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c-Jun N-terminal kinase in pancreatic tumor stroma augments tumor development in mice

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Pancreatic ductal adenocarcinoma (PDAC) is a life-threatening disease and there is an urgent need to develop improved therapeutic approaches. The role of c-Jun N-terminal kinase (JNK) in PDAC stroma is not well defined even though dense desmoplastic reactions are characteristic of PDAC histology. We aimed to explore the role of JNK in PDAC stroma in mice. We crossed Ptf1aCre;KrasG12D/+ mice with JNK1−/− mice to generate Ptf1aCre;KrasG12D/+;JNK1−/− (Kras;JNK1−/−) mice. Tumor weight was significantly lower in Kras;JNK1−/− mice than in Kras;JNK1+/− mice, whereas histopathological features were similar. We also transplanted a murine PDAC cell line (mPC) with intact JNK1 s.c. into WT and JNK1−/− mice. Tumor diameters were significantly smaller in JNK1−/− mice. Phosphorylated JNK (p-JNK) was activated in α-smooth muscle actin (SMA)-positive cells in tumor stroma, and mPC-conditioned medium activated p-JNK in tumor-associated fibroblasts (TAF) in vitro. Relative expression of Ccl20 was downregulated in stimulated TAF. Ccl20 is an important chemokine that promotes CD8+ T-cell infiltration by recruitment of dendritic cells, and the number of CD8+ T cells was decreased in Kras;JNK1−/− mice compared with Kras;JNK1+/− mice. These results suggest that the cancer secretome decreases Ccl20 secretion from TAF by activation of JNK, and downregulation of Ccl20 secretion might be correlated with reduction of infiltrating CD8+ T cells. Therefore, we concluded that inhibition of activated JNK in pancreatic tumor stroma could be a potential therapeutic target to increase Ccl20 secretion from TAF and induce accumulation of CD8+ T cells, which would be expected to enhance antitumor immunity.

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tumor cells and stroma is likely different depending on the context; thus, further examination is needed to better understand the complex interaction.

In the present study, we deleted JNK1 activation in tumor stroma using a s.c. xenograft tumor model transplanted into JNK1 knockout mice and noted that JNK1 depletion in PDAC stroma suppressed tumor progression. Moreover, we examined the effect of JNK inhibition in tumor-associated fibroblasts (TAF) and secretion of related cytokines and chemokines to explore possible underlying mechanisms.

Materials and Methods

Mice. Ptf1aCre+;LSL-KrasG12D/+ mice(21) and JNK1 knockout (JNK1−/−) mice(22,23) were previously described. All mice were maintained in filter-topped cages and fed on autoclaved food and water at the Institute for Adult Diseases, Asahi Life Foundation, and Yokohama City University, Graduate School of Medicine, according to National Institutes of Health (NIH) guidelines. All experiments were approved by the Ethics Committee for Animal Experimentation of the Yokohama City University and the Institute for Adult Diseases, Asahi Life Foundation.

Reagents. We purchased JNK inhibitor SP600125 from Cayman Chemical (Ann Arbor, MI, USA), and transforming growth factor β blockers (TGF-β) from R&D Systems (Minneapolis, MN, USA). Primary antibodies used in immunohistochemical and immunofluorescence assays included rabbit anti-phospho-JNK antibody (1:50; Cell Signaling Technology, Danvers, MA, USA), rat anti-mouse CD45 antibody (1:50; BD Biosciences, San Jose, CA, USA), rabbit anti-mouse F4/80 antibody (1:100; BD Biosciences, San Jose, CA, USA), rat anti-mouse CD11c antibody (1:100; eBioscience, San Diego, CA, USA), rabbit anti-Ki-67 antibody (1:100; Abcam, Cambridge, UK), mouse anti-α-smooth muscle actin (α-SMA) antibody (1:50; Santa Cruz, Santa Cruz, CA, USA), biotin hamster anti-mouse CD11c antibody (1:100; BD Biosciences), rabbit anti-cleaved caspase3 antibody (1:1600; Cell Signaling Technology) and rabbit anti-CD8 antibody (clone 53-6.7; BD Biosciences, Bedford, MA, USA), biotin hamster anti-mouse CD11c antibody (1:100; BD Biosciences, San Jose, CA, USA), rabbit anti-phospho-Stat3 antibody (1:2000; Cell Signaling Technology, Danvers, MA, USA), and rabbit anti-CD8 antibody (clone Vigen A; BD Biosciences) was used for the slides with anti-CD8 antibody (clone 53-6.7; BD Biosciences, Bedford, MA, USA). Primary antibodies used in immunoblotting analysis included rabbit anti-phospho-JNK antibody (1:1000; Cell Signaling Technology), rabbit anti-JNK antibody (1:1000; Cell Signaling Technology), rabbit anti-phospho-Stat3 antibody (1:2000; Cell Signaling Technology) and mouse anti-Stat3 antibody (1:1000; Cell Signaling Technology).

