Blocking NF-κB Is Essential for the Immunotherapeutic Effect of Recombinant IL18 in Pancreatic Cancer

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Abstract

Purpose: We sought to find new immune-based treatments for pancreatic cancer.

Experimental Design: We detected IL18 expression in plasma and specimens from patients with pancreatic cancer. We then investigated whether IL18 had a therapeutic effect for pancreatic cancer in vitro and in vivo and any underlying mechanisms.

Results: Higher plasma IL18 was associated with longer overall survival (OS), but higher IL18 in pancreatic cancer tissues was associated with shorter OS and increased invasion and metastasis. Recombinant IL18 alone had no antitumor effect in the syngeneic mice with orthotopically transplanted tumors and promoted tumors in immunocompromised mice; it also facilitated immune responses in vitro and in vivo by augmenting the activity of cytotoxic T cells and NK cells in peripheral blood and lymph nodes. However, IL18 promoted the proliferation and invasion of pancreatic cancer cells, in vitro and in vivo, through the NF-κB pathway. Nevertheless, by coadministering IL18 with BAY11-7082, an NF-κB inhibitor, we were able to prevent the procarcinous effects of IL18 and prolong the survival time of the mice.

Conclusions: IL18 has both cancer-promoting and cancer-suppressing functions. Although its single-agent treatment has no therapeutic effect on pancreatic cancer, when combined with the NF-κB pathway inhibitor, IL18 improved survival in a murine pancreatic cancer model. Our study implies the possibility of a combinational immunotherapy that uses IL18 and targets NF-κB pathway.

Introduction

The prognosis for pancreatic cancer is poor (1), and effective treatments are sorely needed. Although immunotherapy is a promising modality of cancer treatment (2), specific immunotherapy strategies for pancreatic cancer remain scarce.

IL18 is a uniquely pleiotropic member of the IL1 family. It is synthesized as a 24-kDa precursor protein, cleaved into an 18-kDa mature form by caspase-1, and then secreted (3). IL18 is produced mainly by immune cells and effectively enhances innate and adaptive immune responses (4). IL18 stimulates T cells and enhances Th1 immune responses (5–7). It also has antitumor activity in preclinical models. It increases the serum concentrations of IFNγ, granulocyte macrophage colony-stimulating factor, and soluble Fas ligand (8). IL18 production can account for differential stimulation of natural killer (NK) cell effector functions in vitro by squamous cell carcinoma and may be important for the stimulation of NK cells in vivo (9). A phase I study of recombinant human IL18 (rhIL18) in patients with advanced cancer concluded that biologically active doses of rhIL18 were safe for these patients, in whom rhIL18 increased the expression of activation antigens on lymphocytes and monocytes (10).

However, IL18 also plays a critical role in tumor migration, invasion, and metastasis. Song and colleagues and Jung and colleagues previously demonstrated that IL18 enhances the migration ability of murine melanoma cells (11, 12). IL18 increased adherence to the microvascular walls and induced the production of angiogenic factors and tumor growth–stimulating factors. IL18 promotes melanoma metastasis via VEGF (13). IL18 secreted by microglia (which had been activated by C6 glioma-derived extracellular matrix) enhanced migration of C6 glioma (14). Endogenous IL18 was shown to facilitate gastric cancer cell...
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Translational Relevance
Immunotherapy is a promising cancer treatment modality. Although recombinant IL18 has no therapeutic effect on pancreatic cancer as a single-agent treatment, our study indicated that, when combined with a NF-kB pathway inhibitor, IL18 suppressed pancreatic cancer growth in a mouse model. Our study supports a potential combined immunotherapy strategy that uses IL18 and targets the NF-kB pathway.

Immune escape by suppressing CD70 and increasing gastric cancer cell metastatic ability by upregulating CD44 and VEGF (15). IL18 also increased cell migration directly through filamentous actin (F-actin) polymerization and tensin downregulation in gastric cancer cells (16). Thus, the role of IL18 in cancer progression and metastasis is controversial, and its therapeutic role in pancreatic cancer has not yet been established. Here, we show that IL18 has both cancer-promoting and cancer-suppressing functions. Cotreatment with IL18 and a NF-kB pathway inhibitor had a better immunotherapy effect than IL18 alone.

