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REVIEW

Inflammation and pancreatic cancer: disease promoter and new therapeutic target

Shin Hamada · Atsushi Masamune · Tooru Shimosegawa

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Abstract Chronic inflammation has a certain impact on the carcinogenesis of the digestive organs. The characteristic tissue structure of pancreatic cancer, desmoplasia, results from inflammatory processes induced by cancer cells and stromal cells. Concerning the progression of pancreatic cancer, recent research has clarified the pivotal role of tumor-stromal interaction, which promotes the development of an invasive phenotype of cancer and provides survival advantages against chemotherapeutic agents or immune surveillance. Tumor stromal cells such as pancreatic stellate cells and immune cells establish a microenvironment that protects cancer cells through complex interactions. The microenvironment of pancreatic cancer acts as a niche for pancreatic cancer stem cells from which therapy-resistance and disease recurrence develop. Inhibition of the stromal functions or restoration of the immune reaction against cancer cells has therapeutic benefits that enhance the efficacy of conventional therapies. Some of the recent advances in this field are now under evaluation in clinical settings, but many problems must be overcome to establish a radical therapy for pancreatic cancer. This review summarizes current knowledge about the tumor-promoting stromal functions, immune system modulation and therapeutic strategies targeting tumor-stromal interactions in pancreatic cancer.

Keywords Desmoplasia · Pancreatic stellate cell · Myeloid derived suppressor cell · Epithelial-mesenchymal transition · Cancer stem cell · Sonic hedgehog · Connective tissue growth factor

S. Hamada (✉) · A. Masamune · T. Shimosegawa
Division of Gastroenterology, Tohoku University Graduate School of Medicine, 1-1 Seiryo-machi Aobaku, Sendai, Miyagi 980-8574, Japan
e-mail: hamadas@med.tohoku.ac.jp

Abbreviations

α-SMA	α-Smooth muscle actin
ARB	Angiotensin II type 1 receptor blocker
ATRA	All-trans retinoic acid
CSCs	Cancer stem cells
CTGF	Connective tissue growth factor
DPI	Diphenylene iodonium
ECM	Extracellular matrix
EMT	Epithelial-mesenchymal transition
ERK	Extracellular signal-regulated kinase
HIF1α	Hypoxia-inducible factor 1 alpha
IL	Interleukin
JAK	Janus kinase
JNK	c-Jun NH2-terminal kinase
MAPK	Mitogen-activated protein kinase
M-CSF	Macrophage colony-stimulating factor
MDSCs	Myeloid-derived suppressor cells
MMP3	Matrix metalloproteinase 3
NADPH	Nicotinamide adenine dinucleotide phosphate
p38 MAPK	p38 Mitogen-activated protein kinase
PanINs	Pancreatic intraepithelial neoplasias
PDGF	Platelet-derived growth factor
PEGPH20	PEGylated human recombinant PH20 hyaluronidase
PI3K	Phosphatidylinositol 3-kinase
PSCs	Pancreatic stellate cells
ROS	Reactive oxygen species
Shh	Sonic hedgehog
SPARC	Secreted protein acidic and rich in cysteine
STAT	Signal transducers and activators of transcription
TGF-β	Transforming growth factor-β
Tregs	Regulatory T cells
VEGF	Vascular endothelial growth factor

Introduction

Chronic inflammation is closely related to the carcinogenesis of digestive organs. The pivotal roles of inflammation in the carcinogenesis of various organs are well recognized; *Helicobacter pylori* in gastric cancer, hepatitis virus or non-alcoholic steatohepatitis in hepatocellular carcinoma and colitis-associated colon cancer are typical examples [1, 2]. The role of chronic inflammation in pancreatic carcinogenesis has also been investigated in detail [3]. Chronic pancreatitis causes repeated, acute exacerbations leading to tissue destruction and fibrosis, referred to as the necrosis-fibrosis sequence [4]. A meta-analysis of reports assessing the correlation between chronic pancreatitis and pancreatic cancer described that the pooled relative risk for pancreatic cancer in chronic pancreatitis patients was 13.3 (6.1–28.9, 95 % confidence intervals) [5]. Hereditary pancreatitis is a rare cause of pancreatitis originating from a genetic burden that results in the early onset of pancreatitis and prolonged exposure to inflammatory stimuli. As expected, the pooled relative risk for pancreatic cancer in hereditary pancreatitis patients was higher than that in chronic pancreatitis [5]. A prospective study also revealed that hereditary pancreatitis patients had a significantly higher standardized incidence ratio of pancreatic cancer compared with the general population [6]. Even though these risk factors such as genetic variants of pancreatitis-associated genes and familial history of pancreatic cancer are known, it is still difficult to find an early stage pancreatic cancer [7, 8]. These lines of evidence suggest the indispensable contribution of the inflammatory process to pancreatic carcinogenesis. Indeed, detailed analysis of human pancreatic cancer specimens and the development of pancreatic cancer model mice have highlighted the importance of sustained inflammation, which aggravates the cancer cell behavior, within pancreatic cancer tissue. The tissue structure of pancreatic cancer results from the activation of stromal cells by cancer cells and vice versa, with the result that the wound never heals. This review article summarizes the current knowledge about the inflammatory processes related to pancreatic cancer progression, and possible therapeutic interventions.