Cell culture. A murine pancreatic cancer cell line (K399), established from Ptf1aCre+/−;LSL-KrasG12D/+;Tgfbr2−/−/loxlox mice, was kindly provided by Dr. Iijichi, University of Tokyo. Ptf1aCre+/−;LSL-KrasG12D/+;Tgfbr2−/−/loxlox mice were injected s.c. into the interscapular and interiliac regions of wild-type (WT) or JNK1−/− mice (four separate regions per mouse; n = 2 per group). When tumors became palpable, diameter of tumors was measured periodically (approximately every week) between day 11 and day 68.

Histopathological examination. Pancreatic tissues were fixed in buffered 10% formalin. Embedding in paraffin blocks, preparation of paraffin sections, H&E, and Picro-Sirius Red staining (PSR) were done by Sept Sapie, KK (Tokyo, Japan). Area of each normal acinar and graded pancreatic intraepithelial neoplasia (PanIN) lesion (1, 2, and 3) was measured and the percentage respectively calculated. PSR was scored using the following scale: 0 = absent; 1 = mild; 2 = moderate; 3 = severe.(25).

Immunohistochemical examination. Standard procedures were applied for immunohistochemical examination. In brief, after deparaffinization and rehydration, endogenous peroxidase was blocked with 3% hydrogen peroxide for 20 min at room temperature. For heat-mediated antigen retrieval, with the exception of anti-CD45 antibody, slides were processed for 15 min at 121°C in an autoclave or twice for 10 min each at 500 W in a microwave oven in 10 mM citrate buffer (pH 6.0). Retrieval was performed using the slides with anti-CD45 antibody at 89°C for 10 min. Slides were then incubated with primary antibodies. After incubation, slides were rinsed in PBS (pH 7.5), and stained with DAB. Immunohistochemical examination was performed with the following primary antibodies: rabbit anti-Phospho-JNK1 (1:500; Cell Signaling Technology); rabbit anti-Stat3 (1:500; Cell Signaling Technology); rabbit anti-α-SMA (1:500; Santa Cruz Biotechnology); rabbit anti-CD45 (1:50; Cell Signaling Technology); rabbit anti-cleaved caspase3 (1:500; Cell Signaling Technology); mouse anti-CD8 (clone 53-6.7; BD Biosciences) and rabbit anti-CD11c (clone 53-6.7; BD Biosciences).

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Original Article
JNK in pancreatic tumor stroma

(A) Kras;JNK1+/−  Kras;JNK1−/−

H.E.

(B) PSR

(C) CD45

F4/80

αSMA

Ki-67
with the primary antibodies at the appropriate concentration (described earlier in Reagents) overnight at 4°C. Secondary antibodies, including biotinylated anti-rabbit, anti-mouse, and anti-rat antibodies, were applied for 30 min at room temperature; all secondary antibodies were purchased from Vector Laboratories (Burlingame, CA, USA) and were diluted 1:200. The VECTASTAIN ABC kit (Vector Laboratories) was used according to the manufacturer’s directions, and slides were allowed to react with diaminobenzidine (Muto Pure Chemicals, Tokyo, Japan) solution. Hematoxylin was used as a counterstain. Number of cells staining positive for CD45, F4/80, and SMA were counted and averaged. Ki-67-positive nuclei in PanIN lesions were counted and divided by the total number of nuclei in PanIN lesions. a-SMA-positive areas were measured in pixels by ImageJ(26) and averaged.

