Experimental Therapeutics, Molecular Targets, and Chemical Biology

A Novel Role of Interleukin-13 Receptor α2 in Pancreatic Cancer Invasion and Metastasis

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Abstract

Whereas interleukin-13 receptor α2 chain (IL-13Rα2) is overexpressed in a variety of human solid cancers including pancreatic cancer, we investigated its significance in cancer invasion and metastasis. We used two pancreatic cancer cell lines, IL-13Rα2-negative HPAF-II and IL-13Rα2-positive HS766T, and generated IL-13Rα2 stably transfected HPAF-II as well as IL-13Rα2 RNA interference knocked-down HS766T cells. Ability of invasion and signal transduction was compared between IL-13Rα2-negative and IL-13Rα2-positive cells and tumor metastasis was assessed in murine model for human pancreatic cancer with orthotopic implantation of tumors. IL-13 treatment enhanced cell invasion in IL-13Rα2-positive cancer cell lines but not in IL-13Rα2-negative cell lines. Furthermore, gene transfer of IL-13Rα2 in negative cell lines enhanced invasion, whereas its silencing downregulated invasion of pancreatic cell lines. IL-13Rα2 positive cancer cell lines displayed cachexia and poor prognosis. Invasion and metastasis also correlated with increased matrix metalloproteinase protease activity in these cells. Mechanistically, IL-13 activated extracellular signal-regulated kinase 1/2 and activator protein-1 nuclear factors in IL-13Rα2-positive pancreatic cancer cell lines but not in IL-13Rα2-negative cell lines. Taken together, our results show for the first time that IL-13 can signal through IL-13Rα2 in pancreatic cancer cells and IL-13Rα2 may serve as a prognostic biomarker of invasion and metastasis in pancreatic cancer. [Cancer Res 2009;69(22):8678–85]

Introduction

The natural history of pancreatic cancer is characterized by local invasion of adjacent structures and early metastasis to lymph nodes and liver (1, 2). By the time of diagnosis, most pancreatic cancers are advanced and unresectable; at present, very little effective therapy can be offered to these patients. It is thus critical to understand the mechanism of invasion and metastasis. Cancer invasion and metastasis require the controlled degradation of the extracellular matrix and increased expression of matrix metalloproteinases (MMP; ref. 3). Expression of most MMPs is normally low in tissues, but it is induced when remodeling of extracellular matrix is required. MMPs are associated with tumor invasion and metastasis of malignant tumors with different histogenetic origin (3, 4). Pancreatic cancer expresses different MMPs (e.g., MMP-9, MMP-12, and MMP-14) and their expression affects the malignant phenotype (1, 5, 6). The promoter region of inducible MMP genes show remarkable conservation of regulatory elements including activator protein-1 (AP-1) transcription factors binding sites (7, 8). Mitogen-activated protein kinases (MAPK) are intricately involved in the expression of components involved in MMP promoter induction via AP-1 and its association with c-Jun and c-Fos. In particular, three specific MAPKs have been implicated: extracellular signal-regulated kinase (ERK; of which ERK1 and ERK2 are the most abundant in mammalian cells), stress-activated protein kinase/c-Jun NH2-terminal kinase, and p38 MAPK (9, 10).

Previous studies showed that interleukin-13 receptor α2 chain (IL-13Rα2), a high-affinity receptor of Th2-derived cytokine IL-13, is overexpressed in a variety of human tumors and that it can serve as a biomarker of disease and a target for cancer therapy (11, 12). A bispecific cytokxin targeting receptors for epidermal growth factor and human IL-13 is shown to be effective in animal model of pancreatic cancer (13). IL-13 plays a central role in inflammation and immune responses and binds to two receptor subunits, IL-13Rα1 and IL-13Rα2 (14). IL-13Rα1 is a low-affinity IL-13R, but after binding to IL-13, it recruits IL-4Rα and forms a high-affinity IL-13R complex (type II IL-13R) and mediates signal transduction through the JAK-STAT6 pathway (15). IL-13Rα2, on the other hand, binds IL-13 with high affinity and internalizes, but it does not mediate signal transduction (16). It has been hypothesized that the extracellular domain of IL-13Rα2 may serve as a decoy receptor for the type II IL-13R complex (17, 18). Interestingly, it has been reported that IL-13 can signal through IL-13Rα2 in murine macrophage cell line and that its signaling is STAT6-independent and involves the AP-1 pathway to induce activation of transforming growth factor-β1 activity (19).

