**Interleukin-6 Functions as An Autocrine Invasion Factor of Human Pancreatic Cancer Cells**

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**Abstract:** It was reported that serum interleukin-6 (IL-6) levels were increased in pancreatic cancer patients. However, the relationship between IL-6 expression and invasion of the pancreatic cancer has not yet been clarified. Therefore, the purpose of this study was to determine the effect of IL-6 on tumor cell invasion by the invasion in human pancreatic cancer cell line (PSN-1) and two sub-clones. High invasive clone (5H) and low invasive clone (5L) were established from wild type PSN-1 (WT) after five times passage of invasion assay. The invasive ability of PSN-1 cells was enhanced by IL-6 administration. The invasive ability of 5H cells was approximately two-folds higher than that of 5L cells and WT, and the level of IL-6 in the conditioned medium of 5H cells was significantly higher than those of 5L cells and WT. Furthermore, IL-6 receptor expression was observed in 70% of 5H cells while it was expressed only in 33% of 5L cells. 5H cells were spindle-shaped with long pseudopodia, while 5L cells were relatively round and pseudopodia formation was not observed. Endogenous IL-6 administration induced dose dependent enhancement of invasive ability both in 5H and 5L cells and conferred a spindle-like phenotype to 5L cells, but it did not affect on the cell proliferation in all 5H, 5L and WT cells. Conditioned medium from 5H cells (HCM) enhanced the invasive abilities of both 5H cells and 5L cells and conferred a spindle-like phenotype to 5L cells. The 5L cell-derived conditioned medium (LCM) also showed a similar effect on the invasive ability of both 5H and 5L cells. The effect of HCM on the invasive ability of 5H cells was partially inhibited by addition of anti-human IL-6 neutralizing antibody. In conclusion, overexpression of IL-6 and its receptor may contribute to the highly invasive characteristic of some pancreatic cancer cells, and these data raise the possibility that the regulation of IL-6 ligand/receptor interaction may prove useful in the therapy of this malignancy.

**Keywords:** pancreatic cancer, interleukin-6, interleukin-6 receptor, invasion assay

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**INTRODUCTION**

Pancreatic cancer tends to invade adjacent tissues and organs and metastasize to the liver and lymph nodes even though the primary lesion is relatively small and prognosis after excision is extremely unfavorable compared to that of other cancers of the digestive tract\(^1\). To eluci-
cidate the mechanism of invasion in the pancreatic cancer.

It is clinically well recognized that in pancreatic cancer, even when the primary tumor is small in diameter, it causes marked body weight reduction, indicating cancer cachexia from the early phase of the disease\(^4\). Recently it was reported that IL-6 was closely associated with cancer cachexia\(^5\text{--}7\). Furthermore, it was reported that serum IL-6 levels are highly elevated in pancreatic cancer patients compared to that in patients with cancers of other digestive organs\(^8\), suggesting the involvement of IL-6 in cancer progression, i.e., invasion and metastasis of pancreatic cancer, which is recognized to be biologically highly malignant.

IL-6 is known as a multi-potent cytokine with diverse biological activities on various tissues and cells\(^9\text{--}14\). It was reported to promote proliferation of cancer cells in renal cell carcinoma\(^15\text{,}16\), ovarian carcinoma\(^17\) and cervical carcinoma\(^18\). Furthermore, high levels of IL-6 were detected in the culture supernatant of various cancer cells\(^19\text{--}22\), serum in cancer patients\(^8\text{,}23\text{,}24\) and cancer tissue\(^25\). Taken together, IL-6 might be involved in the highly invasive characteristic of pancreatic cancer; however, the relationship between IL-6 and invasive ability of pancreatic cancer cells has not yet been clarified. Therefore, we investigated how IL-6 affects the invasive ability of pancreatic cancer cells in vitro using a human pancreatic cancer cell line PSN-1. We now report that overexpression of IL-6 and its receptor may contribute to the highly invasive characteristic of some pancreatic cancer cells with morphological changes, and these data raise the possibility that the regulation of IL-6 ligand/receptor interaction may prove useful in the therapy of this malignancy.

**Material and methods**

**Cell culture**

PSN-1 (WT), a cell line established from human pancreatic cancer, cells were kindly provided from the National Cancer Center Research Institute (Tokyo, Japan). Cells were cultured in RPMI1640 medium (Gibco BRL, Grand Island, N.Y.) supplemented with 10 % heat-inactivated fetal calf serum (FCS; Gibco, Auckland, New Zealand), 100 µg/ml of streptomycin and 100 IU/ml of penicillin G (Gibco BRL). Cancer cells were cultured in a humidified atmosphere containing 5 % carbon dioxide at 37°C.