Materials and Methods

Cell culture
Cell lines MIA-PaCa-2 and PANC-1 were purchased and authenticated through STR typing from ATCC. Cell lines LTPA, NK92, and Jurkat were purchased and authenticated through STR typing from Cell Repository, Chinese Academy of Sciences (Shanghai, China). MIA-PaCa-2 and PANC-1 were grown in DMEM (Gibco) and LTPA and Jurkat in 1640 medium, supplemented with 10% FBS (Gibco). 100 U/mL penicillin G, and 100 μg/mL streptomycin (Sigma) at 37°C in a humidified 5% CO2 incubator. NK-92 cells were cultured in MEM-α containing 20% FBS, 1% penicillin/streptomycin, and 100 U/mL recombinant IL2 (Sigma).

Ethical statement
This study was approved by the Human Research Ethics Committee at the Huazhong University of Science and Technology and was carried out in accordance with the principles embodied in the Declaration of Helsinki.

All in vivo animal experiments were approved by the Committee on the Ethics of Animal Experiments of Huazhong University of Science and Technology (Wuhan, China; permit number: 2013-S245). All treatments were carried out according to the U.S. Public Health Service Policy on Humane Care and Use of Laboratory Animals. All surgeries were conducted under sodium pentobarbital anesthesia.

Human tissue and blood samples
Pancreatic cancer tissue microarray (TMA; HPan-Ade160Sur-01) was bought from Shanghai Outdo Biotech. Surgical specimens of pancreatic cancers and adjacent normal pancreatic tissues were collected between January 2010 and June 2014 from 32 patients. Histologically, all 32 cancers were adenocarcinomas. These 32 pairs of specimens were made into another TMA. This study used 112 pairs of pancreatic cancer tissues and adjacent normal pancreatic tissues.

Plasmas were obtained between January 2010 and June 2011 from 104 patients with pancreatic cancer, 35 with benign pancreas tumors, 27 with duodenal cancer, 77 with cholangiocarcinoma, 8 with hepatic cancer, 22 with pancreatitis, and 38 healthy controls. Peripheral blood mononuclear cells (PBMC) were isolated from subjects’ peripheral blood for immune cell detection and plasma for ELISA.

Animals
Female C57BL/6J wild-type mice and female BALB/c nude mice, both 6 weeks old, were purchased from HFK Bioscience. IL18 knockout mice, with a C57BL/6J background, were generated by BGI Ark Biotechnology. The IL18−/− mice were maintained with the original genetic background. All mice were bred in specific pathogen-free conditions in Central Animal Laboratory, Huazhong University of Science and Technology. In all experiments, mice were female and age matched.

Orthotopic transplants of mouse pancreatic cancer cells to Balb/c nude mice, C57BL/6J mice, and IL18−/− C57BL/6J mice
We used 8-week-old wild-type C57BL/6J mice, Balb/c nude mice, and IL18−/− C57BL/6J mice in these experiments. For orthotopic implantation, mice were anesthetized with pentobarbital sodium, and hair was removed from their abdomens. We incised each mouse longitudinally along the abdomen to expose the pancreas, injected 20 μL of the cell suspension into the pancreas, and closed the incision with sutures. Mice then received recombinant mouse IL18 (rmIL18, 1 μg/g/week) or BAY11-7082 (0.1 μg/g/week) as intraperitoneal injections. Each experimental group consisted of five mice. All mice were weighed and checked for signs of distress regularly. Abdominal palpation was used to monitor tumor size. The mice in each group were sacrificed using CO2 narcosis, followed by cervical dislocation at the end of study period or earlier if they appeared moribund or exhibited greater than 15% weight loss. The tumors were evaluated macroscopically and microscopically. Depletion of immune cell subsets was confirmed by flow cytometry performed on spleen cells and abdominal aortic lymph nodes cells following necropsy.

3D cultivation methods
We performed 3D cultivation as described previously (17, 18). Cells were suspended in ice-chilled 6% Matrigel (BD) in complete medium and dispensed at 5 × 10^5 cells in 1,000 μL per well, using 12-well plates on top of a solidified 1% noble agar layer (Difco). Ice-chilled 3% Matrigel in complete medium was used to replenish the medium every 4 days and/or to administer rhIL18 (20 ng/mL). We photographed using an Olympus BX51 microscope.

Subcutaneous transplant model of human pancreatic cancer cells in Balb/c nude mice
We injected 2 × 10^6 transfected cells subcutaneously into the right armpits of female, 6-week-old Balb/c nude mice. The weight of the mice and the diameter of tumors were measured every week. Mice in each group were sacrificed 7 weeks after initiation of treatment. The tumors were evaluated macroscopically and microscopically.