Although IL-13Rα2 has been shown to be overexpressed on certain types of human cancer including glioblastoma, head and neck cancer, kidney cancer, ovarian cancer, medulloblastoma, and Kaposi’s sarcoma (20–24), its role in signal transduction in cancer is unknown. A recent DNA microarray study has shown that IL-13Rα2 gene expression is increased in breast cancer metastasis lesions in lungs (25). However, it is not known whether IL-13Rα2 is also increased in metastatic pancreatic cancer. In addition, the significance of IL-13Rα2 in primary or metastatic cancer is not clear. Whether IL-13Rα2 plays any role in cancer invasion and metastasis is not known.
We have studied the invasion and metastasis of pancreatic cancer cells naturally expressing IL-13Ra2 or in cells in which IL-13Ra2 is introduced by gene transfer. Control cells either lack IL-13Ra2 or their receptor was knocked down by RNA interference (RNAi) transfection. We also examined the mechanism of invasion and IL-13–induced signal transduction in pancreatic cancer cells. Furthermore, we have studied the role of IL-13Ra2 in cancer promotion and metastasis in vivo model of human pancreatic cancer. Our results unequivocally show a novel role of IL-13Ra2 in pancreatic cancer invasion and metastasis and that IL-13Ra2 is directly involved in signaling through the AP-1 pathway. Thus, IL-13Ra2 may be a novel target to inhibit cancer invasion and metastasis.

Materials and Methods

Cell culture and reagents. Pancreatic cancer cell lines (HS766T and HPAF-II) were obtained from the American Type Culture Collection. IL-13 was purchased from PeproTech EC (Frederick Cancer Research Center, National Cancer Institute). Recombinant IL-13-PE38 was generated as described previously (22, 26) and formulated in PBS containing 0.2% human serum albumin.

Stable transfection in pancreatic cancer cells. For IL-13Ra2 knockdown in HS766T cells, retrovirus-mediated RNAi was done using the pSuper RNAi system (Oligoengine) following the manufacturer’s instructions. The target sequence for IL-13Ra2 was 5′-TGGATCATCAGAGAACAAGCC-3′. Retrovirus-mediated expression of short hairpin RNA was done by ViraPort Retroviral Gene Expression System following the manufacturer’s instructions. Briefly, 293T cells were cotransfected with pVPack-VSV-G, pVPack-GP, and pSUPER-retropuro vectors. Following transfection, the medium containing retrovirus was collected, filtered, and transferred onto HS766T cells. Infected cells were selected with puromycin (1 μg/mL) for 7 days. IL-13Ra2 overexpression in HPAF-II was determined as described previously (27).

Reverse transcription-PCR. Total RNA was isolated using RNeasy easy plus kit (Qiagen), and reverse transcription-PCR (RT-PCR) was done using specific primers as described previously (28). β-Actin was used as a housekeeping gene control.

Immunofluorescence. Expression of IL-13Ra2 in pancreatic cancer cells and tissue specimens was observed by immunostaining as described previously (20).

Matrigel invasion assay. Cell invasion was assayed in BD BioCoat Matrigel invasion chambers (BD Biosciences; 24 wells, 8 μm pore size) using 10% fetal bovine serum as a chemotaxtractant. After incubation for 24 to 72 h at 37°C, noninvasive cells were removed from the top surface of the membrane with a cotton swab, and cells on the bottom surface were fixed and stained with H&E. Five random fields per well were counted. Data are shown as mean ± SD.

