REVIEW ARTICLE

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Role of the tumor microenvironment in pancreatic cancer

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Abstract

Pancreatic cancer remains a highly recalcitrant disease despite the development of systemic chemotherapies. New treatment options are thus urgently required. Dense stromal formation, so-called "desmoplastic stroma," plays controversial roles in terms of pancreatic cancer growth, invasion, and metastasis. Cells such as cancer-associated fibroblasts, endothelial cells, and immune cells comprise the tumor microenvironment of pancreatic cancer. Pancreatic cancer is considered an immune-quiescent disease, but activation of immunological response in pancreatic cancer may contribute to favorable outcomes. Herein, we review the role of the tumor microenvironment in pancreatic cancer, with a focus on immunological aspects.

KEYWORDS
immune cell, immunomodulation, pancreatic cancer, tumor-infiltrating lymphocyte, tumor microenvironment

1 | INTRODUCTION

Patients with pancreatic cancer show a dismal prognosis, with 5-year overall survival rates of 7%-8% in both Japan and the USA.[1] Pancreatic-cancer death is estimated to become the second most common cause of cancer death by 2030 in the USA.[2] A lack of symptoms or biomarkers in the early stages of the cancer, an aggressive biological feature in that cancer cells metastasize to distant lesions even from small tumors, and drug resistance as a result of dense fibrous stroma all contribute to poor treatment outcomes of pancreatic cancer. Furthermore, pancreatic cancer is known to create an immune-suppressive microenvironment that results in immune evasion from the host antitumor immune system, leading to rapid cancer progression. Recent studies have shown that the tumor microenvironment of pancreatic cancer, including cancer-associated fibroblasts such as stellate cells, extracellular matrix, various kinds of immune cells, and cytokines released by these cells, participates in controlling tumor growth, invasion, and metastasis by means of close interactions with cancer cells. Hence, preclinical and clinical studies have focused on the tumor microenvironment as a potential novel target that may lead to cure for pancreatic cancer.

The present report reviews the pancreatic tumor microenvironment from the perspective of each stromal component, particularly immune cells. A potential breakthrough therapy targeting the microenvironment is discussed. Figure 1 shows a graphical abstract.

2 | ROLE OF DESMOPLASTIC STROMA IN THE DEVELOPMENT OF PANCREATIC CANCER

Desmoplastic stroma consists of stromal cells and extracellular matrix. The fibroblastic population may comprise 90% of the whole tumor mass of pancreatic cancer.[3] Wu et al[4] reported that the extent of stroma can offer a prognostic factor for patients with solid cancers.