**Invasion assay**

Invasion of cancer cells in vitro was assessed by the method of Albini et al with some modification\(^26\). Namely, Transwell chamber (Costar, Cambridge, MA, U.S.A.) with an 8 µm pore size polycarbonate membrane filter, and coated the upper surface of each filter with 5 µg of Matrigel (reconstituted basement membrane; Collaborative Research, Lexington, MA, U.S.A.) were used. The chamber (upper compartment) was placed in a 24-well culture plate (lower compartment). Cancer cells were resuspended to a final concentration of 5 · 10^5 cells/ml in RPMI1640 medium with 0.1 % FCS. One hundred micro liters of cell suspension was added to the upper compartment. The lower compartment contained 600 µl of RPMI1640 medium with 0.1 % FCS. The plate was incubated for 6 hr at 37°C. After incubation, cancer cells on the upper surface of the membrane were removed by wiping with cotton swabs. The filter was fixed with methanol and stained with hematoxylin and eosin. Cancer cells that had passed through the filter coated with Matrigel to the surface of the lower membrane were counted.
under a microscope at 200 magnification. For each group, the culture was performed in triplicate.

To estimate the effect of IL-6 on cell invasion various concentrations of IL-6 (Gibco BRL, Grand Island, N.Y., U.S.A.) were added to the upper or lower compartment or both compartments (checkerboard analysis). After incubation for 6 hr at 37°C, the cells that had passed through the membrane were counted as described before.

To estimate the effect of HCM and LCM on invasion ability of 5H and 5L cells, cells were suspended to a final concentration of 1 x 10⁶ cells/ml in RPMI1640 medium without FCS. One hundred micro liters of cancer cell suspension was added to the upper compartments. HCM or LCM was added at a concentration of 50 % in RPMI1640 medium without FCS in the lower compartment. To estimate the effect of anti-human-IL-6 neutralizing antibody (Gibco BRL, Grand Island, N.Y., U.S.A.) on invasive ability of 5H and 5L cells, IL-6-removed HCM was added at a concentration of 50 % in RPMI1640 medium without FCS in the lower compartment. After incubation for 12 hr at 37°C, the cells that had passed through the membrane were counted as described before. RPMI1640 medium without FCS was used as the negative control.

Establishment of high and low invasive clones

To examine the characteristics of the invading tumor cells, we separated PSN-1 cells by chemoinvasion assay in the presence of IL-6 (100 ng/ml) in RPMI1640 with 10 % FCS. PSN-1 cells were seeded at 1 x 10⁵ cells/100 µl/well into the upper compartment. IL-6 was added at a concentration of 100 ng/ml to the lower compartment. The cells that passed through the Matrigel-coated 8 µm pore-size membrane during the initial 4 hr were collected (H cells). The cells remaining in the upper compartment for 24 hr were also collected (L cells). We performed this separation five times and established two clones (5H and 5L) (Fig. 1).

ELISA for IL-6

Quantification of IL-6 in the conditioned medium (CM) was performed by IL-6 enzyme-linked immunosorbent assay (ELISA) Kit (Genzyme, Cambridge, MA, U.S.A.). One hundred micro liters of CM was added into the test wells and incubated at 37°C for 30 min. After aspiration of contents, test wells were washed with washing reagent five times. One hundred micro liters of biotinylated antibody were added to each well and the plate was incubated at 37°C for 30 min. After aspiration of contents and five times washes, 100 µl/well of avidin reagent was added into each test well. After incubation at 37°C for 15 min, wells were washed five times with washing reagent. One hundred micro liters of working substrate reagent were added to each well and the plate was incubated at room temperature for 10 min. Then one hundred microliters of stopping solution was added and the absorbance recorded at 450 nm.

Flow cytometric analysis for IL-6 receptor

To examine the expression of IL-6 receptor on 5L and 5H cells, a flow cytometric analysis was performed. Cells were prepared as single cell suspension, and then washed twice with PBS to remove residual IL-6 in the culture medium. Cells were resuspended in PBS with 1 % BSA with 0.1 % sodium azide at a final concentration of 1 x 10⁶ cells/ml. Monoclonal anti-human IL-6 receptor antibody (5 µg/ml) (Sigma Chemical Company, St. Louis, MO, U.S.A.) was added to the cancer cell suspension and the cells were incubated for 30 min at 4°C.
After incubation, cells were washed twice and fluorescence isothiocyanate (FITC)-labeled anti-mouse IgG antibody (Sigma Chemical Company, St. Louis, MO, U.S.A.) was added. After further incubation for 30 min at 4°C, cells were washed twice with PBS and analyzed using a flow cytometer. PBS was used as a negative control against monoclonal anti-human IL-6 receptor antibody.