Protease assay. Proteolytic activity of the pancreatic cancer cells was detected using a fluorescence-based assay. Total cell protein from each pancreatic cancer cell line was collected using protease inhibitor-free lysis buffer (containing 50 mmol/L Tris-HCl, 10 mmol/L CaCl₂, 0.05% Brij-35, and 0.25% Triton X). Protease activity was measured using an EnzChek Protease Assay kit (Molecular Probes) following the manufacturer’s instructions (29). The experiments were done in triplicate and data are shown as mean ± SD.

AP-1 activation assay. Pancreatic cancer cells were stimulated in the presence or absence of IL-13 (10 ng/mL) for 4 h. Nuclear extracts were collected using the Transfactor Extract kit (Active Motif) and tested for DNA-binding activity using the AP-1 family TransAM kit (Active Motif) according to the manufacturer’s instructions. Nuclear extracts were incubated in oligonucleotide-coated plate for 1 h followed by three washings. Primary antibodies against anti c-Fos, FosB, Fra-1, Fra-2, c-Jun, JunB, and JunD were then added in each well for 1 h. After incubation with horseradish peroxidase–conjugated secondary antibody, color was developed and its absorbance measured at 450 nm with a reference wavelength of 655 nm using SpectraMax 5 spectrometer (Molecular Devices). Data are shown as mean ± SD of triplicate determinations.

Western blot analysis. Western blot analysis was done as described previously (26). Antibodies were ERK1/2, stress-activated protein kinase/c-Jun NH₂-terminal kinase, and p38 (Cell Signaling Technology), STAT6 (Santa Cruz Biotechnology), and MMP-9, MMP-12, and MMP-14.
(R&D Systems). Nuclear extracts were used for measurement of STAT6 phosphorylation.

**Measurement of metastasis in orthotopic pancreatic cancer mouse model.** Female nude nu/nu mice between ages 5 and 6 weeks were maintained in a barrier facility on HEPA-filtered racks. All animal studies were conducted under an approved protocol in accordance with the principles and procedures outlined in the NIH Guide for the Care and Use of Laboratory Animals. For orthotopic tumor cell injection, pancreas was carefully exposed, and 2.0 × 10^6 cells were injected into the pancreas. The pancreas was then returned to the peritoneal cavity, the abdominal wall, and the skin was closed with skin clips. Five weeks later, the number of metastatic lesions in the lymph node and liver was determined.

**Protein synthesis inhibition assay.** *In vitro* cytotoxic activity of IL-13 cytotoxin (IL-13-PE38) was measured by the inhibition of protein synthesis as described earlier (26). All assays were done in quadruplicate and data are shown as mean ± SD.

**Statistical analysis.** The data were analyzed for statistical significance using Student’s t test and ANOVA. Survival curves were generated by Kaplan-Meier method and compared by using the log-rank test.

**Results**

**Expression of IL-13Ra2 in pancreatic cancer cell lines.** We studied two pancreatic cancer cell lines to examine the effect of IL-13 on cancer invasion and metastasis. The cell lines tested included HPAF-II cells, a cell line lacking the IL-13Ra2 that was transfected with an IL-13Ra2 cDNA, and the HS766T cell line that naturally expresses this receptor chain was selected. In addition, IL-13Ra2 was silenced in HS766T cell line using RNAi. The expression of IL-13Ra2 after gene transfer or RNAi knockdown was confirmed by semiquantitative RT-PCR and immunofluorescence assays. Both analyses showed markedly increased IL-13Ra2 mRNA levels in IL-13Ra2–transfected HPAF-II cells, whereas RNAi knock-down of IL-13Ra2 in HS766T cells showed only <10% of original expression of mRNA levels (Supplementary Fig. S1). In contrast, the mRNA for two other subunits of IL-13R complex, IL-13Ra1 and IL-4Ra, was unaltered in these cell lines.