Liver metastasis model of human pancreatic cancer cells in Balb/c nude mice
Human pancreatic cancer cells (2 × 10^6) were suspended in serum-free medium and then injected into the spleen involucra of female 8-week-old Balb/c nude mice. The mice underwent
Figure 1.
Expression of IL18 in plasma and tissue samples of patients with pancreatic cancer. A, clustering analyses of screened cytokines in plasma from patients with pancreatic cancer and health controls (n = 3 for both). B, ELISA IL18 expression in plasma of indicated cancer is shown. ELISA IL18 expression in plasma of pancreatic cancer patients before and after surgery. Patients were divided into those above (IL18 high) and those below (IL18 low) the average value (508 pg/mL). Kaplan-Meier analysis for OS of the IL18 high and IL-18 low groups is shown. C, statistical analysis of IL18 expression in pancreatic cancer tissues and adjacent pancreas tissues. D, quantitative analysis of IL18 expression in tumors from patients with and without invasion and/or metastasis. E, quantitative analysis of IL18 expression grouping according to NCCN pancreatic cancer staging. F, patients were divided into those above (IL18 high) and those below (IL18 low) the average value. Kaplan-Meier analyses of their OS is shown.
splectomy 20 minutes after injection. Mice were weighted once a week and were sacrificed at the end of study period or earlier if they appeared moribund or exhibited greater than 15% weight loss. Their livers were evaluated macroscopically and microscopically.

Figure 2.
IL18 increases cytolytic activities of T cells (Tc) and NK cells. A, PBMCs isolated from healthy volunteers were treated with rhIL18 (20 ng/mL) for 24 hours; flow cytometry was used to detect the proportions of immune cells and statistically compared with the negative control (NC) group (n = 5 for both groups). B, immune cells isolated from spleen and aortic lymph nodes of C57BL/6J mice were injected intraperitoneally. rmIL18 and untreated C57BL/6J mice were subjected to flow cytometry; results were statistically compared (n = 5). C, IL18−/− C57BL/6J mice and control mice were subjected to flow cytometry to detect the proportions of immune cells in spleens and aortic lymph nodes; results were statistically compared (n = 5). D, indicated pancreatic cancer cells and T cells or NK cells were subjected to 4-hour 51Cr release assay; rhIL18-treated group and negative control group were compared (n = 6 for both groups, **, P < 0.01, * P < 0.05). E, plasma isolated from blood of rmIL18 were intraperitoneally injected into C57BL/6J mice; IL18−/− C57BL/6J mice and control mice were subjected to ELISA to detect the relative expression of IFN-y (n = 5). F, we established orthotopic transplants in wild-type C57BL/6J mice. The mice were divided into two groups: LTPA negative control (NC) group (n = 5, intraperitoneal injection 20 μL PBS per week) and LTPA rmIL18 group (n = 5, intraperitoneal injection 1 μg/g rmIL18 per week). ELISA was used to detect the relative expression of IFN-y in plasma isolated from the mice.

Statistical analyses
Results for continuous variables are presented as means ± SD unless stated otherwise. Treatment groups were compared with independent sample by t tests. Pair-wise multiple comparisons

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used one-way ANOVA (two sided). \( P < 0.05 \) was considered statistically significant. All analyses were performed using SPSS v.17.0 software (SPSS Inc.).

More detailed materials and methods can be found in Supplementary Materials and Methods.

Results

IL18 is higher in plasma of pancreatic cancer patients and is associated with longer survival

We screened 89 cytokines in plasma samples from three patients with pancreatic cancer and three healthy controls, using the Human Cytokine Array. We found that six cytokines (IL18, CD30, CD23, CD40, angiopoietin-1, and activin A) were overexpressed and eight cytokines (VEGF, TGF\( \beta \), eotaxin-2, MIG, SDF-\( \alpha \), TNF\( \beta \), I-309, TNF\( \alpha \), and IL7) were underexpressed in the plasma of PC patients compared with the healthy controls. IL-18 was the most up-regulated cytokine in the plasma of pancreatic cancer patients (\( P < 0.001 \); Fig. 1A). Furthermore, higher plasma levels of IL18 were found in pancreatic cancer patients than in patients with benign pancreatic tumors, patients with pancreatitis, or healthy subjects or in patients with other common gastrointestinal malignancies, including hepatic cancer, cholangiocarcinoma, duodenal carcinoma, gastric carcinoma, and colorectal carcinoma (Fig. 1B). Plasma IL18 levels taken 3 days before surgery and those taken 3 days after surgery differed significantly (Fig. 1B). Postoperative IL18 levels were about one third of preoperative levels (Fig. 1B). Interestingly, patients with higher IL18 plasma levels (\( n = 49 \), median survival: undefined) had a slightly longer overall survival (OS) than did patients with lower levels (\( n = 42 \), median survival: 19.5 months; Fig. 1B).