The extracellular matrix consists of a variety of materials such as collagen, integrin, laminin, fibronectin, glycosaminoglycan,
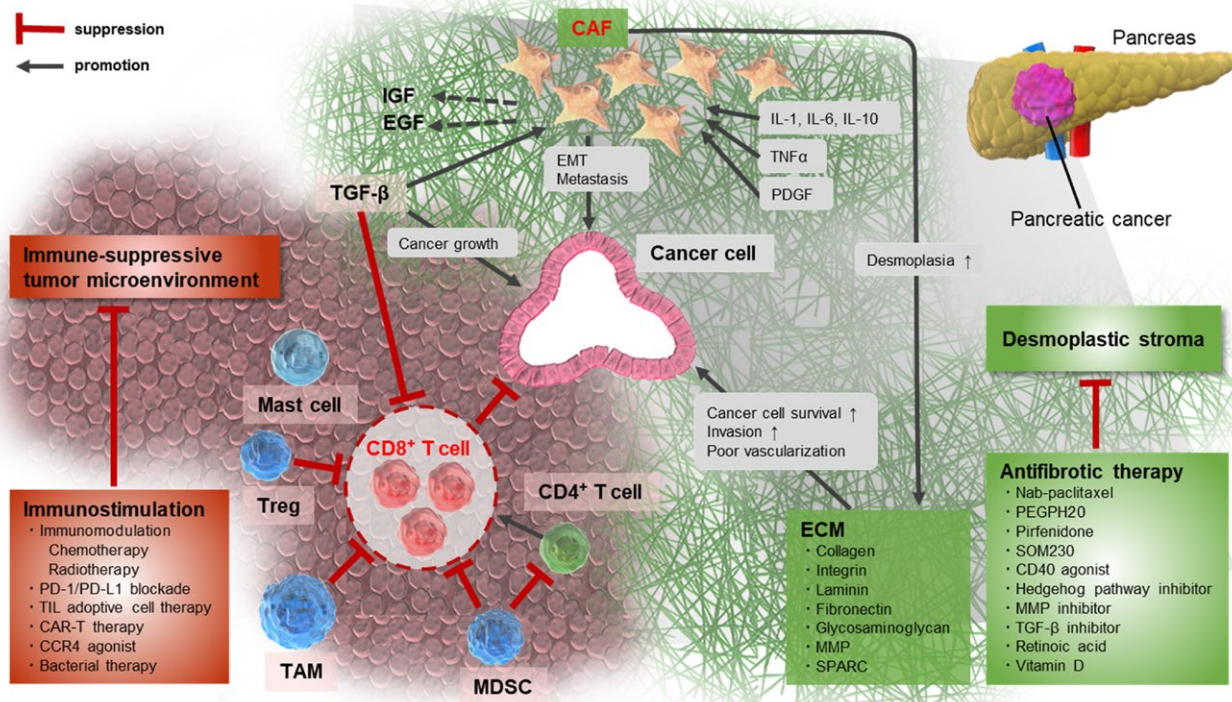
FIGURE 1 Schematic of the tumor microenvironment in pancreatic cancer. The tumor microenvironment in pancreatic cancer contributes to tumor progression in a multifaceted way. Cancer-associated fibroblasts (CAFs) and the extracellular matrix (ECM) comprise the desmoplastic stroma and enhance cancer growth, invasion, and metastasis in direct or indirect ways. In contrast, immune-suppressor cells such as regulatory T cells (Treg), myeloid-derived suppressor cells (MDSC), and tumor-associated macrophages (TAM) inhibit CD8^{+} T cells, which play a key role in the antitumor immune response, by establishing an immunosuppressive tumor microenvironment. Cytokines secreted by CAFs, immune cells, or other components mediate these processes. Antifibrotic therapy, immunotherapy, induction of immunomodulation, and bacterial therapy may improve the unfavorable tumor microenvironment associated with pancreatic cancer. CAR-T, chimeric antigen receptor T cell; CCR4, chemokine receptor type 4; EGF, epidermal growth factor; EMT, epithelial mesenchymal transition; IGF, insulin-like growth factor; IL, interleukin; MMP, matrix metalloproteinase; PD-1, programmed cell death protein 1; PDGF, platelet-derived growth factor; PD-L1, programmed cell death ligand 1; PEGPH20, pegvorhyaluronidase alfa; SPARC, secreted protein acidic and rich in cysteine; TGF-β, transforming growth factor β; TIL, tumor-infiltrating lymphocyte; TNF-α, tumor necrosis factor α

matrix metalloproteinase (MMP), and secreted protein acidic and rich in cysteine (SPARC).[5] Under normal circumstances, the extracellular matrix conserves cellular polarity, proliferation, and migration while inhibiting dysplasia.[6] In contrast, dysregulated integrin subunits, seen in the basement membrane in pancreatic cancer tissue, contribute to cancer-cell survival and invasiveness.[7,8] Hyaluronan, a glycosaminoglycan, is deposited in high concentration in the extracellular matrix of pancreatic cancer.[9] Once hyaluronan binds to its receptor, CD44, subsequent interactions prolong cancer-cell survival and promote cancer cell growth.