**IL-13 signals through IL-13Ra2 in promoting pancreatic cancer cell invasion.** The invasion ability of each pancreatic cancer cell line was examined using a Matrigel invasion assay. We observed that the number of invaded cells was unaltered between mock and IL-13Ra2–transfected HPAF-II cells without any stimulation. However, when cells were stimulated with 20 ng/mL IL-13, cell invasion significantly increased in HPAF-II IL-13Ra2–transfected cells compared with the mock control ($P = 0.002$; Fig. 1A). To determine the kinetics of cell invasion, HPAF-II cells were incubated with IL-13 for various periods and cell invasion was determined. IL-13 stimulation increased cell invasion in IL-13Ra2–transfected cells at three time points examined. Cell invasion was highest at 72 h compared with vector alone–transfected (mock) HPAF-II cells (130.5 ± 45.9 versus 405 ± 9.89; $P < 0.0003$; Supplementary Fig. S2). To confirm these observations, we examined the invasion ability of mock and IL-13Ra2–silenced HS766T pancreatic cancer cell lines in response to IL-13. IL-13Ra2–silenced cells were found to be significantly less invasive compared with the vector alone–transfected HS766T cells ($P = 0.0002$; Fig. 1B). These results suggest that IL-13 signals through IL-13Ra2 and that this receptor is involved in pancreatic cancer invasion.

**IL-13 increases protease production by pancreatic cancer cells through IL-13Ra2.** Because cancer cells inherently degrade extracellular matrix as the first step of cancer invasion and metastasis, we measured protease activity in untransfected pancreatic cancer cells, cells transfected with an IL-13Ra2 cDNA, and cells where IL-13Ra2 expression was knocked down. IL-13 treatment significantly increased protease activity in IL-13Ra2–transfected HPAF-II cells but not in mock HPAF-II cells ($P = 0.0002$ at 10 ng/mL and $P < 0.0001$ at 100 ng/mL. IL-13 compared with untreated control; Fig. 1C). Similarly, IL-13 treatment significantly increased protease activity in mock HS766T cells expressing IL-13Ra2 but not in IL-13Ra2–silenced HS766T cells ($P = 0.002$ at 10 ng/mL and $P = 0.0005$ at 100 ng/mL. IL-13 compared with untreated control; Fig. 1D).

**IL-13 induces MMP expression through IL-13Ra2 in pancreatic cancer cell lines.** To examine the mechanism of IL-13 enhanced pancreatic cancer cell invasion, we performed
RT-PCR analyses for MMPs. We examined the levels of mRNA of three different types of MMPs: MMP-9 (gelatinase B), MMP-12 (mem- 
talloelastase), and MMP-14 (membranetype1MMP), whicharerelated to pancreatic cancer invasion. Expression of MMP-9, MMP-12, and MMP-14 was either not detected or unchanged in mock and IL-13Ra2-transfected HPAF-II cells without IL-13 stimulation. However, on IL-13 stimulation, mRNAs of all three MMPs were induced in IL-13Ra2-transfected HPAF-II cells but not in mock 
HPAF-II cells (Fig. 2A). Similarly, IL-13 induced MMP mRNAs in mock HS766T (IL-13Ra2-positive) cells but not in IL-13Ra2- 
silenced HS766T cells (Fig. 2B).

We also performed Western blot analysis to confirm MMP protein 
regulation by IL-13 in mock and IL-13Ra2-transfected HPAF-
II cells. Similar to the RT-PCR results, IL-13 up-regulated MMPs in IL-13Ra2-positive cells but not in IL-13Ra2-negative HPAF-II pancreatic cancer cells (Fig. 2C).

IL-13 mediates activation of AP-1 transcription factor through IL-13Ra2. As AP-1 is possibly involved in the regulation of MMP gene expression, we examined whether IL-13 can activate AP-1 nuclear factors in pancreatic cancer cell lines. Activation of the c-Fos, c-Jun, and Fra-2 family of AP-1 transcription factors was examined. IL-13 stimulation did not activate AP-1 transcription factor in IL-13Ra2-negative mock HPAF-II cells. However, in IL-13Ra2-transfected HPAF-II cells, IL-13 doubled the degree of activation compared with mock cells (Fig. 3A). Similarly, IL-13 caused activation of AP-1 nuclear factors, c-Fos, c-Jun, and Fra-2, in mock HS766T cells but not in IL-13Ra2-silenced HS766T cells (Fig. 3B).