IL18 is higher in pancreatic cancer tissues and correlates with increased metastasis and shorter survival

Immunohistochemical staining of TMAs showed that IL18 was mainly localized in the cytoplasm of pancreatic cancer cells (Supplementary Fig. S1B). IL18 expression in tissues differed significantly between normal pancreas and cancerous pancreatic tissues (Fig. 1C). However, IL18 levels did not differ significantly among patients with different grades of pancreatic cancer (Supplementary Fig. S1D). IL18 expression was significantly higher in tumors from patients with lymph node involvement and/or distant metastasis than in those with no lymph node involvement and distant metastasis (Fig. 1D). Patients with more poorly differentiated tumors by NCCN staging (19) had higher IL18 expression (Fig. 1E). Importantly, we found that patients with higher IL18 levels (\( n = 40 \), median survival: 18 months) had a slightly shorter OS compared with the patients with lower IL18 levels (\( n = 26 \), median survival: 43 months; Fig. 1F and Supplementary Fig. S1E).

Thus, higher IL18 levels in plasmas and tumor tissues are associated with different prognoses. These results also raised the possibility that IL18 has different roles in immune cells and pancreatic cancer cells.

Figure 3.

IL18 has no therapeutic effect in the syngeneic mice with pancreatic cancer orthotopic transplants but promotes tumors in the immunocompromised mice with orthotopic transplants. We used wild-type C57BL/6J mice (A) or Balb/c nude mice (C) for our models. The mice were divided into two groups, LTPA negative control (NC) group, which received 20 \( \mu \)L PBS intraperitoneally per week (\( n = 5 \)) and LTPA rmIL18 group (\( n = 5 \), i.p. 1 \( \mu \)g/g rmIL18 per week). Weight changes of C57BL/6J mice (A) and Balb/c nude mice (C) with orthotopic transplants are shown. Kaplan-Meier analyses are shown in C57BL/6J mice (B) and Balb/c nude mice (D) with transplants. *, \( P < 0.05 \).
IL18 increases cytolytic activities of T cells and NK cells

We then studied whether IL18 has a modulatory effect on the immune system. PBMCs isolated from healthy volunteers were cocultured with rhIL18 for 24 hours in 6-well plates. Flow cytometry was used to investigate changes of immunophenotype and activation status in PBMCs. Notably, the proportions of T and NK cells were significantly increased (Fig. 2A). Next, rmIL18 was injected i.p. into C57BL/6J mice daily for 7 days. Immune cells from spleen and aortic lymph nodes were isolated for flow cytometry analysis to investigate changes in immunophenotype and activation status. Proportions of T and NK cells were significantly increased (Fig. 2B). We then knocked out the IL18 gene from C57BL/6J mice and compared the main immune cell subpopulation in the spleens and aortic lymph nodes of IL18−/− C57BL/6J mice with those of wild-type C57BL/6J mice. Proportions of T and NK cells decreased in IL18−/− C57BL/6J mice, but the proportions of B cells, Th cells, and dendritic cells (DC) were little changed (Fig. 2C). IL18 promoted the cytotoxic activity of the T cells and NK cells (Fig. 2D). Finally we found that IFNγ was increased in IL18-treated group but decreased in IL18−/− C57BL/6J mice (Fig. 2E). Recombinant IL18 increased IFNγ expression in the syngeneic mice with orthotopically transplanted tumors (Fig. 2F).