Tissue structure of pancreatic cancer and its cellular origin

Pancreatic cancer is recognized as a less-enhanced tumor by contrast-enhanced computed tomography or magnetic resonance image, reflecting poorly the vascularized tissue structure [9]. Microscopic images of pancreatic cancer specimens show a characteristic tissue structure, desmoplasia. Typically, a dense fibrotic stroma surrounding cancer cells is observed, accompanied by sparse blood vessels [10]. The formation of desmoplasia results from the continuous inflammation evoked by pancreatic cancer cells, activated stromal cells and immune cells. These tumor components establish an inflammatory network within pancreatic cancer, leading to the protection of cancer cells from exogenous anticancer drugs and immune surveillance [11]. Inflammatory cytokines and downstream signals contribute to this process, such as mitogen-activated protein kinase (MAPK) or Akt pathways [10, 12]. Among stromal cells, pancreatic stellate cells (PSCs) play a central role in the development of desmoplasia [13]. In the normal pancreas, PSCs remain in a quiescent state, carrying vitamin A-containing lipid droplets. Similar cells have been found in the peri-sinusoidal space of the liver's hepatic stellate cells [14]. The activation of PSCs by inflammatory signals results in the proliferation of PSCs, extracellular matrix (ECM) protein production, α-smooth muscle actin (α-SMA) expression and inflammatory cytokine secretion [15]. The increased expression of secreted protein acidic and rich in cysteine (SPARC) in the tumor stroma is correlated with a poor prognosis, suggesting that ECM proteins have a role in promoting tumors [16]. The interaction of PSCs with cancer cells and other types of stromal cells form a feed-forward loop of inflammation within the pancreatic cancer, leading to the desmoplasia.

In addition to desmoplasia, the pancreatic cancer tissue is infiltrated by a wide variety of immune cells. Assessment of these cells clarified that pancreatic cancer tissue is enriched with immunosuppressive cells. These cells include myeloid-derived suppressor cells (MDSCs) and regulatory T cells (Tregs), which suppress cytotoxic T cell functions. MDSCs are immature myeloid derived precursor cells of granulocytes, dendritic cells and macrophages. MDSCs produce reactive oxygen species (ROS), arginase and nitric oxide that increase the intra-tumoral oxidative stress [17]. The infiltration of MDSCs in pancreatic cancer tissue leads to the establishment of antigen-specific T-cell tolerance, which enables cancer cells to escape from immune surveillance [18]. In addition, a higher ratio of tumor-infiltrating Tregs to $CD4^+$ T-cells was significantly associated with shorter survival in pancreatic cancer patients [19], suggesting that immune suppression within the tumor greatly contributes to the pancreatic cancer progression. Another type of immune cell, the mast cell, was also reported to be involved in the pancreatic cancer progression. Mast cells were originally thought to mediate type I hypersensitivity by releasing chemical mediators in allergic diseases [20]. Immunohistochemical analysis for the quantification of mast cells in pancreatic cancer

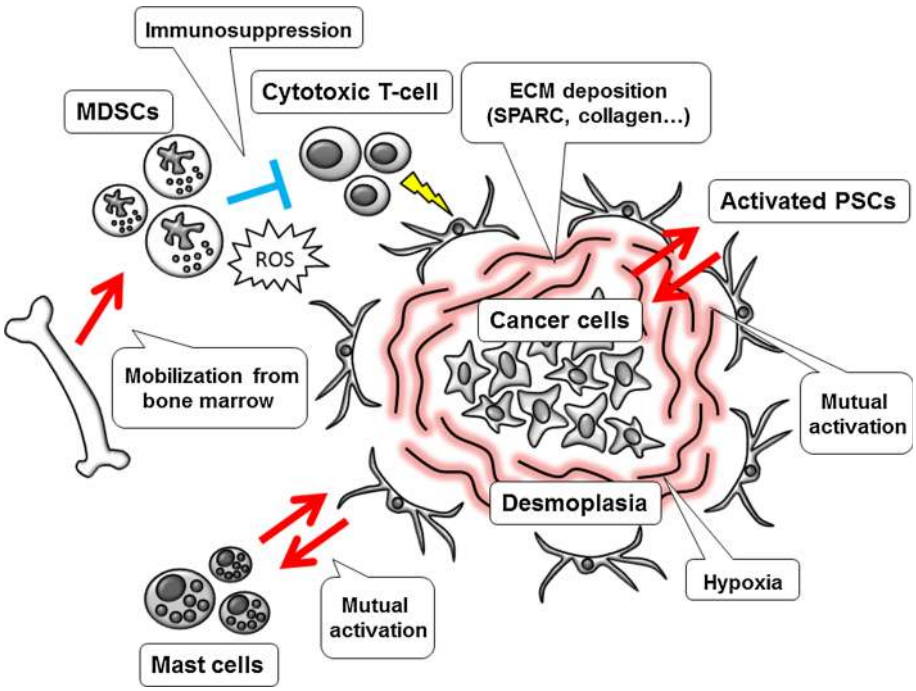

Fig. 1 A schematic view of the tissue structure of pancreatic cancer

revealed that the presence of higher numbers of mast cells in the intra-tumoral border zone was an independent prognostic factor for overall survival [21].

These characteristic tissue structures affect the tumor microenvironment in a manner conducive to the invasive growth of pancreatic cancer. Desmoplasia and poor vascularity hamper the blood supply, leading to severe hypoxia within the tumor. Hypoxia stabilizes the transcriptional factor hypoxia-inducible factor 1 alpha (HIF1α), which promotes the transcription of HIF1α target genes such as vascular endothelial growth factor (VEGF) or matrix metalloproteinase 3 (MMP3) [22, 23]. Adaptation to the hypoxic condition itself increases the malignant potential of pancreatic cancer cells. The up-regulation of VEGF and interleukin (IL)-6 enhanced the invasive growth of pancreatic cancer cells under the hypoxic condition [24]. Another report described that hypoxia induced a typical oncomiR, miR-21, in pancreatic cancer cells, which contributed to the sustained cell proliferation under hypoxia [25]. Furthermore, a recent report described that hypoxia regulated the susceptibility of cancer cells to lysis by cytotoxic T-cells, indicating its involvement in the modulation of immune function [26]. This effect was mediated by the induction of miR-210, one of the miRNAs induced by hypoxia [27]. Coordinated knockdown of miR-210 target genes *PTPN1*, *HOXA1*, and *TP53I11* recapitulated the blunted susceptibility to lysis by cytotoxic T-cells, clarifying the novel mechanism by which hypoxia yields an immunosuppressive effect in cancer cells. Interestingly, co-culture of PSCs could induce miR-210 expression in pancreatic cancer cells in an HIF1α-independent manner, suggesting an unknown tumor-stromal interaction [28]. These characteristic tissue structures and cellular components of pancreatic cancer are summarized in Fig. 1.