Stromal cells in pancreatic cancer comprise cancer-associated fibroblasts (CAFs), endothelial cells, and immune cells. Pancreatic stellate cells are a subset of CAFs.[10] CAFs are a major component of pancreatic cancer stroma, derived from different kinds of progenitor cells such as fibroblasts, pancreatic stellate cells, and epithelial, endothelial, and mesenchymal stem cells.[11,12] CAFs express α-smooth muscle actin (α-SMA), a well-known and reliable marker of CAF, stromal cell-derived factor-1α, fibroblast activation protein, and fibroblast specific protein-1.[3,11] CAFs are activated by transforming growth factor β (TGF-β), sonic hedgehog, tumor necrosis factor α (TNF-α), platelet-derived growth factor (PDGF), and interleukin (IL)-1, -6, and -10.[11,13] TGF-β regulates tumor growth, differentiation, and immune cell function.[14] TGF-β initially plays a tumor-suppressive role, but enhances tumor growth as cancer progresses.[6,15] TGF-β1 enhances the ability of CAFs to form abundant filopodia, which allows CAFs to migrate into cancer cell nests.[13] CAFs are stimulated by several types of mediators such as C-C motif chemokine ligand 2 (CCL2), hepatocyte growth factor (HGF), and fibroblast growth factor (FGF).[11] However, some growth factors including insulin-like growth factor (IGF), epidermal growth factor (EGF), and TGF-β are derived from CAFs.[6,14] CAFs induce desmoplasia through the secretion of collagen types I and III, fibronectin, proteoglycans, and glycosaminoglycans, leading to increased mechanical pressure in the extracellular matrix, which may promote cancer-cell migration and inhibit vascularization.[3] CAFs provide cancer cells with nourishment under low-glucose conditions.[11] CAFs also contribute to epithelial-to-mesenchymal transition (EMT), cancer invasion, angiogenesis, and metastasis.[16,17]

3 | ROLE OF IMMUNE CELLS IN THE TUMOR MICROENVIRONMENT OF PANCREATIC CANCER

3.1 | Immune-suppressive tumor microenvironment in pancreatic cancer

Pancreatic cancer is thought to be immune-quiescent, as a variety of immune-suppressive mechanisms can inhibit antitumor immunity.[18] Low expression of major histocompatibility complex (MHC) class I molecules on pancreatic cancer cells inhibits T-cell activation.[19] CD8^{+} T cells are activated by interaction with antigen presented by MHC class I molecules on antigen-presenting cells. Cytotoxic T cells, representing activated CD8^{+} T cells, attack cancer cells by secreting perforin and granzyme and expressing Fas ligand. CD8^{+} T cells also express immune checkpoint molecules, which restrain T-cell functions, inducing immune exhaustion. CD8^{+} T cells play a central role in eliciting antitumor immune responses, but their function in the tumor microenvironment is impaired as a result of several immune-suppressing mechanisms. Neoantigens show high immunogenicity arising from genetic mutations present in cancer cells.[20] These oligopeptides of eight to 12 amino acids are presented on MHC class I receptors. Neoantigens are expressed by most pancreatic cancers, but effective immune responses fail to be generated, probably due to the immunosuppressive tumor microenvironment.[21]

Transforming growth factor β excreted by pancreatic cancer cells or extracellular matrix also restrains immune cell function.[22,23] Cancer cell-derived indoleamine 2,3-dioxygenase, a tryptophan-metabolizing enzyme, results in effector T cells becoming deficient in tryptophan, inducing immunological tolerance.[24] In a mouse model, major immune-suppressor cell lines including regulatory T cells (Tregs), myeloid-derived suppressor cells (MDSCs), and tumor-associated macrophages (TAMs) have been seen in pancreatic tissue even in the early stages of carcinogenesis.[25] Tregs were identified as CD4^{+} CD25^{+} immune-suppressive cells in 1995.[26] The transcriptional factor Foxp3 was shown to be a master regulator of Treg function in 2003.[27] Tregs comprise 5%-10% of peripheral CD4^{+} T cells in healthy hosts, whereas higher concentrations of Tregs were reported in patients with cancers, including pancreatic cancer.[28,29] Tregs maintain immune cell homeostasis by controlling self-reactive T cells. The immune-suppressing mechanisms induced by Tregs are as follows: suppression of effector T cells by secreting immune-suppressive cytokines such as TGF-β or competing for IL-2; induction of effector T-cell apoptosis by cytotoxic enzymes such as granzyme B; and inhibition of dendritic cell maturation and function.[30] In pancreatic cancer tissue, abundant Tregs are present.[6] Cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), which is constantly expressed on Tregs, plays a central role in suppressing antigen-presenting cells. MDSCs induce immune evasion by inhibiting both innate and adaptive antitumor immunity in pancreatic cancer.[21] Pancreatic-cancer patients with higher levels of circulating MDSCs correlated with poorer overall survival.[31] TAMs are macrophages that comprise a major component of immune cells in the tumor microenvironment. TAMs contribute to immune suppression as well as promoting angiogenesis. Cytokines such as CC chemokine, a protein belonging to the CXC chemokine family called stromal-derived factor 1, and vascular endothelial growth factor attract TAMs into the tumor microenvironment.[32] TAMs support cancer progression by secreting a variety of growth factors.[33] IL-10 secreted from TAMs contributes to establishment of the immunosuppressive tumor microenvironment by preventing dendritic cell-mediated antitumor immune responses.[34] Peranzoni et al[35] reported that macrophages inhibit CD8^{+} tumor-infiltrating lymphocytes (TIL).