IL-13 signaling through IL-13Ra2 activates the MAPK but not the STAT6 pathway. We next examined MAPK activity, which acts upstream of the AP-1/MMP pathways. There are three main molecules that make up the MAPK pathway: ERK1/2, stress-activated protein kinase/c-Jun NH2-terminal kinase, and p38 MAPK. Our Western blot analyses to evaluate the activities of these kinases indicated that ERK1/2 was activated by IL-13 stimulation only in IL-13Ra2-transfected HPAF-II cells but weekly in control mock cells (Fig. 3C). In contrast, stress-activated protein kinase/c-Jun NH2-terminal kinase and p38 MAPK were not activated by IL-13 stimulation (data not shown). We confirmed the effects of IL-13 and signaling through IL-13Ra2 on MAPK activities using mock and IL-13Ra2-silenced HS766T cells. Similar to HPAF-II cells, IL-13 activated ERK1/2 only in mock HS766T (IL-13Ra2-positive) cells but not in RNAi HS766T cells (Fig. 3D). In contrast, stress-activated protein kinase/c-Jun NH2-
terminal kinase and p38 MAPK were not activated by IL-13 stimulation (data not shown).

We also examined the STAT6 pathway for activation by IL-13 in 
type II IL-13–positive cells (21). As expected, IL-13 induced phos- 
phorylation of STAT6 in HPAF-II cells; however, phosphorylation was lower in IL-13Ra2-transfected HPAF-II cells (Supplementary 
Fig. S3A). Conversely, STAT6 phosphorylation was increased in 
IL-13Ra2-silenced HS766T cells compared with mock HS766T.

Figure 3. IL-13 increases the expression of MMPs through activation of AP-1 nuclear factors and ERK1/2 in IL-13Ra2-positive pancreatic cancer cells. Activities of AP-1 family members c-Fos, c-Jun, and Fra-2 were determined in mock and IL-13Ra2-transfected HPAF-II cells (A) and mock and IL-13Ra2-silenced HS766T cells (B) after incubation with IL-13 (10 ng/mL) or vehicle for 4 h. AP-1 activity in nuclear extracts (2 μg) was measured and expressed as a ratio of IL-13 treated 
and vehicle groups. Western blot analysis for phosphorylated ERK1/2 in mock and IL-13Ra2-transfected HPAF-II (C) and mock and IL-13Ra2-silenced HS766T cells (D). Pancreatic cancer cells were treated with IL-13 (10 ng/mL) or vehicle for 1 h, and total cell protein was collected. Mean ± SD. Representative of three experiments.
IL-13Ra2 promotes pancreatic cancer metastasis and cachexia in nude mice with orthotropic human pancreatic tumors. To confirm a role for IL-13Ra2 in pancreatic cancer cell invasion and metastasis, we developed an orthotopic pancreatic cancer mouse model using HPAF-II and HS766T cells. The size of the primary pancreatic tumor was similar in mock and IL-13Ra2–transfected HPAF-II groups. However, mice transplanted with IL-13Ra2–transfected HPAF-II cells developed a significantly higher number of lymph node and liver metastases compared with mock HPAF-II tumors (Table 1). Two mice transplanted with IL-13Ra2–transfected tumors developed severe ascites, which was bloody in one mouse. A representative picture of the abdomen and liver is shown in Fig. 4A. The mouse transplanted with mock HPAF-II showed no metastasis in the abdominal cavity. However, the mouse transplanted with IL-13Ra2–transfected HPAF-II showed many lymph nodes and liver metastases (blue arrow).