Immunofluorescence examination. Slides were deparaffinized and rehydrated. For heat-mediated antigen retrieval, slides were processed for 15 min in an autoclave at 121°C. Slides were incubated with the primary antibodies at the appropriate concentrations (see Reagents) overnight at 4°C. Alexa Fluor® 488- or 594-conjugated secondary antibodies (1:500; Life Technologies, Carlsbad, CA, USA) were applied for 1 h at room temperature, protected from light, and covered with mounting medium containing DAPI (Vector Laboratories). Images were taken using a fluorescence microscope (BZ-9000; Keyence, Osaka, Japan).

Immunoblotting analysis. Cell lysates were prepared using a tissue protein extraction reagent (Thermo Fisher Scientific). Immunoblotting analysis was carried out on the lysates, which were separated by SDS-PAGE and transferred to Immobilon-P membranes (Merck Millipore). The membranes were incubated in PVDF blocking reagent for Can Get Signal immunostain (Toyobo, Osaka, Japan) at room temperature for 1 h to block non-specific reactions, overnight at 4°C with primary antibodies at the appropriate concentrations (see Reagents). Then, HRP-conjugated secondary antibodies were applied to the membrane for 1 h and photographs of the image were taken on LAS-3000 (Fujifilm, Tokyo, Japan) using ECL prime Western blotting detection reagent (GE Healthcare, Chicago, IL, USA).

Quantitative RT-PCR (qRT-PCR). RNA was extracted from TAF and pancreatic tissues using ISOGEN2 (Wako). Extracted RNA was reverse transcribed to cDNA using a high-capacity RNA-to-cDNA kit (Thermo Fisher Scientific). qRT-PCR, using the cDNA, was carried out with fast SYBR green master mix (Thermo Fisher Scientific) according to the manufacturer’s directions in 96-well plates on a 7900HT Fast Real-Time PCR System (Applied Biosystems, Waltham, MA, USA). Relative expression levels of each gene were calculated using the delta Ct method normalizing the data based on the endogenous reference (GAPDH). Primer sequences are shown in Table S1.

Pancreatic acinar isolation and collagen culture. The pancreas was removed, washed twice with ice-cold PBS, minced, and digested with collagenase I (37°C with shaker). Digested pancreatic pieces were washed and pipetted through a mesh. Acinar cells were pelleted (200 g for 2 min at 4°C) and resuspended in medium (1% FBS and 1 μg/mL dexamethasone). Acinar cells were cultured with or without TGF-α (50 ng/mL) or SP600125 (10 μM).

Statistical analysis. Significant differences were detected using Student’s t-test. P-values < 0.05 were considered statistically significant.

Results

Effect of JNK1 deletion in Kras-mediated pancreatic tumors. To analyze the effect of JNK1 in Kras-mediated pancreatic tumors, we crossed Ptf1aCre+/+ mice with LSL-KrasG12D+/+ and JNK1−/− mice to generate Ptf1aCre+/+;LSL-KrasG12D+/+;JNK1−/− mice. Littermates, Ptf1aCre+/+;LSL-KrasG12D+/+;JNK1−/− (Kras;JNK1−/−) mice, were used as controls (Fig. 1a).

There were no phenotypical differences between Kras;JNK1−/− and Ptf1aCre+/+;LSL-KrasG12D+/+;JNK1−/− mice (data not shown). Mice were killed at 6 months of age and ratio of pancreas weight to bodyweight (pancreas/B.W., %) was measured. In Kras;JNK1−/− mice, the pancreas/B.W. ratio was significantly smaller than in Kras;JNK1+/− mice (P = 0.001) (Fig. 1b). Interestingly, despite this result, we noted no histopathological differences between Kras;JNK1−/− and Kras;JNK1+/− mice. There were no significant differences in the rate of PanIN formation (Fig. 2a); therefore, we hypothesized that JNK1 deletion did not have any effects on PanIN formation. To confirm this hypothesis, we harvested acinar cells from WT mice and cultured them in collagen with or without TGF-α and/or JNK inhibitor SP600125. Stimulation of the acinar cells by TGF-α resulted in induction of acinar-to-ductal metaplasia (ADM), both with or without SP600125 (Fig. 3a). Furthermore, acinar cells harvested from JNK1−/− mice could not avoid ADM (Fig. 3b). These results suggest that JNK1 is non-essential for ADM and PanIN formation.