Inflammation and mouse model of pancreatic cancer

The role of chronic inflammation in pancreatic carcinogenesis was also confirmed by a mouse model of pancreatic cancer. Several key mutations were confirmed in pancreatic cancer such as a mutation of *Kras*, inactivating mutations of tumor suppressor *p16*, *p53* and *Smad4* [29], which were applied to establish a mouse model of pancreatic cancer. Pancreas-specific expression of constitutively active mutant *K-ras* (G12D) using *PDX-1* or *p48* promoter-driven Cre-loxP system resulted in the development of pre-neoplastic lesions, pancreatic intraepithelial neoplasias (PanINs) [30]. However, the development of invasive pancreatic cancer was rare in this mouse model, suggesting the requirement of an additional insult. Conditional knockout of *p16* or the expression of loss of function mutant *p53*, which were frequently observed mutations in high-grade PanINs [29], accelerated the progression to invasive pancreatic cancer [31, 32].

Interestingly, conditional expression of pancreatic cancer-promoting gene mutations in addition to the oncogenic *K-ras* recapitulates the formation of a stromal structure similar to that found in human pancreatic cancer. For example, the above-mentioned mouse model that expressed

K-ras (G12D) and *p53* (R172H) in the pancreas (KPC mouse) developed pancreatic cancer accompanied by a prominent stromal matrix and decreased blood vessels [33]. In another study, conditional expression of *K-ras* (G12D) and conditional knockdown of type II transforming growth factor-β (TGF-β) receptor impaired the growth-suppressive TGF-β signal in mouse pancreas. This mouse model also developed well-differentiated pancreatic cancer accompanied by a dense fibrotic stroma [34]. These observations indicate that cumulative gene mutations within cancer cells trigger the activation of stromal cells and inflammatory reactions, which leads to the characteristic tissue structure of pancreatic cancer. Several factors released from pancreatic cancer cells were proven to activate stromal cells, which will be described in detail in the following section.

In addition to these genetic changes in the pancreas, non-specific inflammation itself also accelerates the development of invasive pancreatic cancer. The pancreas-specific expression of constitutively active mutant *K-ras* (G12V) using an inducible Cre-loxP system enabled the expression of mutant *K-ras* in adult mice. The expression of mutant *K-ras* in adult pancreas failed to develop PanINs, but the addition of caerulein-induced chronic pancreatitis significantly promoted PanIN and invasive pancreatic cancer formation [35]. This study confirmed that inflammation in the adult pancreas has the potential to overwhelm the protective machinery against carcinogenesis. The following study further clarified the effect of cancer-promoting inflammation in this mouse model. It was found that the expression of constitutively active mutant *K-ras* (G12V) triggered cellular senescence, as evidenced by the β-galactosidase staining and p16Ink4a expression in PanIN lesions, referred to as oncogene-induced cellular senescence. The induction of chronic pancreatitis using caerulein inhibited the oncogene-induced cellular senescence in this mouse model, which identified the tumor-promoting downstream mechanism of inflammation. The withdrawal of caerulein led to the reversal of cellular senescence, indicating the importance of continuous inflammation. In addition, this attenuation of cellular senescence was also reversed by the administration of Sulindac, a nonsteroidal anti-inflammatory drug, suggesting anti-inflammatory treatment could have tumor suppressive potential [36]. The contribution of immune cells to pancreatic cancer progression was also confirmed by a mouse model. In the conditional *K-ras* (G12V) expression mouse model, mast cell infiltration was observed around the PanIN lesions. The growth of an ortho-topically implanted tumor was significantly retarded in mast cell-deficient $Kit^{w\text{-}sh/w\text{-}sh}$ mice. A rescue experiment using mast cells from wild-type bone marrow restored the tumor growth, which revealed the requirement of mast cells during the progression of pancreatic cancer [37]. These results demonstrated the mutual activation between cancer cells and stromal cells, forming a feed-forward loop that perpetuates the inflammation in pancreatic cancer. Thereafter, dissection of each signaling pathway and therapeutic intervention uncovered the intriguing cell-to-cell interactions between cellular components within the tumor that are indispensable for the disease progression.

Detailed mechanism of PSC activation in pancreatic cancer

Since the continuing inflammation plays a pivotal role during pancreatic carcinogenesis, the key signaling pathways that lead to stromal cell activation and pancreatic fibrosis were extensively studied. The detailed mechanism of pancreatic fibrosis was identified by studies on the pancreatitis-associated fibrosis. Among pancreatic stromal cells, PSCs attracted great attention due to their central role in pancreatic fibrosis, and multiple cytokines and growth factors have been reported to activate PSCs. For example, platelet-derived growth factor (PDGF), TGF-β, angiotensin II and tumor necrosis factor-α are well-known activators of PSCs [38–41]. These ligands activate downstream signaling pathways that promote cell proliferation, survival, migration and the ECM production of PSCs. Pancreatic cancer cells could be a source of these ligands, as confirmed by the neutralizing antibody-based inhibition of each ligand such as PDGF or TGF-β in a co-culture of PSCs with pancreatic cancer cells [42]. The activation of a wide variety of signaling pathways participates in the PSC activation, such as extracellular signal-regulated kinase (ERK), c-Jun NH2-terminal kinase (JNK), p38 mitogen-activated protein kinase (p38 MAPK), Janus kinase-signal transducers and activators of transcription (JAK-STAT) and phosphatidylinositol 3-kinase (PI3K) pathways [43–47]. Pharmaceutical inhibition of these pathways effectively attenuated PSC activation, but the off-target effects of each compound and redundant activation of PSCs hampered clinical application. Following these studies, increased ROS production within PSCs was found to play an important role during fibrogenesis. Activated PSCs express key components of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, which is one of the major ROS-generating enzymes [48, 49]. Inflammatory cytokines such as PDGF, angiotensin II or IL-1β increased ROS production within PSCs in a similar manner, resulting in the activation of MAPK pathways. Treatment with diphenylene iodonium (DPI) and apocynin, which are inhibitors of NADPH oxidase, effectively inhibited PSC activation [48].