3.2 | Tumor-infiltrating immune cells in pancreatic cancer

Cancer cells are surveyed by the host immune system, which eliminates cancer in the first phase. Cancer and immunity are then balanced in the next phase. In the last immune-evasion phase, cancer appears in the human body. This theory of immunoediting was developed only recently.[36] TILs are considered to reflect immunoediting.[37] TILs are observed in several tumor types, including colorectal cancer, gastric cancer, hepatocellular carcinoma, bile duct cancer, and pancreatic cancer, which are reported to have prognostic value.[38] Regarding pancreatic cancer, Fukunaga et al[39] first reported that both CD4^{+} and CD8^{+} TILs are associated with longer postoperative survival. Hwang et al[40] showed that the Foxp3^{+}/granzyme B^{+} ratio correlated with both disease-free and overall survival for patients with left-sided pancreatic cancer. Furthermore, recent studies have shown that mast cells also affect tumor growth by enhancing angiogenesis, cancer-cell proliferation and invasion.[41] Mast-cell infiltration of tumors predicts poor long-term outcomes for colorectal cancer, hepatocellular carcinoma, colorectal cancer liver metastasis, and pancreatic cancer patients.[42–44] Kato et al[45] showed that semaphorin-4D (Sema4D), a membrane-bound or secreted protein involved in the regulation of antitumor immunity, was demonstrated in TILs in pancreatic cancer tissue.

3.3 | Immunomodulation induced by drugs or X-ray in pancreatic cancer

Favorable effects might be achieved by removing immunosuppressive mechanisms in pancreatic cancer. Recent studies showed that certain types of chemo- and radiotherapy contribute to the activation of antitumor immune response. Cancer cells treated with cyclophosphamide, doxorubicin, oxaliplatin, or mitoxantrone are reported to undergo immunogenic cell death. In addition, 5-fluorouracil- or gemcitabine-treated cancer cells also become immunogenic.[46–48] Radiotherapy also induces immunogenic cell death. The "abscopal effect", a phenomenon by which metastatic lesions outside the irradiation field show reductions in size, suggests some form of underlying immunological response.[49] Homma et al[50] reported that neoadjuvant chemoradiotherapy (NACRT) consisting

of gemcitabine plus S-1 followed by 30 Gy radiation for pancreatic cancer enhances both CD4^{+} and CD8^{+} TILs. Furthermore, abundant CD8^{+} TILs or scarce Foxp3^{+} TILs after NACRT are associated with favorable long-term outcomes.[50,51] MHC class I chain-related gene A and gene B (MICA/B), a ligand of transmembrane protein, natural killer group 2 member D, is able to activate CD8^{+} T cells and γδT cells, as well as NK cells. Murakami et al showed that damage-associated molecular patterns (DAMP) such as MICA/B, calreticulin, and heat-shock protein 70 (Hsp70) were overexpressed after NACRT, and MICA/B was associated with favorable TIL status, suggesting MICA/B as an important regulator of immunomodulation.[51] Moreover, proteomic analysis of pancreatic cancer treated with NACRT showed that marginal-zone B- and B1-cell-specific protein (MZB1) expression was associated with abundant CD8^{+} TIL and longer survival.[52] These results indicate that immunogenic cell death induced by chemoradiation plays a pivotal role in pancreatic cancer.

4 | CANCER STROMA-TARGETING THERAPY FOR PANCREATIC CANCER

Depletion of the desmoplastic stroma has led to better chemotherapy delivery and drug response in preclinical models of pancreatic cancer.[53–55] Antifibrotic therapy therefore appears to represent a promising strategy in the treatment of pancreatic cancer.