Histopathological analysis of tumor stroma showed no significant differences in the amount of PSR-stained collagen fibers (Fig. 2b), the number of infiltrated CD45- and F4/80-stained cells, and the area of a-SMA-stained lesions (Fig. 2c). Therefore, JNK1 does not play an important role in the development of tumor stroma. However, the number of Ki-67 stained cells in PanIN lesions was significantly greater in Kras;JNK1−/− mice than in Kras;JNK1+/− mice (P < 0.05) (Fig. 2c). These results suggest that JNK inhibition reduces tumor growth without changing tumor structure. These results are in accordance with previous reports showing that the JNK inhibitor, SP600125, inhibits pancreatic cancer growth in vivo(27) and has a therapeutic effect on murine pancreatic ductal adenocarcinoma.(9)

JNK activation in Kras-mediated pancreatic tumor stroma. As the effect of JNK on pancreatic cancer cells has already been reported,(27,28) we focused on pancreatic tumor stroma to...
determine whether it has an effect on tumor progression. We noted that not only PanIN cells but also stromal cells were partially stained with phosphorylated-JNK (p-JNK) antibodies (Fig. 4a). This finding indicates that JNK is activated in the pancreatic tumor microenvironment. For further investigation, we transplanted K399 cells s.c. onto the backs of WT or JNK1+/− mice. In this model, transplanted K399 cells had intact JNK1 activation whereas other components, such as fibroblasts, immune cells, and endothelial cells, lacked JNK1. We measured the diameter of each s.c. tumor for 68 days, and the sizes of xenografts transplanted into JNK1+/− mice were significantly smaller than those in WT mice after 38 days (Fig. 4b). As the majority of p-JNK-stained cells in stroma were spindle-shaped, we considered them fibroblasts (Fig. 4a, arrowhead). To elucidate the p-JNK-stained cell types, we carried out co-immunofluorescent staining for p-JNK with α-SMA or F4/80, and both stains colocalized with p-JNK staining (Fig. 4c,d). Although both fibroblasts and macrophages were stained with p-JNK, the tumor stroma was rich in α-SMA-stained cells compared with F4/80-stained cells. Therefore, we focused on fibroblasts for further investigation.

**p-JNK activation in tumor-associated fibroblasts and their expression of related cytokines and chemokines.** We established TAF derived from Kras-mediated murine pancreatic tumors and stimulated them with K399-conditioned medium. p-JNK activation in TAF lysate was evaluated by immunoblotting analysis, and we found that p-JNK was activated by K399-conditioned medium after 15 and 30 min (Fig. 5a). Prolonged JNK activation was also observed at 24 h after stimulation (Fig. 5a). This JNK activation is not K399-conditioned medium specific; the conditioned medium produced by another murine pancreatic cancer cell line (EPPK1, established from Ptf1aCre+/LSL-KrasG12D+ mice, materials and methods are shown in Doc. S1) could also activate JNK in TAF (Fig. 5b). These results suggest that the cancer secretome activated JNK in TAF directly. To investigate the role of JNK activation in TAF, we next examined the expression of cytokines and chemokines that have been reported to be related to pancreatic ductal adenocarcinoma progression, including chemokine ligand (Ccl)2, Ccl20, chemokine (C-X-C motif) ligand (Cxcl) 1, Cxcl12, Cxcl10, Cxcl12, interleukin (IL)-1β, IL-6, TGF-β1, tumor necrosis factor-α (TNF-α), bone morphogenetic protein (BMP), CD40 ligand (CD40L), granulocyte/macrophage-colony-stimulating factor (GM-CSF, SF2), granulocyte colony-stimulating factor (CSF3), epidermal growth factor (EGF), IL-2, IL-4, IL-12β, IL-13, IL-17A, Nodal, TNF-related apoptosis-inducing ligand (Trail), and vascular endothelial growth factor C (VEGFC) (Figs 5b, S2). Expression of Ccl20 and Cxcl1 were downregulated by K399 condition medium, and SP600125 had a rescue effect. Furthermore, Ccl20 expression was significantly upregulated in Kras;JNK1+/− pancreatic tumors compared with Kras;JNK1+/+ tumors. In contrast, Cxcl1 expression was not significantly upregulated in Kras;JNK1+/− tumors (Fig. 6a). Ccl20 expression is reported to be