After detailed examinations of pancreatitis-associated fibrogenic mechanisms, many studies focused on the

mechanism of pancreatic cancer-specific desmoplasia formation. Sonic hedgehog (Shh) is a secreted ligand that regulates the developmental process of digestive organs. The Shh is highly expressed in the endoderm of the gut tube during embryogenesis, while its expression is absent in pancreatic precursor cells [50]. A Shh-lacking pancreatic bud forms the pancreas and Shh expression is almost undetectable in adult pancreas. However, pancreatic cancer tissue and their precursor lesions express high levels of Shh compared with a normal pancreas [51]. A recent report described that Shh from orthotopically implantated pancreatic cancer cells induced desmoplasia formation in the pancreas of athymic nude mice [52]. In this study, Shh promoted the proliferation of PSCs and increased the expression of α-smooth muscle actin, Vimentin and desmin. Shh also facilitated the invasion of human myofibroblasts through matrigel, suggesting it has a substantial role as an activator of stromal cells.

Another ligand, connective tissue growth factor (CTGF), is also involved in the induction of desmoplasia. In pancreatic cancer, the expression of CTGF is increased compared with normal pancreas. CTGF altered the functions of other growth factors or integrins by directly binding to those molecules [53, 54]. CTGF interacted with α5β1 integrin promoting the activation of PSCs. This signal increased the cell adhesion and migration of PSCs, leading to PSC activation [55]. CTGF was supplied from activated PSCs themselves, which were activated by exogenous stimulants such as ethanol [56]. Another report described that pancreatic cancer cells could also become a source of CTGF in pancreatic cancer tissue [57]. This evidence suggests that CTGF plays an important role in the sustained activation of PSCs in pancreatic cancer.

In addition to the specific signaling pathways, tumor microenvironment could also activate PSCs. For example, a hypoxic condition affects the stromal cell functions. Hypoxia was reported to stimulate type I collagen production from PSCs, which were typical fibrogenic processes seen in activated PSCs. Hypoxia also promoted the migration of PSCs, which was partly mediated by VEGF secreted from the PSCs themselves [58]. Since increased fibrogenesis leads to the maintenance of desmoplasia, these mechanisms persistently maintain the hypoxic condition within the tumor. At the same time, solid stress by the desmoplasia resulted in increased intra-tumoral pressure due to the unregulated deposition of ECM and continuous proliferation of cancer cells and stromal cells [59]. Externally applied pressure also activated PSCs via the increased production of ROS within PSCs [60]. These PSC-activating signaling pathways and environmental factors are summarized in Fig. 2.

Increased metastatic potential by tumor-stromal interaction

The tumor-promoting role of stromal cells was experimentally confirmed in vitro by co-culturing PSCs with pancreatic cancer cells or conditioned medium derived from the culture supernatant of PSCs. Proliferation, cellular migration, invasion and soft-agar colony formation of human pancreatic cancer cell lines BxPC3 and Panc-1 were increased by the PSC-conditioned medium [61]. This study also confirmed that the co-injection of PSCs with BxPC3 into immuno-deficient mouse pancreas increased the size of ortho-topically implanted primary tumors. Since the co-

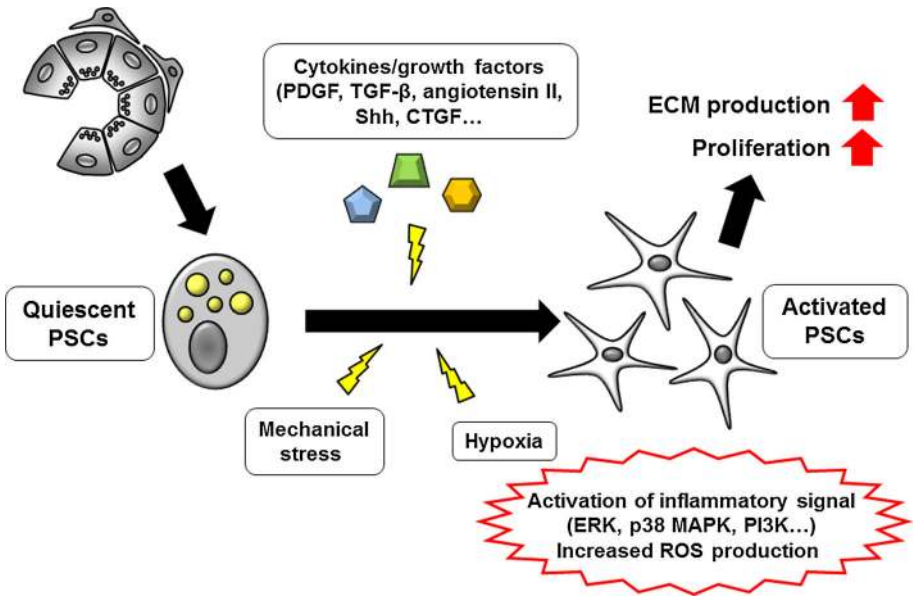

Fig. 2 Summary of PSC-activating stimuli and environmental factors

injection of PSCs also promoted metastatic invasion, the interaction between pancreatic cancer cells and PSCs was assumed to promote metastasis. Another group reported similar results using an alternative human pancreatic cancer cell line, MiaPaCa-2, the orthotopic injection of which in mouse pancreas with PSCs increased the tumor size and distant metastasis [62]. In this report, PSC-conditioned medium inhibited cancer cell apoptosis in addition to increasing proliferation and cellular migration. These results suggested that PSCs affect the biological behavior of pancreatic cancer cells by secreting soluble factors. The activation of cell growth or of a survival-enhancing signal, such as ERK or Akt pathway, was detected in the pancreatic cancer cells treated by PSC-conditioned medium in a previous study [61]. While PSCs promote the invasive growth of cancer cells, they inhibit normal cell function at the same time. Co-culture of the RIN-5F pancreatic beta-cell line with PSCs induced apoptosis, suggesting a cell-context specific effect of PSCs [63].