Therapeutic strategies to target CAFs in pancreatic cancer include treatments that reduce the abundance of stroma in pancreatic cancer, such as nab-paclitaxel, pegvorhyaluronidase alfa (PEGPH20), pirfenidone, SOM230 and CD40 agonists,[53,56–59] and that reduce CAFs proliferation, including hedgehog pathway inhibitors, multi-MMP inhibitors, TGF-β inhibitor, retinoic acid[53,60–62] or vitamin D receptor activation to reprogram CAFs to a quiescent phenotype.[63]

Chemotherapy combining nab-paclitaxel with gemcitabine has recently become the standard regimen for patients with metastatic pancreatic cancer, significantly prolonging overall survival in the MPACT trial, which was an international, multicenter, open-label, randomized phase III study.[64] Exploratory analysis was carried out to gain insight into the role of SPARC expression as a predictor of survival, because nab-paclitaxel was reported to decrease CAFs and increase microvessel density, attributed to increased drug concentration in tumors treated by nab-paclitaxel in preclinical models.[65–67] However, stromal and tumor levels of SPARC as measured by immunohistochemistry showed no correlation with overall survival.[68]

Metastatic pancreatic ductal adenocarcinoma (PDAC) is characterized by excessive accumulation of hyaluronan (HA) in the tumor microenvironment, elevating interstitial pressure and impairing perfusion. Preclinical studies have shown that PEGPH20 degrades HA, thereby increasing drug delivery.[53] A randomized phase II study of PEGPH20 plus nab-paclitaxel/gemcitabine (PAG) compared with nab-paclitaxel/gemcitabine (AG) in patients with untreated metastatic pancreatic ductal adenocarcinoma (the HOLO202 trial) showed the largest improvement in progression-free survival among patients with hihg-HA tumors who received PAG.[69]

SOM230, a next-generation somatostatin analogue, re-sensitized pancreatic cancer cells to chemotherapeutic drugs by inhibiting CAF secretory activity through inhibition of the mammalian target of rapamycin/eukaryotic translation initiation factor 4E binding protein 1 pathway.[58] SOM230 has already been approved by the Food and Drug Administration for the treatment of Cushing's pituitary tumors, and clinical trials in the setting of pancreatic ductal adenocarcinoma are thus anticipated. In another approach, Beatty et al tested the combination of agonist CD40 antibody with gemcitabine chemotherapy in a small cohort of patients with unresectable PDAC, resulting in tumor regressions in some patients. They demonstrated that CD40-activated macrophages rapidly infiltrated tumors, which became tumoricidal, and facilitated the depletion of tumor stroma.[59]

The first successful approach to reduce CAF proliferation that led to depletion of tumor stroma and better gemcitabine delivery and prolonging survival in initial preclinical studies, was achieved through inhibition of sonic hedgehog signaling.[54] The results of that study paved the way for clinical trials. Various hedgehog- pathway inhibitors were tested in a phase II trial in the setting of advanced solid tumors, including pancreatic cancer. Unfortunately, this very promising approach in targeting the proliferation of CAFs using hedgehog-pathway inhibitors failed in phase II trials.[70] Other preclinical studies indicated that sonic hedgehog signaling inhibition resulted in tumor progression even though desmoplasia was decreased.[71,72] Moreover, clinical research using pancreatic cancer patient specimens demonstrated that high stromal density was associated with longer survival.[73] Given these results, part of the components of desmoplastic stroma work as tumor-restraining rather than as tumor-promoting.[74] A similar lesson has been learned from multi-MMP inhibitors, which did not improve survival among patients with PDAC in clinical trials, despite encouraging preclinical data. Recent data, however, have shown that some MMPs are protective against cancer and others are not, so non-selective inhibition also cancels the protective effects of some MMPs. Furthermore, initial clinical trials were faulty in that inhibitors were tested in late-stage cancers, whereas animal data were obtained during cancer initiation. Timing has to be taken into consideration, and entry criteria for clinical trials should be early-stage of cancer patients in order to match animal data.[60]

In a mouse model, a TGF-β antagonist suppressed metastasis without any adverse effects.[75] In another report, TGF-β inhibition reduced pancreatic cancer stroma in an orthotopic pancreatic-cancer mouse model, suggesting TGF-β inhibition as a potential treatment for controlling stroma density.[62] In a phase Ib clinical trial, the TGF-β inhibitor galunisertib was given in combination with gemcitabine to patients with advanced or metastatic pancreatic cancer.[76] The response rate with TGF-β inhibition therapy was 42.9% with acceptable safety.