![Fig. 3. Impact of c-Jun N-terminal kinase1 (JNK1) deletion on induction of acinar-to-ductal metaplasia (ADM).](#) (a) Acinar cells were isolated from WT mouse pancreas and cultured with or without transforming growth factor (TGF)-β (50 ng/mL) or SP600125 (10 μM). (b) (Left) Acinar cells were isolated from the pancreas of WT or JNK1+/− mice and cultured with or without TGF-α (50 ng/mL). (Right) Percentage of ADM was measured. Results are mean ± SD (WT, n = 10; JNK1+/−, n = 4) (*P* = 0.97, Student's *t*-test) (n.s., not significant).
regulated through activated signal transducers and activator of transcription 3 (Stat3), and JNK signaling is considered to be a negative regulator of Stat3 activation. Therefore, we investigated the involvement of the JNK-Stat3 pathway by immunoblotting analysis, and we found that phosphor-Stat3 (p-Stat3) was certainly downregulated in TAF stimulated by K339 conditioned medium (Fig. 5c). Ccl20 is one of the chemokines that recruits dendritic cells (DC). In addition, it has been reported that DC are responsible for cross-priming CD8+ T cells, which are cytotoxic effector cells. Therefore, we focused on these immune cells for further investigation.

Ccl20 upregulation and recruitment of CD8+ T cells in Kras;JNK1−/− mice. To determine the role of Ccl20 in Kras;JNK1−/− pancreatic tumors, we carried out qRT-PCR analysis for CD11c and perforin. CD11c is a marker for DC, and perforin is a pore-forming protein that induces apoptosis and destroys cells and plays important roles in the cytotoxic activity of CD8+ T cells and natural killer cells. Although CD11c and perforin expression were not statistically different in Kras;JNK1−/− mice compared with Kras;JNK1+/+ mice, CD11c expression showed a tendency of slight upregulation and perforin expression showed a strong tendency of upregulation in Kras;JNK1−/− mice (Fig. 6a). Immunohistochemical analysis showed CD11c-stained cell infiltration in the tumor stroma, and there was a tendency for a larger number of CD11c-stained cells infiltrating in Kras;JNK1−/− pancreatic tumors (Fig. 6b). To elucidate whether cytotoxic T-cell recruitment was accelerated in Kras;JNK1−/− pancreatic tumors, we carried out immunohistochemical staining using an anti-CD8 antibody. Numbers of CD8-stained cells and cleaved caspase-3-positive PanIN cells were significantly greater in Kras;JNK1−/− mice than in Kras;JNK1+/+ mice (P < 0.05) (Fig. 6c, d), which should be another reason, in addition to the numerous Ki-67-stained cells in PanIN lesions, that the pancreas/B.W. ratio of Kras;JNK1−/− mice was smaller than in Kras;JNK1+/+ mice. To summarize our results, in pancreatic tumor stroma, p-JNK in TAF is activated by the cancer secretome, which downregulates Ccl20 expression. This leads to a decrease in the recruitment of DC and CD8+ T cells, thereby resulting in accelerated tumor growth (Fig. 7).