Morphological study
For morphological study cells were seeded into a 35 mm plastic dish (Coning, Coning, N.Y., U.S.A.) at the final concentration of 2 × 10³ cells/dish and incubated in RPMI1640 medium without FCS for 24 hr. After removal of the supernatant, cells were washed three times in PBS and observed by microscope. To estimate the effect of endogenous IL-6 or HCM, cells were incubated in 2 ml of RPMI1640 medium with 100 ng/ml of IL-6 or 50 % of HCM in 50 μl of RPMI1640 medium for next 24 hr.

Cell growth
Cells (5 × 10³ cells/well) of 5H and 5L cells were seeded on a 96-well plate (Coning, Coning, N.Y., U.S.A.) at the final concentration of 2 × 10³ cells/dish and incubated in RPMI1640 medium without FCS for 24 hr. After removal of the supernatant, cells were washed three times in PBS and observed by microscope. To estimate the effect of endogenous IL-6 or HCM, cells were incubated in 2 ml of RPMI1640 medium with 100 ng/ml of IL-6 or 50 % of HCM in 50 μl of RPMI1640 medium for next 24 hr.
ing, N.Y., U.S.A.) and incubated with or without 100 ng/ml of IL-6 or 50 % of HCM in 50 μl of RPMI1640 medium for various hours. The number of cells was counted by MTT assay27).

Preparation of cancer cell-derived conditioned medium
Cancer cells were seeded into a 100 mm plastic dish (Coning, Coning, N.Y., U.S.A.) and incubated in RPMI1640 medium with 10 % FCS to a semi confluent monolayer state. After removal of the supernatant, cells were washed three times with PBS. Then, the cells were incubated in 10 ml of RPMI1640 medium with 0.5 % bovine serum albumin (BSA; Sigma Chemical Company, St.Louis, MO, U.S.A.) for 24 hr. The supernatant was collected from PSN-1, 5L and 5H cells. Supernatants were centrifuged at 1,000 r.p.m. for 30 min, dialyzed against RPMI1640 medium without FCS for 24 hr, filtered through a micro filter (pore size 0.22 μm; Millipore, Bedford, MA, U.S.A.) and stored at - 20°C until use as CM derived from PSN-1 (PCM), 5H (HCM) and 5L (LCM). To examine the effects of anti-human-IL-6 neutralizing antibody (Gibco BRL, Grand Island, N.Y., U.S.A.) on the invasion ability of 5H cells with HCM, we prepared anti-IL-6 antibody treated HCM. On the addition of anti-human-IL-6 neutralizing antibody to the invasion assay system, IL-6 that bound with the antibody might promote invasion of 5H cells. Therefore, we removed as much IL-6 from HCM as possible using immunoprecipitaion methods. Protein A-agarose conjugates (Calbiochem, Cambridge, MA, U.S.A.) were pre-blocked with both BSA and HCM to reduce non-specific binding of immunoglobulin and some motility factors in HCM. Anti-human-IL-6 neutralizing antibody was added to the conjugates and incubated for 4 hr at 4°C. Standard rabbit IgG was used as a control. Conjugates were washed three times with PBS, and HCM was added. After incubation for 24 hr at 4°C, the supernatant was collected. The supernatant was dialyzed against RPMI1640 medium, filtered through a micro filter and stored at - 20°C until used as anti-IL-6 antibody treated HCM. The concentration of IL-6 in anti-IL-6 antibody treated HCM was measured by ELISA methods.

Statistical analysis
Data were analyzed statistically using Student’s t test. A p-value less than 0.05 was considered significant.