IL18 has no therapeutic effect in the syngeneic pancreatic cancer orthotopic transplants in mice but promotes tumor in transplanted immunocompromised mice

First, we investigated whether IL18 could be used as a therapeutic agent for pancreatic cancer by testing it on orthotopic pancreatic cancer transplants in wild-type C57BL/6J mice and Balb/c nude mice. Although IL18 had no effect on survival of tumor-bearing C57BL/6J mice (Fig. 3A and B), we found that IL18 accelerated tumor growth and shortened survival in Balb/c nude (Balb/c-nu) mice that received human pancreatic cancers established as xenografts. Long-term follow-up showed that IL18 treatment shortened OS of tumor-bearing mice (Fig. 3C and D). Balb/c-nu mice lack thymus glands and therefore have defective T-cell functions and B-cell maturity. In these immunocompromised mice, the effect of IL18 is possibly limited to tumor cells, and its tumor-promoting effects could be elucidated. In contrast, in the immunocompetent mice, IL18 appeared to act on both tumor and immune cells. Its lack of effect on tumors in these mice suggests that IL18 might have two opposite effects.

IL18 promotes pancreatic cancer cell proliferation, invasion, and metastasis

Our above-mentioned findings suggested that IL18 might play an important role in pancreatic cancer proliferation and tumor growth. We therefore generated stable MIA-PaCa-2 and Panc-1 cell lines that upregulated and downregulated IL18 (respectively), using a lentiviral delivery system. Upregulated IL18 (IL18U) and downregulated IL18 (IL18D) were verified by Western blot analyses (Supplementary Fig. S3A). We found that upregulated IL18 promoted pancreatic cancer cell proliferation, whereas downregulated IL18 suppressed pancreatic cancer cell proliferation (as shown by the CCK-8 assay and 3D culture system; Fig. 4A and B and Supplementary Fig. S3B). Moreover, rhIL18 promoted pancreatic cancer cell proliferation in both concentration- and time-dependent manners (Fig. 4A and B and Supplementary Fig. S3C). Next, we established subcutaneous transplants of human pancreatic cancer cells in Balb/c nude mice and found that IL18U promoted tumor growth, whereas IL18D suppressed tumor growth (Fig. 4C and Supplementary Fig. S4A). In the IL18U group, the xenografts grew faster and the mice developed cachexia sooner; in the IL18D group, the xenografts grew slowly, and the mice remained a healthy state. Microscopic evaluation of the xenografts showed higher Ki67, PCNA, and IL18 levels in the IL18U group and lower levels in the IL18D group (Supplementary Fig. S3D and S3E). Protein analysis confirmed IL18 overexpression in the IL18U group xenografts (Supplementary Fig. S3F).

Because the tissue array IHC showed IL18 expression levels to correlate with local invasion and metastasis, we investigated the role of IL18 on pancreatic cancer cell invasiveness. Upregulated IL18 promoted pancreatic cancer cell invasion and migration, whereas IL18 downregulation suppressed cancer cell invasion and migration (Fig. 4D and Supplementary Fig. S4B and S4D). rhIL18 also promoted pancreatic cancer cell invasion and migration (Fig. 4D and Supplementary Fig. S4C). Next, we established a liver metastasis model of human pancreatic cancer cells in Balb/c nude mice as described previously (20). This model showed that IL18 promoted metastasis and reduced OS of metastasis-bearing mice (Fig. 4E). Histology analysis of serial whole-liver sections showed more micrometastases in the IL18U group, and fewer micrometastases in the IL18D group, compared with the controls (Fig. 4E and Supplementary Fig. S4E).

We treated IL18U cells with IL18-binding protein (IL18BP) and the IL18D cells with rhIL18. IL18BP suppressed IL18U’s promotion of pancreatic cancer cell proliferation and invasiveness; rhIL18
IL18 promotes proliferation and invasion of pancreatic cancer cells via the NF-κB pathway

To elucidate the mechanisms underlying IL18’s promotion of tumor cell proliferation, invasion, and metastasis, we analyzed the mRNA microarray data and found that IL18 was associated with the NF-κB pathway (Supplementary Fig. S6A). Stimulation of PANC-1 cells with rhIL18 (20 ng/mL) for 120 minutes or longer increased p-Iκα/κB. Stimulation of PANC-1 cells with rhIL18 for 15 minutes increased p-IκBα expression and IκBα expression began to decrease after at least 240-minute rhIL18 treatment (Fig. 5A, left). Stimulation of PANC-1 cells with rhIL18 and BAY11-7082 for 120 minutes did not increase p-IκBα expression, but p-Iκα/κB expression continued to increase (Fig. 5A, right). A dual-luciferase assay also showed IL18 to directly activate the NF-κB pathway in PANC-1 cells (Fig. 5B, top). rhIL18 promoted the translocation of NF-κB from the plasma into the nuclei in PANC-1 (Fig. 5B, bottom) and MIA-PaCa-2 cells (Supplementary Fig. S6C). An electrophoretic mobility shift assay also confirmed that IL18 directly activated the NF-κB pathway (Supplementary Fig. S6B).