Discussion

We demonstrated here that JNK activation is important to promote pancreatic tumor progression, not only in tumor cells but
also in tumor stroma, especially in fibroblasts; however, the initiation and formation of PanIN was not affected by inhibition of JNK in vitro or knockout of JNK1 in vivo. Toste et al. have reported that gemcitabine treatment activates JNK and p38 signaling pathways in pancreatic cancer-associated fibroblasts (CAF). These gemcitabine-treated CAF secrete multiple inflammatory mediators, resembling a senescence-associated secretory phenotype that is considered to promote tumor progression. Additionally, inhibition of stress-associated MAPK signaling suppresses induction of the senescence-associated secretory phenotype in CAF. Therefore, inhibition of JNK has therapeutic potential for PDAC patients by targeting both the tumor cells and the stromal component, and it could be a promising choice for both monotherapy and combination therapy with current chemotherapeutic regimens.

Fig. 5. Effect of activated phosphorylated c-Jun N-terminal kinase (p-JNK) in tumor-associated fibroblasts (TAF). (a) TAF, fibroblasts derived from Kras-mediated murine pancreatic cancers, were stimulated by K399-conditioned medium and harvested at the indicated times (top). Immunoblotting analyses of stimulated TAF lysate with anti-p-JNK and anti-JNK antibodies are shown. (b) TAF were stimulated by control medium or K399-conditioned medium with or without SP600125 (20 μM) and harvested after 8 h. Relative mRNA expression of indicated cytokines and chemokines is shown. (c) TAF were stimulated by K399-conditioned medium and harvested at the indicated times (top). Immunoblotting analyses of stimulated TAF lysate with anti-p-Stat3, anti-Stat3, anti-p-JNK and anti-JNK antibodies are shown.

Fig. 6. Chemokine ligand (Ccl)20 expression, CD11c positive cells and CD8+ T cell recruitment in pancreatic tumor tissue. (a) mRNA was isolated from pancreatic tumors of Kras;JNK1+/- and Kras;JNK1/− mice. Relative mRNA expression of chemokine (C-X-C motif) ligand (Cxcl)1, Ccl20, Ccl21, and perforin are shown (n = 4 in Kras;JNK1+/- group and n = 3 in Kras;JNK1−/− group) (Cxcl1, P = 0.21; Ccl20, *P < 0.05; Ccl21, P = 0.60; perforin, P = 0.16; Student’s t-test) (n.s., not significant). (b) (Left) Immunohistochemical staining using anti-CD11c antibody is shown (magnification, ×400; scale bar, 50 μm). (Right) Number of antibody-stained cells was counted and averaged. Results are mean ± SEM of five random views (n = 2 in each group) (P < 0.37, Student’s t-test). (c) (Left) Immunohistochemical staining using anti-CD8 antibody is shown (magnification, ×400; scale bar, 50 μm). (Right) Number of antibody-stained cells was counted and averaged. Results are mean ± SEM of five random views (n = 3 in Kras;JNK1+/- group and n = 5 in Kras;JNK1−/− group) (*P < 0.05, Student’s t-test).
(A) 

**Cxcl1**

| Kras;JNK1+/− | Kras;JNK1−/− |
|----------------|----------------|
| 1              | 1.5            |

n.s.

**Ccl20**

| Kras;JNK1+/− | Kras;JNK1−/− |
|----------------|----------------|
| 12             | 14.5           |

* |

**CD11c**

| Kras;JNK1+/− | Kras;JNK1−/− |
|----------------|----------------|
| 1              | 1.5            |

n.s.

**Perforin**

| Kras;JNK1+/− | Kras;JNK1−/− |
|----------------|----------------|
| 20             | 18             |

n.s.

(B) 

**CD11c**

| Kras;JNK1+/− | Kras;JNK1−/− |
|----------------|----------------|
| [Image]        | [Image]        |

| Kras;JNK1+/− | Kras;JNK1−/− |
|----------------|----------------|
| [Image]        | [Image]        |

(C) 

**CD8**

| Kras;JNK1+/− | Kras;JNK1−/− |
|----------------|----------------|
| [Image]        | [Image]        |

| Kras;JNK1+/− | Kras;JNK1−/− |
|----------------|----------------|
| [Image]        | [Image]        |

(D) 