After these studies, the detailed mechanisms how PSCs exacerbate the malignant phenotype of pancreatic cancer cells were examined. When the orthotopically implanted pancreatic cancer cells and PSCs co-injected tumors in immunodeficient mouse developed metastatic nodules in the liver, diaphragm, lung and mesentery, PSCs were also detected in those metastatic nodules with cancer cells [64]. This finding suggested that PSCs were capable of enhancing the colonization and survival of cancer cells in the metastasized organ. The cancer cell-stimulated PSCs were also able to migrate through the endothelium, which meant PSCs extravasate from blood vessels as well as cancer cells. Furthermore, a specific population of PSCs was found to facilitate exclusively the invasive growth of pancreatic cancer cells. In pancreatic cancer patients, the expression of stromal CD10 expression was correlated with shorter patient survival. Since the expression of CD10 was observed in the areas showing strong α-SMA expression, this subpopulation of PSCs was thought to express CD10. Isolated $CD10^+$ PSCs revealed a higher capacity to promote pancreatic cancer cell invasion compared with $CD10^-$ PSCs. This functional difference between $CD10^+$ and $CD10^-$ PSCs was attributed to the increased production of MMP3 in $CD10^+$ PSCs, which was confirmed by the attenuation of the invasion-promoting ability in MMP3-knockdown PSCs [65].

Interaction between pancreatic cancer cells and PSCs also affects the cancer cell phenotypes. Among various phenotypic changes, epithelial-mesenchymal transition (EMT) was shown to play an important role during the establishment of distant metastasis or invasion toward surrounding organs by mobilizing cancer cells from the site of origin [66]. Loss of cell polarity, cell-to-cell adhesion, decreased expression of epithelial markers and increased expression of mesenchymal markers are typical characteristics of cancer cells undergoing EMT. A wide variety of growth factors and cytokines such as TGF-β, bone morphogenetic protein and VEGF induced EMT [67–69]. A recent report described that indirect co-culture with PSCs and PSC-conditioned medium caused EMT-compatible phenotypic changes in pancreatic cancer cells. The human pancreatic cancer cell lines Panc-1 and SUIT-2 indirectly co-cultured with PSCs showed decreased cell-to-cell adhesion and a scattered appearance. Along with these changes, the epithelial markers E-cadherin and cytokeratin 19 were decreased, whereas the mesenchymal marker Vimentin and EMT-inducing transcriptional factor Snail were increased [70]. Interestingly, inhibition of the typical EMT inducer TGF-β [67] by adding neutralizing antibody to the PSC-conditioned medium failed to attenuate the EMT-inducing effect, suggesting an unknown EMT-inducing mechanism in the tumor-stromal interaction. The effects of the metabolic status or mechanical stress in the tumor microenvironment, and direct cell-to-cell interaction between cancer cells and stromal cells also remain to be clarified so far in vivo.

Cancer stem cell-related phenotypes and tumor-stromal interaction

In normal organs, tissue stem cells supply the proper cellular components by strictly regulated mechanisms. Cancer stem cells (CSCs) are the counterpart of normal tissue stem cells, which give rise to the cancer cells at various degrees of differentiation and reconstruct entire populations of cancer cells [71]. CSCs are capable of self-renewing and are resistant to conventional chemotherapy and radiation, leading to the re-growth of therapy-resistant tumors and recurrence after surgery. CSCs play an indispensable role in pancreatic cancer progression and therapy-resistance [72–74]. Several CSC-containing cell fractions were identified in pancreatic cancer, based on cell surface markers such as $CD44^+CD24^+ESA^+$ or $CD133^+$ [75, 76]. However, the regulatory factors of the CSC-related phenotypes have not been clarified. The EMT phenotype is also one of the CSC-related phenotypes, recognized as migrating CSCs that establish distant metastasis and cause postoperative recurrence [77]. Based on these findings, the induction of EMT-compatible phenotypes by co-culture with PSCs [70] and the co-localization of PSCs with cancer cells within the metastatic foci [64] suggested the possible contribution of tumor-stromal interaction to the maintenance of CSC-related phenotypes.

As reported previously, co-injection of PSCs with pancreatic cancer cells into immunodeficient mice accelerated tumor growth [61, 62]. Since increased tumorigenicity is

another trait of CSCs, detailed analysis of the pancreatic cancer cell phenotypes under the influence of PSCs was carried out. Indirect co-culture of pancreatic cancer cell lines Panc-1 and AsPC-1 with PSCs enhanced the spheroid formation in low-adhesion coated plates, also a feature of CSCs. Along with this change, the expression of CSC-related genes was up-regulated in pancreatic cancer cells co-cultured with PSCs [78]. The up-regulated genes included *ABCG2*, *Nestin* and *LIN28*. *ABCG2* is a member of the ATP-binding cassette transporter, whose expression was elevated in side population cells enriched with CSCs [79]. *Nestin* is an intermediate filament expressed in pancreatic progenitor cells that increased pancreatic cancer cell invasion and metastasis [80]. *LIN28* is an RNA-interacting protein that plays a crucial role in the maintenance of embryonic stem cell functions [81]. These observations indicate the pivotal role of PSCs in maintaining CSC-related phenotypes and forming a CSC niche. Indeed, another study reported that PSCs promote the self-renewal of CSCs, by creating a paracrine niche for pancreatic cancer cells. Nodal-expressing PSCs promoted this effect, which was mediated by paracrine Nodal/Activin signaling [82].