RESULTS
Effect of IL-6 on invasive ability of PSN-1 cells
The number of PSN-1 cells passing through the membrane in the absence of IL-6 was 35.0 ± 17.4 /field in the invasion assay system (Table 1). When IL-6 was added only to the lower compartment at concentration of 1, 10, and 100 ng/ml, the number of invading cells was 43.2 ± 18.8, 52.5 ± 18.9 and 70.3 ± 9.8 /field, respectively, and exogenous IL-6 induced

<table>
<thead>
<tr>
<th>PSN-1 cells IL-6 in upper compartment (ng/ml)</th>
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<tr>
<td>IL-6 in lower compartment (ng/ml)</td>
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<tr>
<td>(field)         0  1  10  100</td>
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<tr>
<td>0            35.0 ± 17.4  54.6 ± 21.4  49.0 ± 20.7  36.4 ± 10.6</td>
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<tr>
<td>1            43.2 ± 18.8  49.8 ± 19.5  45.4 ± 8.8  50.2 ± 16.3</td>
</tr>
<tr>
<td>10           52.5 ± 18.9  70.0 ± 27.9  61.4 ± 16.2  38.3 ± 11.0</td>
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<tr>
<td>100          70.3 ± 9.8  102.5 ± 30.1  110.2 ± 22.8  86.8 ± 14.0</td>
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Table 1. Effect of IL-6 on invasive ability of PSN-1 cells
significant migration effect in a dose-dependent manner \((p < 0.005)\) (Table 1). In contrast, when IL-6 was added only to the upper compartment, the number of cells passing through the membrane was not changed. When IL-6 was added to the both compartments, the number of cells was not affected by the IL-6 concentration in the upper compartment. That is, the invasive ability of PSN-1 cells is dependent on IL-6 concentration in the lower compartment.

**Establishment of two clones from PSN-1 by chemoinvasion assay in the Presence of IL-6**

The number of cells passing through the membrane was 43 \(\pm\) 12.7 /field in 5H cells and 18 \(\pm\) 5.8 /field in 5L cells (Fig. 2). The invasive ability of 5H cells was significantly higher than that of 5L cells \((p < 0.0001)\). The concentration of IL-6 in the conditioned medium was 27760 \(\pm\) 660 mg/ml in 5H cells, which was approximately 16-fold higher than that in 5L cells of 1690 \(\pm\) 20 mg/ml (Fig. 3). The expression of IL-6 receptor on 5H and 5L cells was examined by flow cytometric analysis. The expression was detected in 70 \(\pm\) 14 % of 5H cells and 33 \(\pm\) 12 % of 5L cells (Fig. 4). 5H cells were spindle-shaped with long pseudopodia, while 5L cells were relatively round and devoid of pseudopodia (Fig. 5a, b).

**Effect of IL-6 on 5H and 5L cells**

On addition of 100 mg/ml of IL-6 to 5H and 5L cells, the number of invading cells were increased significantly (from 62.5 \(\pm\) 17.3 /field to 101.1 \(\pm\) 21.2 /field in 5H cells \((p < 0.001)\) and 27.8 \(\pm\) 4.8 /field to 41.1 \(\pm\) 6.6 /field in 5L cells \((p < 0.001)\). When IL-6 was added to 5H

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**Fig. 2.** Invasion assay of 5H and 5L cells: Cancer cells were seeded at 5 \(\times\) 10^4 cells/100 \(\mu\)l/well into the upper compartment of the chamber. The lower compartment contained 600 \(\mu\)l of RPMI1640 medium with 0.1 % FCS. The number of invading tumor-cells was counted after 6-hour incubation under a microscope at \(\times\) 200 magnification. The number of cells passing through the membrane was 43 \(\pm\) 12.7 /field in 5H cells and 18 \(\pm\) 5.8 /field in 5L cells. The invasive ability of 5H cells was significantly higher than that of 5L cells \((p < 0.0001)\). The values are the mean \(\pm\) standard deviation of invading cell numbers from triplicate examinations. N.S., not significant; FCS, fetal calf serum.

**Fig. 3.** Level of IL-6 in HCM and LCM: The level of IL-6 in HCM measured by ELISA method was 27760 \(\pm\) 660 pg/ml, approximately 16-fold higher than that in LCM of 1690 \(\pm\) 20 pg/ml. HCM; conditioned medium derived from 5H cells, LCM; conditioned medium derived from 5L cells.
cells, the invasive ability of 5H cells was enhanced in dose-dependent manner \((p < 0.01)\). Those enhancements were greater in 5H cells than 5L cells (Fig. 6). IL-6 also caused same morphological changes as 5H cells in 5L cells (Fig. 7). In contrast, there was no significant difference in growth rates between 5H and 5L cells after 48 hr incubation with or without IL-6 administration (data not shown).