5 | IMMUNOTHERAPY FOR PANCREATIC CANCER

William Coley, known as the father of cancer immunotherapy, noticed that bacterial infection induced notable tumor shrinkage in patients with osteosarcoma. He started bacterial therapy using bacteria or bacterial components, called "Coley's toxin" in 1891, and significant treatment effects were observed in patients with sarcoma.[77–79] The antitumor effect induced by this bacterial therapy is attributable to activation of the immune system followed by inflammation. Although bacterial therapy was replaced with chemotherapy and radiotherapy from the early 20th century, the achievements of bacterial therapy have recently been rediscovered because of marked developments of immunotherapy.

Anti-CTLA-4 and anti-PD-1 antibody are both immune checkpoint inhibitors that activate tumor-specific $CD8^+$ T-cell responses. Krummel et al reported CTLA-4 is a restricting factor for T cells in 1995.[80] CTLA-4 antibody therapy was the first immunotherapy drug to significantly prolong overall survival in patients with metastatic melanoma.[81] In recent years, a new immune checkpoint inhibitor therapy, targeting CTLA-4 for pancreatic cancer, showed limited efficacy.[21,82–84] However, in a tumor microenvironment in which immune reaction has been stimulated, checkpoint inhibition may be more effective. Tumeh et al[85] reported that tumor response to anti-programmed cell death protein 1 (anti-PD-1) therapy depended on pre-existing $CD8^+$ TILs in melanoma patients. Considering that $CD8^+$ TILs in pancreatic cancer stroma were induced by NACRT, PD-1 blockade may be effective in combination with NACRT. In addition, programmed death-ligand 1 (PD-L1)-positive responses in more than half of the cancer cells within tumors indicated good response to PD-1 inhibitor therapy in patients with non-small cell lung cancer.[86] PD-L1 overexpression in pancreatic cancer cells is thus possibly predictive of the response to anti-PD-1 therapy.

Tumor-infiltrating lymphocyte adoptive cell therapy has been developing since the 1980s. TILs extracted from resected specimens were stimulated and cultured in vitro, then transfused into patients. Rosenberg and Restifo[87] reported that the objective response rate for TIL adoptive cell therapy in melanoma patients ranged from 34% to 56%. Although the efficacy of TIL adoptive cell therapy for pancreatic cancer has not yet been reported,[88] this immunotherapy appears to have functional potential as TILs are likely to be a prognostic factor. Sakellariou-Thompson et al[89] recently noted that $CD8^+$ TILs derived from pancreatic cancer tissue could be grown with the aid of a 4-1BB agonist, suggesting the clinical feasibility of TIL adoptive cell therapy.

Chimeric antigen receptor T-cell (CAR-T) therapy has shown high remission rate for patients with acute lymphoblastic leukemia.[90] Cultured T cells transferred with the CAR gene using a retroviral or lentiviral vector are reinjected into the host. CAR-T therapy directly stimulates cell-mediated immunity, and can thus induce stronger antitumor immune reaction than antibody therapy.[91] Several studies of CAR-T therapy for pancreatic cancer are under way.[88]

Targeting immunosuppressive cells may be promising. Tumor-infiltrating Tregs in melanoma patients highly express chemokine receptor type 4 (CCR4), a potential target for Treg depletion. CCR4 antibody has been shown to remove effector-type Treg both in vivo and in vitro.[92] Mogamulizumab, a humanized anti-CCR4 antibody therapy for solid tumors, is under clinical study.[93]

Bacterial therapy may become a potential immunotherapy for pancreatic cancer. *Salmonella typhimurium* A1-R has been shown to be effective in patient-derived xenograft mouse models of pancreatic cancer.[94] In addition, *S. typhimurium* A1-R enhanced $CD8^+$ TILs in a syngeneic pancreatic cancer mouse model, suggesting activation of host antitumor immunity.[95]

6 | CONCLUSIONS

The tumor microenvironment in pancreatic cancer contributes to tumor growth, invasion, and metastasis in a multifaceted way, including immune evasion. New immunotherapies or cancer stroma-targeting therapies have potential to induce a cure for pancreatic cancer.

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