CXC4 receptor mRNA in hepatocellular carcinoma and lack of inducibility of its ligand a-chemokine hIRH/SDF-1a/PBSF *in vitro*. Int. J. Oncol., *5*: 927–934, 1999.
30. Mitra, P., Shibuta, K., Mathai, J., Shinoda, K., Banner, B. F., Mori, M., and Barnard, G. F. CXC4 mRNA expression in colon, esophageal and gastric cancers and hepatitis C infectioned liver. Int. J. Oncol., *5*: 917–925, 1999.