**Effect of HCM on 5H and 5L cells**

After administration of HCM to culture dishes the number of invading cells was increased significantly from 1.7 \(\pm\) 1.2 /field to 24.4 \(\pm\) 14.1 /field in 5H cells \((p < 0.001)\) and from 0.6 \(\pm\) 0.7 /field to 38.3 \(\pm\) 25.8 /field \((p < 0.001)\) in 5L cells (Fig. 8). However, on addition of LCM the number was increased only to 15.6 \(\pm\) 16.0 /field \((p < 0.05)\) in 5H cells and to 5.6 \(\pm\) 5.8 /field \((p < 0.05)\) in 5L cells. The promoting effect of LCM on invasive ability was less than that of HCM. Furthermore, HCM also caused same morphological changes as 5H cells in 5L cells (Fig. 9a, b). Fifty % HCM had no significant effect on 5H and 5L cell proliferation after 48 hr incubation \((p < 0.05)\) (data not shown).

**Effect of anti-IL-6 antibody treated HCM on invasive ability of 5H cells**

By means of immunoprecipitation with anti-human-IL-6 neutralizing antibody and protein A-agarose conjugates, the IL-6 concentration in HCM was decreased from 9.72 \(\pm\) 0.05 ng/ml to 1.22 \(\pm\) 0.02 ng/ml \((p < 0.001)\) (data not shown). The number of 5H cells that invaded on addition of natural HCM was 76.4 \(\pm\) 31.5 /field. Whereas, the number of invading 5H cells on addition of IL-6-removed HCM was blunted to 39.6 \(\pm\) 15.8 /field. The promoting effect of HCM on invasion ability of 5H cells was significantly inhibited by removal of IL-6 in HCM \((p < 0.05)\) (Fig. 10).

**DISCUSSION**

It is extremely important to clarify whether the factor is chemotactic or chemokinetic in
Fig. 5.  

**a.** Appearance of 5H cells: The cancer cells were seeded into a 35 mm plastic dish at a final concentration of $2 \times 10^3$ cells/dish and incubated in RPMI1640 medium without FCS for 24 hr. In morphological observations of 5H cells, typical cells were spindle-shaped with long pseudopodia. Scale bar = 10 μm

**b.** Appearance of 5L cells: The cancer cells were seeded into a 35 mm plastic dish at a final concentration of $2 \times 10^3$ cells/dish and incubated in RPMI1640 medium without FCS for 24 hr. 5L cells were relatively round and pseudopodia formation was not observed. Scale bar = 10 μm
investigating cancer cell invasion. In this study the invasion assay using the Boyden chamber showed that the number of invading PSN-1 cells was not affected by the IL-6 concentration in the upper compartment that directly acts on the cells, but it was dependent on IL-6 concentration in the lower compartment. Therefore, IL-6 may have chemotactic activity on PSN-1 cells.

Since IL-6 production in the pancreas is markedly higher than that in other organs28), cancer cells may individually invade the normal pancreatic parenchyma. This is a very important finding, since it is consistent with a clinical observation of pancreatic cancer that cancer cells are observed individually in the interstitially distant from main tumor without forming a colony. It is also well known that the main tumor consists of heterogeneous cells with various degrees of differentiation in the pancreatic cancer29). Therefore, it is important to investigate which clone of PSN-1 cells respond to the chemotactic effect of IL-6. In the present study, two sub-clones based on a difference in invasive ability were established in the presence of IL-6 by the chemoinvasion assay. The biological behavior of these two sub-clones were investigated by examining IL-6 production and IL-6 receptor expression. The 5H cells exhibited higher expression of IL-6 and its receptor than 5L cells. On morphological examination, 5H cells were exhibited spindle-shaped with long pseudopodia suggesting their high invasive ability. Furthermore, to estimate the direct effect of IL-6 on each clone the changes of invasive ability of these clones were investigated under three different concentrations of IL-6 administration. As a result, the invasive ability was enhanced in a dose-dependent manner in all clones. In addition, 5L cell morphology was changed to that similar to 5H cells. Thus, it was concluded that IL-6 is closely involved in the invasive ability of PSN-1 cells.