IL-13Ra2 promotes pancreatic cancer metastasis and cachexia in nude mice with orthotropic human pancreatic tumors. To confirm a role for IL-13Ra2 in pancreatic cancer cell invasion and metastasis, we developed an orthotopic pancreatic cancer mouse model using HPAF-II and HS766T cells. The size of the primary pancreatic tumor was similar in mock and IL-13Ra2–transfected HPAF-II groups. However, mice transplanted with IL-13Ra2–transfected HPAF-II cells developed a significantly higher number of lymph node and liver metastases compared with mock HPAF-II tumors (Table 1). Two mice transplanted with IL-13Ra2–transfected tumors developed severe ascites, which was bloody in one mouse. A representative picture of the abdomen and liver is shown in Fig. 4A. The mouse transplanted with mock HPAF-II showed no metastasis in the abdominal cavity. However, the mouse transplanted with IL-13Ra2–transfected HPAF-II showed many lymph nodes and liver metastases (blue arrow).

Similarly, in another tumor model, numerous lymph nodes and liver metastases were observed in animals transplanted with mock IL-13Ra2–positive HS766T tumors. However, no metastasis was observed in animals transplanted with IL-13Ra2–silenced HS766T cells (Fig. 4B). H&E staining of original tumor, lymph node, and liver metastases were suggestive of highly proliferative and metastatic pancreatic tumors (Supplementary Fig. S4-A-C).

Severe cachexia and peritoneal metastasis were observed in all mice transplanted with mock HS766T cells. However, none of the mice transplanted with IL-13Ra2–silenced HS766T cells developed cachexia or peritoneal metastasis (Table 1).

We also examined survival of immunodeficient mice transplanted with both tumors. Interestingly, the median survival time of mice harboring IL-13Ra2–transfected HPAF-II tumors was significantly shorter compared with mock HPAF-II tumors (P = 0.0078; Fig. 4C). Conversely, the median survival time of mice harboring IL-13Ra2–silenced HS766T tumors was significantly longer compared with mock HS766T tumors (P = 0.0007; Fig. 4D). When tumor-bearing mice were treated with IL-13-PE38, an IL-13Ra2–targeted agent, from day 4 after tumor transplantation for 7 days,

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**Table 1. Cancer metastases in orthotopic pancreatic cancer mouse model developed from mock and IL-13Ra2–transfected HPAF-II cells and mock and IL-13Ra2–silenced HS766T cells**

<table>
<thead>
<tr>
<th>Mouse no.</th>
<th>Body weight (g)</th>
<th>Tumor size (cm³)</th>
<th>Lymph node metastasis</th>
<th>Liver metastasis</th>
<th>Ascites</th>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>23.1</td>
<td>1.19</td>
<td>2</td>
<td>0</td>
<td>−</td>
</tr>
<tr>
<td>2</td>
<td>18.9</td>
<td>1.32</td>
<td>3</td>
<td>0</td>
<td>−</td>
</tr>
<tr>
<td>3</td>
<td>26.5</td>
<td>1.47</td>
<td>3</td>
<td>0</td>
<td>−</td>
</tr>
<tr>
<td>4</td>
<td>24.4</td>
<td>1.09</td>
<td>1</td>
<td>0</td>
<td>−</td>
</tr>
<tr>
<td>5</td>
<td>23.1</td>
<td>1.32</td>
<td>0</td>
<td>0</td>
<td>−</td>
</tr>
<tr>
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<td>23.6</td>
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<tr>
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<tr>
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<td>0.13</td>
<td>1.3</td>
<td>0.0</td>
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<tr>
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<td>+</td>
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<td>−</td>
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<td>17</td>
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<td>18.2*</td>
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<tr>
<td>Average</td>
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*P = 0.0023.
†P = 0.04 compared with mock HPAF-II.
‡P = 0.02.
§P < 0.0001 compared with α2-silenced HS766T.
IL-13-PE38 significantly prolonged survival of mice harboring IL-13Rα2–transfected HPAF-II tumors \( (P = 0.008) \) and mock HS766T tumors \( (P = 0.0074) \) but not of mice harboring mock HPAF-II tumors and IL-13Rα2–silenced HS766T tumors.

**Increased expression of IL-13Rα2 in metastatic pancreatic cancer lesions.** Next, we examined the IL-13Rα2 expression levels in pancreatic tumors and in metastatic lesions that had developed in mice with HS766T tumor cells. The expression of IL-13Rα2 mRNA was higher in lymph node metastasis compared with original pancreatic cancer lesion (Fig. 5A).