Next, we examined whether IL18 promoted pancreatic cancer cell invasion and proliferation through the NF-κB pathway. NF-κB siRNA and the NF-κB pathway inhibitors, BAY11-7082 and curcumin, completely counteracted the cancer-promoting effect of IL18 (Fig. 5C and D and Supplementary Fig. S6D). NF-κB is a transcriptional factor that orchestrates the expression of many genes involved in inflammation, oncogenesis, and apoptosis. We examined the main target genes of the NF-κB pathway of IL18U, IL18D, and negative control (NC) cells by real-time PCR and found that IL18 mainly affected MMP-3, MMP-9, cyclin-D1, and c-myc expression (Supplementary Fig. S6E). Upregulation of IL18 or adding rhIL18 increased the expression of MMP-3, MMP-9, cyclin-D1, and c-myc, whereas si-NF-κB, BAY11-7082, or curcumin inhibited expression of MMP-3, MMP-9, cyclin-D1, and c-myc in pancreatic cancer cells (Fig. 5E). Consistently, MMP-3, MMP-9, cyclin-D1, and c-myc were increased in the IL18U group of the subcutaneous transplanted tumors in Balb/c nude mice (Fig. 5E). We also found IL18 increased the recruitment of NF-κB to the MMP3, MMP9, c-myc, and cyclin-D1 promoters (Fig. 5F).

Combination of the recombinant IL18 and NF-κB pathway inhibitor has a therapeutic effect on pancreatic cancer

We established orthotopic transplants in C57BL/6j mice and in IL18+/− C57BL/6j mice using syngeneic LTPA mouse pancreatic cancer cells and and IL18 knockdown (KD) LTPA cells. Four groups of mice with tumor transplants were investigated: (i) C57BL/6j mice with LTPA cells; (ii) C57BL/6j mice with IL18KD LTPA cells; (iii) IL18+/− C57BL/6j mice with LTPA cells; and (iv) IL18+/− C57BL/6j mice with IL18KD LTPA cells. The results showed that the C57BL/6j mice with IL18KD cells had the longest survival time, whereas IL18+/− C57BL/6j LTPA cells had the shortest survival time (Fig. 6A) and also developed more liver and lung micrometastases (Fig. 6B). This result suggests that IL18 might suppress pancreatic cancer by acting on the systemic immune system but also meanwhile promote tumor growth and metastases via the NF-κB pathway in pancreatic cancer cells.

We therefore treated C57BL/6j mice orthotopically transplanted with LTPA cells with recombinant IL18 and NF-κB inhibitor, in four groups: (i) control group, (ii) IL18 only, (iii) BAY11-7082 only, and (iv) IL18 + BAY11-7082. The IL18 + BAY11-7082 group had the longest survival time of the four groups. In contrast, OS did not significantly differ between the control and IL18-only groups. Thus, IL18 had an antitumor therapeutic effect on pancreatic cancer only when combined with an NF-κB pathway inhibitor (Fig. 6C).

Discussion

IL18 is one of several proinflammatory/immunostimulatory cytokines that have been investigated as therapeutic agents for cancer. Clinical trials have shown that rhIL18 can be given safely and is biologically active in patients (21, 22). However, these studies only focused on the IL18’s promotive effect on the immune system and did not examine its effect on tumor cells. IL18 is increased in pancreatic cancer tissues and patient plasma. Higher IL18 levels in patient plasma correlated with a longer survival time, but higher IL18 levels in cancer tissues correlated with shorter survival time. In ovarian cancer, tumor cells predominantly release the biologically inactive 24-kDa pro-IL18 (23), and only this form is detectable in the ascites of patients (24). We therefore chose an antibody specific to mature form IL18 in pancreatic cancer patients’ plasma and tissues directly. Actually, pancreatic cancer cells predominantly release the biologically active 18-kDa mature IL18. We found that IL18 had no therapeutic effect in orthotopic transplants in wild-type C57BL/6j mice but promoted transplanted tumors in Balb/c nude mice. This implies a complex relationship between IL18 and pancreatic cancer.