The tumor microenvironment also affects the CSC-related phenotypes. The hypoxia resultant from the desmoplasia could increase the expression of the putative CSC marker CD133 in pancreatic cancer cells. Hypoxia also increased the expression of CXC chemokine receptor 4, which was highly expressed in an invasive subpopulation of CSCs in a previous study [76]. Other stem cell markers, such as EZH2, Oct4 and Nanog were induced by the hypoxic condition in pancreatic cancer cells [24]. In addition to the induction of these CSC-related genes, resistance to radiation was supported by hypoxia. Hypoxia facilitates glycolysis in cancer cells, leading to the activation of the pentose phosphate pathway, which increases the antioxidant capacity [83]. Inhibition of the HIF1α function by the specific inhibitor PX-478 increased pancreatic cancer cell killing by radiation, suggesting some contribution of hypoxia to the radioprotection [84]. Since resistance against radiation is another CSC-related phenotype, desmoplasia-derived hypoxia contributes to the maintenance of CSCs as a part of the niche for CSCs. Together with the increased metastatic ability, these CSC-related phenotypes supported by tumor-stromal interaction promote disease progression such as the dissemination of metastatic foci and selection of a refractory population against therapeutic intervention. The effects of tumor-stromal interactions on the metastatic capacity of pancreatic cancer cells and CSC-related functions are summarized in Fig. 3.

Modulation of the immune system in pancreatic cancer

According to a recent report, establishment of the primary pancreatic cancer requires approximately 10 years of cumulative gene mutations, and 5 more years are needed to develop metastatic disease [85]. In other words, cancer

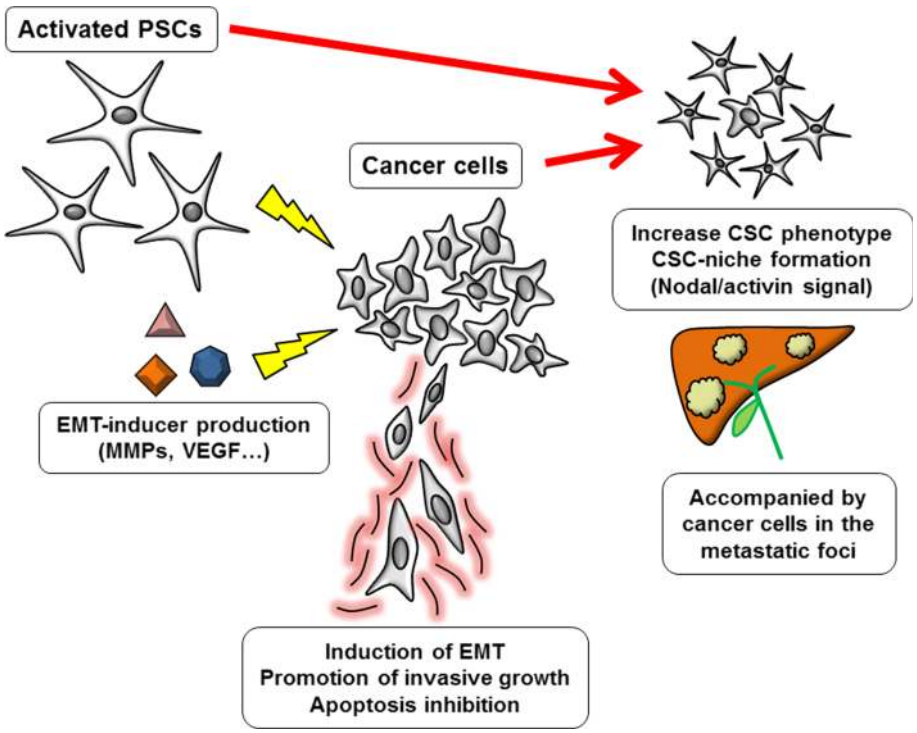

Fig. 3 EMT induction and CSC-related phenotype maintenance mechanisms by PSCs

cells have escaped from the host immune surveillance for the same period. Wide varieties of immunosuppressive changes contribute to this process, including attenuation of the immune reaction against cancer cells and the induction of immunosuppressive cells around the pancreatic cancer tissue. The analysis of a mouse model that conditionally expresses *K-ras* (G12D) in the pancreas revealed that even at low-grade PanIN, $CD45^+$ leukocyte infiltration was observed, which gradually increased along with the progression of PanIN. In contrast, the effector T-cell response was lost within the pancreas, suggesting immunosuppression. Infiltrating leukocytes expressed Gr-1 and CD11b, showing the characteristics of MDSCs [86]. These results indicated that pancreatic carcinogenesis mobilizes bone marrow-derived cells from the early stage of disease to establish an immunosuppressive microenvironment. The immunosuppressive status could be reflected by systemic changes. MDSCs were detectable in peripheral blood samples, and their amount correlated with the prognosis of patients. In cancer-bearing patients, the MDSC level in blood samples was significantly elevated, and was also an independent prognostic factor for survival [87].

PSCs also affect the immune cell functions during pancreatic carcinogenesis. The differentiation of myeloid-derived cells to MDSCs was promoted by pancreatic cancer-associated PSCs via the activation of the STAT3 pathway [88]. This study clarified the contribution of cytokines derived from PSCs such as IL-6, VEGF and macrophage colony-stimulating factor (M-CSF) to the induction of MDSCs. The induction of MDSCs by PSC-derived cytokines and chemokines offers survival advantages to pancreatic cancer cells, enabling escape from immune surveillance. In addition to the induction of MDSCs, activated PSCs sequestered cytotoxic T-cells and reduced their interaction with cancer cells. Immunohistochemical analysis of pancreatic cancer tissue revealed that the density of the $CD8^+$ T-cell infiltrate in pancreatic cancer tissue was significantly reduced compared with ampullary carcinoma and cholangiocarcinoma. An increased $CD8^+$ T-cell infiltrate around cancer cells was correlated with improved survival after surgery, suggesting this immune reaction against cancer cells directly affects the clinical outcome [89]. This study clarified the detailed mechanism of immunosuppression by PSCs using a KPC mouse model. As described previously, the pancreatic cancer tissue of KPC mice was accompanied by desmoplasia, which could be reversed by the administration of all-trans retinoic acid (ATRA) [90]. The treatment with ATRA enhanced the $CD8^+$ T-cell infiltrate around the cancer cells, suggesting activated PSCs sequester CD8+ T-cells preventing access to the cancer cells. Finally, T-cell migration toward activated PSCs was found to be mediated by CXCL12 from PSCs [89].