**Increased sensitivity of metastatic pancreatic cancer to IL-13R–directed cytotoxin.** We assessed mRNA levels for IL-13Rα2 in primary and metastatic tumors of mice treated with IL-13-PE38. Treatment with IL-13-PE38 decreased the expression of IL-13Rα2 mRNA in both lymph node metastasis and pancreatic tumor compared with nontreated mice due to cell death of high IL-13Rα2–expressing cells. However, IL-13Rα2 expression was still higher in lymph node metastasis compared with primary pancreatic tumor (Fig. 5A).

To further confirm higher IL-13Rα2 expression in metastatic lesions, we treated single-cell suspensions of tumor cells derived from primary and lymph node metastatic lesions with various concentrations of IL-13-PE38 and cytotoxicity was determined. As shown in Fig. 5B, the sensitivity of lymph node metastatic cells to IL-13-PE38 in vitro was significantly higher compared with primary tumor, indicating higher levels of IL-13Rα2 in metastatic tumors \( (P = 0.008) \) at 1 ng/mL.

**Discussion**

We show for the first time that IL-13Rα2 is involved in human pancreatic cancer invasion and metastasis but not in proliferation and that IL-13 mediates signal transduction through IL-13Rα2 in...
cancer cells. These conclusions were drawn from multiple observations: (a) IL-13 significantly enhanced tumor invasion in IL-13Ra2–positive pancreatic cancer cells but not in IL-13Ra2–negative cells in vitro; (b) IL-13Ra2–positive pancreatic tumors metastasized to liver, lymph nodes, and the peritoneal cavity when transplanted onto the pancreas; (c) metastatic tumors had higher levels of IL-13Ra2 compared with primary tumor; (d) IL-13 induced MMPs in IL-13Ra2–positive but not in negative pancreatic tumor cell lines; and (e) IL-13 induced activation of ERK1/2 MAPK and AP-1 transcription factors. Thus, our study supports a previous observation that IL-13Ra2 is elevated in breast cancer metastasis and unequivocally shows directly that IL-13Ra2 is involved in pancreatic cancer invasion and metastasis (25).

Multiple mechanisms seem to be operational in IL-13–induced pancreatic cancer invasion and metastasis through IL-13Ra2. IL-13 has been shown to act as an autocrine growth factor in pancreatic cancer cells in vitro. In addition, endogenous expression of IL-13 and IL-4R in primary pancreatic ductal adenocarcinoma is shown to facilitate lymph node metastasis (30). In our current study, IL-13 induced expression of MMPs only in IL-13Ra2–positive pancreatic cancer cells but not in IL-13Ra2–negative cells, indicating that IL-13Ra2 plays a major role in metastasis, as activation of proteases is the first step in invasion and metastasis. We also show that IL-13 activates AP-1 in pancreatic tumor cells including the c-Fos, c-Jun, and Fra-2 family members. Interestingly, other cytokines such as IL-1 and tumor necrosis factor-α also increase MMP production in many cell types and involve the AP-1 pathway (31, 32). In addition, we show that IL-13 induces ERK1/2 MAPK expression in pancreatic cancer cells, which is consistent with the observation that many stimuli including growth factors and cytokines activate the ERK1/2 MAPK pathway contributing to increased proliferation rates of tumor cells (33). Lee and colleagues have reported that IL-13 selectively induces ERK1/2 activation in a STAT6–independent manner in the lungs of mice (34). Although these reports did not attribute this effect to IL-13Ra2, they support our results that IL-13 activates ERK1/2 in the MAPK pathway through IL-13Ra2. These results suggest that MAPK may play a key role in IL-13–induced tumor progression through AP-1 pathway.

Interestingly, IL-13 decreased STAT6 phosphorylation in IL-13Ra2–positive cells compared with IL-13Ra2–negative cells. This observation is similar to our previous study, which showed that gene transfer of IL-13Ra2 in CHO-K1 cells expressing IL-4Ra and IL-13Ra1 decreased activation of STAT6 by IL-13 treatment (16). This is because the IL-13Ra2 chain binds IL-13 with high affinity and consequently prevents optimal binding of IL-13 to other IL-13R proteins, resulting in inhibition of signaling through type II IL-13R.