**Cleaved caspase3**

| Kras;JNK1+/− | Kras;JNK1−/− |
|----------------|----------------|
| [Image]        | [Image]        |

| Kras;JNK1+/− | Kras;JNK1−/− |
|----------------|----------------|
| [Image]        | [Image]        |
Ccl20 expression is considered to be regulated through nuclear factor kappa B (NF-κB), CCAAT/enhancer-binding proteins, activator protein 1 (AP-1), specificity protein 1 (Sp-1), and activated Stat3. In this study, it is interesting that whereas AP-1 is one of the downstream elements of the JNK signaling pathway, Ccl20 expression was downregulated in JNK-activated TAF, and downregulated Stat3 activation should be the mechanism responsible for Ccl20 downregulation. It is still not clear why the Stat3 pathway but not AP-1 is the dominant regulator of Ccl20 expression in TAF.

Despite our findings showing that Ccl20 expression correlated with the upregulation of DC markers and cytotoxic T-cell infiltration, which would be expected to induce tumor apoptosis and to increase antitumor effects, there are several reports showing that Ccl20 has supportive rather than suppressive effects on PDAC cells. Campbell et al. reported that Ccl20 promotes PDAC cell invasion, and Liu et al. reported that Ccl20, derived from tumor-associated macrophages, enhances PDAC cell growth and metastatic ability. Moreover, Rubie et al. showed that Ccl20 was significantly upregulated in human PDAC tissue and associated with advanced T stage based on the UICC TNM classification. However, in other carcinomas, such as esophageal, gastric, ovarian, and colorectal cancers, upregulation of Ccl20 expression and infiltration of DC and CD8 + T cells represent a positive prognostic factor for survival. The present study shows, for the first time, that Ccl20 may exhibit antitumor effects by recruiting DC and CD8 + T cells to pancreatic tumor stroma. Tumor immunity has recently been highlighted as a therapeutic target, and nivolumab, (an antibody against programmed death 1 receptor) targeting an immune checkpoint, has recently become widely available for the treatment of melanoma and other carcinomas. Accumulation of antitumor immune cells in the tumor stroma is expected to increase the efficacy of immune checkpoint therapy; thus, JNK inhibition could be a favorable choice in combination with immune checkpoint therapy.

In summary, results from the present study suggest that activated JNK suppresses Ccl20 expression in TAF and inhibits infiltration of antitumor immune cells (DC and CD8 + T cells). JNK inhibition rescues Ccl20 expression in TAF and increases the number of antitumor immune cells. Thus, JNK is an attractive target for PDAC treatment.

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Disclosure Statement

Authors declare no conflicts of interest for this article.

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Supporting Information

Additional Supporting Information may be found in online Supporting Information tab for this article:

Doc. S1. Supplementary materials and methods: Cell culture, tumor-associated fibroblast (TAF) stimulation by K399-conditioned medium to examine prolonged JNK activation, TAF stimulation by EPPK1 conditioned medium to examine prolonged JNK activation, Supplementary materials and methods: Cell culture, tumor-associated fibroblast (TAF) stimulation by K399 conditioned medium to examine prolonged JNK activation, TAF stimulation by EPPK1 conditioned medium to examine prolonged JNK activation, TAF stimulation by EPPK1-conditioned medium and harvested at the indicated times (top), to evaluate the long-term effect. (b) TAF were stimulated by EPPK1-conditioned medium and harvested at the indicated times (top) (EPPK1: murine pancreatic cancer cell line established from Ptf1aCre;LSL-KrasG12D mice). Immunoblotting analyses of stimulated TAF lysate with anti-p-JNK and anti-JNK antibodies are shown.

Fig. S1. (a) Tumor-associated fibroblasts (TAF) were stimulated by K399-conditioned medium and harvested at the indicated times (top), to evaluate the long-term effect. (b) TAF were stimulated by EPPK1-conditioned medium and harvested at the indicated times (top) (EPPK1: murine pancreatic cancer cell line established from Ptf1aCre;LSL-KrasG12D mice). Immunoblotting analyses of stimulated TAF lysate with anti-p-JNK and anti-JNK antibodies are shown.