Interaction between PSCs and another type of immune cell also contributes to the pancreatic cancer progression. Analysis of a pancreatic cancer model mouse revealed the contribution of mast cells during pancreatic carcinogenesis, and detailed mechanisms of their activation were examined thereafter. Co-culture of mast cells with PSCs led to mast cell activation, characterized by tryptase and tumor necrosis factor-α release in the culture supernatant [91]. In turn, mast cell-derived IL-13 and tryptase promoted the proliferation of PSCs, leading to further fibrogenesis. Interestingly, a previous report described that IL-13 negatively regulated immune surveillance against cancer cells by inducing MDSCs [92, 93]. These findings were connected by recent research, which described the mast cell's novel role in enhancing the immunosuppressive functions of MDSCs [94]. Taken together, complex interactions between cancer cells, PSCs and immune cells produce an immunosuppressive microenvironment that inhibits the elimination of cancer cells by the host immune reaction. A schematic view of the interaction between cancer cells, PSCs and immune cells is shown in Fig. 4.

Therapy-resistance and stromal cells

The prognosis of pancreatic cancer remains dismal, despite improvements in imaging studies and therapeutic strategies. According to the Japan Pancreatic Cancer Registry, the 5-year survival of pancreatic cancer patients doubled over the past 30 years, but is still less than 20 % [95]. This clinical manifestation of pancreatic cancer is largely due to the low possibility for curative surgical resection ($\sim$20 %) and resistance to alternative therapies such as chemotherapy or radiation for unresectable disease [96]. As summarized in this review, stromal cells and host immune cells establish a formidable fortress protecting the pancreatic cancer cells after a long period of complex interactions. Since conventional therapy such as gemcitabine treatment itself could be a selection pressure for a therapy-resistant new population of cancer cells [97], novel strategies that overwhelm these machineries are urgently required. Therapy-resistant evolution is a relatively rare phenomenon in normal cells such as PSCs or immune cells due to the intact genomic regulation and absence of oncogenic mutations. Therefore, targeting tumor-promoting stromal cells or the immune cell function is being considered as an alternative therapeutic target in pancreatic cancer.

For the attenuation of desmoplasia, PSC-targeting interventions have been extensively studied. Strategies to inhibit the functions of PSCs are generally divided into two approaches of specific inhibition of PSC-activating pathways and broad inhibition of PSC functions. The first approach became available by the identification of PSC-

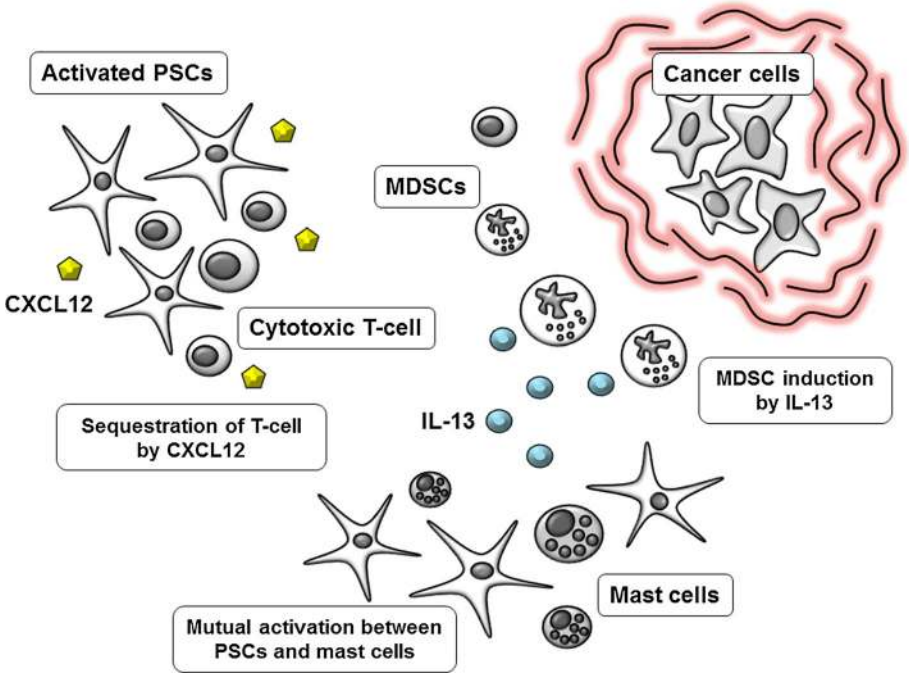

Fig. 4 Establishment of the immunosuppressive microenvironment by PSCs, MDSCs and mast cells

activating pathways such as the Shh pathway or CTGF pathway [52, 55]. The Shh inhibition was achieved by the discovery of small molecules such as cyclopamine that modulated the downstream signal mediators of the Shh pathway [98]. A desmoplasia-recapitulating mouse model of pancreatic cancer, the KPC mouse, develops gemcitabine-resistant pancreatic cancer [33]. The pancreatic tumors continued to grow despite gemcitabine administration. Isolated cancer cells from KPC mice tumors were sensitive to gemicitabine indicating the contribution of desmoplasia to this resistance. The administration of IPI-926, an oral inhibitor of the Shh pathway, reduced the desmoplasia in the tumors of KPC mice, and transiently increased the vascularity of the pancreatic cancer tissue [33]. Combination therapy of IPI-926 and gemcitabine prolonged the survival of KPC mice, suggesting the possibility of tumor stroma-targeting therapy in addition to the conventional chemotherapies. Similarly, conditional expression of mutant *K-ras* (G12D) with TGF-β receptor type II knockout in pancreas also led to desmoplasia-containing pancreatic cancer development, in which elevated CTGF expression was detected [99]. This CTGF induction was mediated by the Cxc chemokine signal, and inhibition by the Cxc receptor inhibitor SB225002 repressed CTGF expression in mouse tumors and prolonged the survival. Another study used a monoclonal antibody against CTGF (FG-3019) in combination with gemcitabine in KPC mice. The administration of FG-3019 improved the response to the gemcitabine, accompanied by the decreased expression of an X-linked inhibitor of apoptosis [100]. In this study, combination therapy of cytidine deaminase inhibitor and gemcitabine, which increased the intra-tumoral gemcitabine concentration without affecting the tumor microenvironment, failed to show a beneficial effect. Based on these results, gemcitabine resistance due to desmoplasia might be mediated by the tumor microenvironment.