It has been hypothesized that IL-13Ra2 chain serves as a decoy receptor for type II IL-13R complex, as extracellular domain of IL-13Ra2 inhibits IL-13–induced STAT6 activation in murine and human cell lines (16, 18). However, in our study, IL-13 mediated signaling through IL-13Ra2, indicating that IL-13Ra2 does not function as a decoy receptor in pancreatic cancer cells. IL-13Ra2 involved the AP-1 but not the STAT6 pathway of signaling. Thus, IL-13 signals though JAK/STAT6 pathway when it binds to type II IL-13R but involves AP-1 and MAPK when it binds to IL-13Ra2. Thus, receptor configuration determines the pathway of signal transduction through the IL-13R complex (Supplemental Fig. S5).

In the mouse metastatic model, whereas the size of primary tumor in the pancreas was not changed, HS766T tumor cells induced significantly higher peritoneal metastasis compared with HS766T tumor cells in which IL-13Ra2 expression was dramatically inhibited by RNAi-mediated gene silencing. These results indicated that the ability of cell invasion and metastasis mainly depends on IL-13Ra2 expression. This conclusion was further supported by IL-13Ra2 gene transfer in HPAF-II cells that dramatically enhanced cancer metastasis to lymph nodes and the liver compared with mock-transfected cells. Again, the size of primary tumor in the pancreas was not changed between mock and IL-13Ra2–transfected cells. Thus, we speculate that IL-13Ra2 may promote cancer invasion and metastasis but not affect primary tumor growth. We have reported previously that overexpression of IL-13Ra2 slowed tumor growth after initial implant at the s.c. site in immunodeficient mice (35). IL-13Ra2 induced activation of innate immune response that prevented tumor growth. However, in the present study, IL-13Ra2 overexpression did not affect primary pancreatic tumor growth when implanted orthotopically but favored tumor metastasis. These results suggest that IL-13Ra2 can activate innate immunity in mice at s.c. site causing rejection of tumor, but this response seems to be restricted at the s.c. site, as orthotopic tumors remained unchanged. This is an intriguing observation and is a topic of further investigation in our laboratory between IL-13Ra2 and tumor immune response.

Consistent with our hypothesis that IL-13Ra2 promotes tumor metastasis, we observed a higher expression of IL-13Ra2 in metastatic lesions compared with primary pancreatic tumors in the mouse metastatic model. Similarly, Minn and colleagues reported higher expression of IL-13Ra2 mRNA in lung metastasis compared with the parent breast cancer cells (25). Based on this and our observations, we speculate that IL-13Ra2–overexpressing tumors are more prone to invade as these cells produce more proteases and MMPs. Consequently, these cells metastasize more vigorously, resulting in a higher numbers of IL-13Ra2–expressing tumor cells in metastatic lesions compared with cells displaying reduced or lack IL-13Ra2 expression.

It is of considerable interest to note that the mice implanted with IL-13Ra2–expressing HST766T pancreatic tumors dramatically survived for a shorter period (median survival time, 47 days) compared with mice with same tumor (109 days) but significantly reduced IL-13Ra2, as it was knocked down by RNAi transfection before implantation. In addition, when these mice with IL-13Ra2–expressing pancreatic tumors were systemically treated with IL-13R–directed cytotoxin, they survived significantly longer compared with untreated control mice (P < 0.0074). These observations indicate that IL-13Ra2 expression may serve as a prognostic biomarker of pancreatic or other cancers and this conclusion should be further tested. In addition, IL-13Ra2 may serve as a new therapeutic target for prevention of invasion and metastasis for not only pancreatic cancer but also other cancers that express IL-13Ra2. Finally, as pancreatic cancer expresses IL-13Ra2, it provides a new target for receptor-directed pancreatic cancer therapy.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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