To determine whether other factors are involved in the invasive ability of these three clones, 5H cell-derived conditioned medium (HCM) that contained a high level of IL-6 and 5L cell-derived conditioned medium (LCM) that contained a low level of IL-6 were prepared. On the invasive assay, not only HCM but also LCM significantly enhanced 5H cells invasion. The degree of enhancement of invasive ability of 5H cells was less in LCM than HCM but there was no significant differences. As shown in Fig. 6, the number of invading 5H cells significantly increased by IL-6 administration in a dose-dependent manner. However,
Fig. 7. Morphology of 5H and 5L cells after addition of IL-6: 5H or 5L cells were seeded at $2 \times 10^5$ cells/well into a 35 mm plastic dish and incubated in RPMI1640 with 0.5 % BSA for 24 hr. The cells were incubated in 2 ml of RPMI1640 with 0.5 % BSA with 100 ng/ml of IL-6. 5L cells became 5H cell-like in shape.

a. 5H cells, b. 5L cells. Scale bar = 10 μm
there were no significant differences between the degrees of enhancement caused by 10 ng/ml and 1 ng/ml of IL-6, and between that caused by 100 ng/ml and 10 ng/ml of IL-6. Therefore, the promotion effect of HCM on the invasive ability of 5H cells did not significantly differ from that of LCM because the concentration of IL-6 in HCM (27.76 ± 0.66 ng/ml) was only approximately 16-fold higher than that in LCM (1.69 ± 0.02 ng/ml). Thus the invasive ability of 5H cells which posses the overexpression of IL-6 receptor was not significantly changed by differences in IL-6 concentration in these levels, it may be depend on the number of IL-6 receptors on 5H cells saturated by IL-6 contained in LCM and the majority of IL-6 in HCM may remain as an unbounded form. In contrast, both HCM and LCM may be able to enhance the invasive ability of 5L cells, which have low number of IL-6 receptor. HCM enhanced the invasive ability of 5L clone significantly higher than LCM and the effect of enhancement was similar to that in 5H cells caused by HCM. This finding suggested the presence of another motility factor except IL-6 in HCM that promotes the invasive ability of 5L cells. One possibility is the production of soluble IL-6 Receptor (sIL-6R). It was reported that increased IL-6 production after stimulation by IL-1 or TNFa result in complex formation with sIL-6R in human fibroblasts. This IL-6/sIL-6R complex can bind to gap130, which is the important signal transducing receptor component shared in IL-6 family, without IL-6R and can induce intracellular signal. Aberrant overexpression of IL-6/sIL-6R complex in HCM may induce strong invasive activity in 5L cells. We removed IL-6 from HCM by adding anti-human IL-6 neutralizing antibody, and then added this IL-6 removed HCM to 5H cells and compared invasive ability before and after removal of IL-6. Although the invasive ability of 5H cells was significantly reduced after adding HCM lacking IL-6, the invasive ability remained significantly higher than that of 5H cells without the addition of HCM. Sakurai et al. reported pancreatic cancer-derived motility factor (PDMF) as a factor that promotes the invasive ability of pancreatic cancer cells. They reported that this factor chemotactically as well as chemokinetically acts on pancreatic cancer cells, unlike IL-6 and TGFβ1, mainly in paracrine fashion. Considering the presence of PDMF in evaluating the results of this study, 5H cells may produce
Fig. 9.  

a. Morphology of 5L cells before addition of HCM: 5L cells were seeded at $2 \times 10^3$ cells/well into a 35 mm plastic dish and incubated in RPMI1640 with 0.5 % BSA for 24 hr. The cells were incubated in 2 ml of 50 % HCM with 0.5 % BSA. Scale bar = 10 $\mu$m

b. Morphological change of 5L cells after addition of HCM: HCM caused a morphological change in 5L cells to 5H cell like formation. Scale bar = 10 $\mu$m
PDMF as well as IL-6 to a greater extent than 5L cells. Invasive ability of PSN-1 cells may be exhibited by, at least, these two migration factors. But further study is necessary to analyze the relationship of these cascades.

When 5L cells were cultured in the presence of HCM, round cell morphology of 5L cells changed to 5H cell-like morphology of spindle shape with long pseudopodia. Since 5H cells already have such morphology and the same result was acquired after addition of IL-6 alone, IL-6 involves the effect to induce these morphological changes. Indeed, Tamm et al. reported that in ductal carcinoma cells of the breast, IL-6 reduced intercellular adhesion with change of cell morphology and colony formation ability and up-regulated cancer cell motility. This report supports our results.

Close association of IL-6 with strong invasive ability of pancreatic cancer was shown in this study. Nakase et al. showed that the serum IL-6 level is markedly elevated after pancreatic surgery regardless of whether the tumor was malignant or benign compared to serum IL-6 levels after surgery on other organs. This finding suggested that cancer cells are very likely to be induced to invade by the overexpressed IL-6 around the primary lesion if IL-6 is chemotactically involved in pancreatic cancer invasion. Therefore, these data raise the possibility that the regulation of IL-6 ligand/receptor interaction may prove useful in the therapy of this malignancy.

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