The second approach, broad inhibition of the PSC functions, has also been studied in detail. A wide variety of agents have been identified that inhibit ECM production or the proliferation of PSCs, such as plant-derived polyphenol (green tea polyphenol, ellagic acid and curcumin) [101–103], NADPH oxidase inhibitor [48] and angiotensin II type 1 receptor blocker (ARB) [104]. Among these agents, ARB has been used clinically as an antihypertensive drug with acceptable feasibility and safety. The administration of candesartan, one of the ARBs, decreased pancreatic inflammation and fibrosis in Wistar Bonn/Kobori rats, a model rat of chronic pancreatitis [105]. A retrospective study revealed that patients with pancreatic cancer who received angiotensin I-converting enzyme inhibitors and ARBs showed better prognoses [106], suggesting that inhibition of the PSC function by ARBs might yield therapeutic benefits. This hypothesis was assessed by the administration of olmesartan to subcutaneous-tumor bearing immunodeficient mice, the tumors derived from the co-injection of the human pancreatic cancer cell line AsPC-1 and PSCs. Olmesartan significantly retarded the growth of the subcutaneous tumors, accompanied by decreases in the expression of α-SMA and collagen deposition in the tumors [107]. Delayed administration of olmesartan also had a growth suppressive effect on the tumors, suggesting that PSCs were required for the maintenance of tumor growth, as well as for tumor implantation.

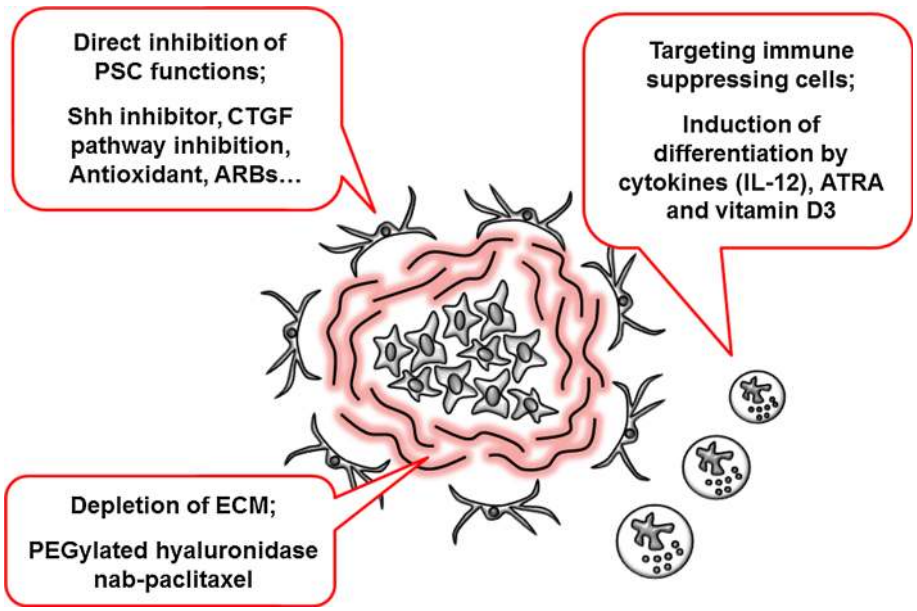

Fig. 5 Summary of therapeutic strategies targeting tumor-stromal interaction

The extracellular component of pancreatic cancer tissue or immune cells could be an additional therapeutic target. A recent report suggested that an abundant stromal matrix component, hyaluronan, accumulates in pancreatic cancer tissue [108]. The enzymatic degradation of hyaluronan by PEGylated human recombinant PH20 hyaluronidase (PEGPH20) improved the vascular patency and chemotherapeutic delivery in tumors of KPC mice. The combination of PEGPH20 with gemcitabine extended the survival of the tumor-bearing mice by enhancing the effect of gemcitabine. Excess ECM components such as hyaluronan in desmoplasia increase the interstitial fluid pressure, leading to a compromised vascular function in the pancreatic tissue. Combined treatment of KPC mice tumors with gemcitabine and PEGPH20 demonstrated decreased expression of α-SMA, that is, tissue remodeling [109]. In addition, a recently approved chemotherapeutic agent, nab-paclitaxel, revealed a tumor-stroma depleting effect that led to an increased intra-tumoral gemcitabine concentration, improving the antitumor activity [110, 111]. These studies indicate that depletion of the ECM component could be an attractive therapeutic target. Tumor-infiltrating immune cells were also targeted by direct elimination or by inducing differentiation to attenuate immunosuppressive functions. Injection of effector T-cells targeting $CD11b^{+}Gr1^{+}$ MDSCs in tumor-bearing mice efficiently inhibited tumor growth, suggesting the host immune reaction could suppress tumor growth in the absence of MDSCs [112]. Another study reported that IL-12 induced the differentiation of MDSCs and decreased nitric oxide synthase expression [113]. As inducers of MDSC differentiation, ATRA and vitamin D3 were also clinically tested in metastatic renal cell carcinoma and head and neck squamous cell carcinoma patients [114, 115]. The therapeutic strategies targeting tumor-stromal interaction in pancreatic cancer are summarized in Fig. 5.

Closing remarks

The tumor-stromal interaction plays a pivotal role during the progression of pancreatic cancer, which, by the time it has become apparent, has probably existed more than a decade. Targeting tumor-stromal interaction by inhibiting PSC functions, depleting ECM components and restoring the host immune reaction seems promising, but several problems still exist that must be addressed before the establishment of radical therapy. For example, a clinical trial using IPI-926 in combination with gemcitabine has been halted due to the increased mortality compared with the control group, suggesting simple inhibition of the desmoplasia might be insufficient to cure pancreatic cancer. Further study is necessary to improve the clinical outcomes of pancreatic cancer patients, by clarifying the complex interactions between pancreatic cancer cells and the stromal components.

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Conflict of interest The authors declare that they have no conflict of interest.

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