Next, we studied the effect of IL18 on the malignant biologic behavior of pancreatic cancer cells in the absence of an immune system. Several studies showed the direct effect of IL18 on tumor suppression IL18D’s inhibition of pancreatic cancer cell proliferation and invasiveness (Figs. 4F and Supplementary Fig. S5).
cells. IL18 has prometastatic effects in melanoma patients and may promote clinical aggressiveness of human myeloid leukemia (13). IL18 also promoted hepatoma cell metastasis and migration (25). We found that IL18 promoted the proliferation and invasion of pancreatic cancer cells in vitro and in vivo but had no effect on apoptosis, drug resistance, or stem cell–like properties in pancreatic cancer. Furthermore, IL18 seems to be a key regulator of proliferation and invasion of pancreatic cancer cells. We investigated the mechanisms involved in the promotion of proliferation and invasion of pancreatic cancer cells by IL18. A previous study showed that IL18 directly increased the migration of gastric cancer cells through F-actin polymerization and decreased tensin levels (16). IL18 enhanced melanoma cell migration via the generation of reactive oxygen intermediates and the MAPK pathway (12), and IL18 might facilitate stomach cancer cell immune escape by suppressing CD70 and increasing metastatic ability by increasing CD44 and VEGF (15). We verified these mechanisms and confirmed that IL18 promotes tumor cell proliferation and invasion.

Figure 6.
Combined recombinant IL18 and NF-κB pathway inhibitor have a therapeutic effect on pancreatic cancer. A, we used a mouse pancreatic cancer cell line LTPA and IL18KD/LTPA to establish orthotopic transplants in C57BL/6J mice and IL18−/− C57BL/6J (n = 5). Left, weight change curve. The other three groups were compared with the LTPA/C57BL/6J group (P < 0.05); right, Kaplan–Meier analysis of the different treatments. B, whole liver (left) and lung (right) serially sectioned and micrometastases calculated (H&E staining). C, we used a mouse pancreatic cancer cell line, LTPA, to establish orthotopic transplants in C57BL/6J mice, which were treated with rmIL18 and/or BAY11-7082 as indicated (n = 5). Weight change curve is shown (left). The other three groups were compared with the negative control group (P < 0.05). Right, Kaplan–Meier analyses of survival times of groups with different treatments.
in pancreatic cancer through an underlying mechanism, which bioinformatics analysis indicates through the NF-κB pathway. In addition, inhibition of NF-κB though siRNA, the NF-κB pathway inhibitor BAY11-7082, or curcumin counteracted this tumor promotion effect. NF-κB is a ubiquitously expressed proinflammatory transcription factor that regulates the expression of more than 500 genes involved in cellular transformation, survival, proliferation, invasion, angiogenesis, metastasis, and inflammation. The NF-κB pathway is a potential target for pharmacologic intervention (26, 27).

IL-18 can regulate both innate and adaptive immune responses through its effects on T cells (4, 7, 28), NK cells (29, 30), macrophages (31), and DCs (32). Systemic administration of IL-18 had little antitumor activity in several preclinical animal models (33). IL-18 appears to act predominantly as a costimulatory cytokine; its optimal use in cancer immunotherapy may be in combination with other immunostimulatory cytokines, vaccines, or mAbs. Our research showed that IL-18 increases the proportion of antitumor immune cells (especially cytotoxic T cells and NK cells) and increases the cytolytic activity of the cytotoxic T cells and NK cells. Thus, IL-18 shows a potential therapeutic effect on pancreatic cancer. Unexpectedly, IL-18 had no therapeutic effect on orthotopic transplants in C57BL/6j mice. This might be explained by IL-18 promoting tumor cell proliferation and invasion in pancreatic cancer through the NF-κB pathway. BAY11-7082, a potential antitumor agent that blocks the NF-κB signaling pathway, counteracted the tumor-promoting effect of IL-18 (34–36). Finally, we showed that coadministrating of IL-18 and a NF-κB pathway inhibitor has an antitumor effect on pancreatic cancer.

In conclusion, IL-18 has both cancer-promoting and cancer-suppressing effects. Single-agent treatment with recombinant IL-18 has no therapeutic effect. However, when combined with an NF-κB pathway inhibitor, IL-18 is a potential immunotherapeutic agent against pancreatic cancer. Our study supports a potential combined immunotherapy strategy that uses IL-18 and targets the NFκB pathway.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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