New Potential Therapeutic Strategy of Pancreatic Cancer via Targeting the Tumor Microenvironment

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Abstract
Pancreatic cancer is one of the most lethal types of cancers, with an overall five-year survival rate of less than 5%. Radical surgical resection remains a potential cure for this refractory disease but only a small number of patients could be benefit from surgery. The interactions between pancreatic cancer cells and stromal cells induce the formation of immunosuppressive and desmoplastic tumor environment and contribute to tumor progression, chemoresistance, and immune escape. In this review, we describe majority of the acellular and cellular components of tumor microenvironment and specifically review the importance of them in TME. Additionally, we discuss the most promising therapeutic strategies for targeting those components or reshaping the pancreatic tumor stroma. Finally, current clinical trials and novel investigational agents for pancreatic cancer targeting components of the microenvironment are also briefly evaluated.

Introduction
Pancreatic Ductal Adenocarcinoma (PDAC) is a highly aggressive human malignancy with the fourth leading cancer-related death in the United States currently. As is reliably predicted, PDAC will probably overtake the position of breast, prostate, and colon cancers to become the second leading cause of cancer death, following only lung cancer in the next decade [1]. Even though surgical resection with negative margins in combination with adjuvant therapy serves the only potentially curative therapy, approximately 70% to 80% of PDAC patients are inoperable because of local advanced or metastatic diseases [2,3]. Recently, the utilization of FOLFIRINOX or nab-paclitaxel plus gemcitabine in neoadjuvant therapy of borderline resectable pancreatic cancer and metastatic cancer is likely to improve overall survival in some patients to a limited extent [4,5]. However, 5-year survival rate in pancreatic cancer patients is lower than 10%, and considering the lack of effective treatment, there is no significant progression over the last 40 years [2].

The poor prognosis is multifactorial, not only the highly aggressive nature and complex mutational of the tumor is contributed to this refractory disease, but also the unique tumor microenvironment adds the fuel to the flame [6]. Tumor microenvironment is complex mixture of cellular and acellular components, which contains cancer-associated fibroblasts, myofibroblasts, pancreatic stellate cells, immune cells, collagen, fibronectin, extracellular matrix and multiple soluble factors in stoma [7]. Compared with other solid tumors the tumor microenvironment of pancreatic cancer is characterized by dense fibrosis, significant desmoplastic response, and abundant infiltration of immune cells [7]. The aforementioned features may pertain an essential role in the aspects of initiating malignancy, inducing immunosuppression, promoting tumor progression and metastasis, as well as delivering drug resistance. With the further exploration of constituents and functions of tumor microenvironment being crucial to the biology of PDAC, there could be potentially valuable presentation on targeting tumor microenvironment therapy and subsequent clinical developments have been attempted [8]. Hence, this review serves to delineate the current developing application of target therapy for tumor microenvironment to seek for a potentially more effective therapeutic strategy in pancreatic cancer.

Targeting tumor stroma
Hyaluronic acid (HA): Hyaluronic Acid is one of several glycosaminoglycans that comprise the ECM, abundant in the PDAC and correlated with a poor prognosis. In pancreas cancer, high HA content is present as high as 90% of PDAC tissue [9]. Along with fibrotic collagens, HA contributes to the TME component of PDAC thus leading to elevated interstitial fluid, which is known to induce chemoresistance via impairing delivery of systematic therapy to the tumor core. Besides HA also binds to CD44 and RHAMM cell surface receptors to activate downstream cell signaling pathways...
that promote cancer cell survival, adhesion, proliferation, migration, and invasion [10]. Based on those roles, HA is considered a potential therapeutic target to remodel the tumor microenvironment and to enhance the effectiveness of systemic therapy [10,11]. Subsequent mouse studies demonstrated that PEGPH20 (a PEGylated recombinant human hyaluronidase), is functioned to normalize intra-tumor interstitial fluid pressures, relieve vascular collapse, and enhance intra-tumor chemotherapy delivery [12]. In a Phase I clinical trial, combination PEGPH20 with gemcitabine-based chemotherapy improved both progression free and overall survival in advanced PDAC cases (NCT01453153). These promising results led to the randomized phase II trial, PEGPH20 in combination with gemcitabine and nab-paclitaxel for treatment of metastatic pancreatic cancer compared with gemcitabine and nab-paclitaxel alone [13-15]. In spite of a significant increase of thromboembolic events in PEGPH20 arm, the triplet arm with PEGPH20 yielded a significantly higher objective response rate (52% vs. 24%) and longer DFS (9.2 vs. 4.3 months; HR, 0.39; P=0.05) than gemcitabine and nab-paclitaxel [16]. These findings were even more evidenced in patients that had high levels of HA expression. The patients with high level of HA in tumor presented a more significant improvement in PFS (9.2 with PEGPH20 vs. 5.2 months, (HR, 0.45; 95% CI, 0.17 to 1.22; p=0.11) [17]. Considering this promising result, a phase III study has been initiated to select patients with high HA PDACs and anticoagulation is now administered in conjunction with PEGPH20 to precontrol the side effects of PEGH20 [18].

Focal adhesion kinase (FAK): Focal Adhesion kinase (FAK) is non-receptor tyrosine kinases which include FAK1 and PYSK2 (FAK2) is implicated in cancer migration, proliferation and survival [19]. Hyperactivated FAK in neoplastic PDAC cells contributes to fibrotic remodeling of ECM and immunosuppression of tumor microenvironment. Besides, it also functions to regulate pro-inflammatory pathway activation and to promote epithelial-mesenchymal transition [20]. In a KPC mouse model of PDAC, high level of FAK activity was observed and associated with CD8+ cytotoxic T cell infiltration, what’s more, the addition of FAK inhibitor VS-4718 reduced tumor fibrosis and decreased numbers of tumor infiltrating immunosuppressive cells [21]. FAK inhibitors were also shown to enhance sensitivity of immunotherapy such as PD-1 antagonists or CTLA-4 antibodies. While no clinical trials have yet demonstrated a reliable response to single-agent FAK inhibition in PDAC [22]. Recently several trials of PDAC treatment with FAK inhibitor are active, and a phase I study of combination of FAK inhibitor and PD-1 antagonist is underway (phase I, NCT02546531).

Matrix metalloproteinase (MMPs): Matrix Metalloproteinases (MMPs) are a family of proteolytic enzymes that regulate the tumor cell invasion potential and migration [23]. Overexpression of MMPs in PDAC is responsible for tumor progression, invasion, and metastasis [24]. Preclinical study demonstrated that when stimulated the secretion and activation of MMPs by TGFβ1, there is markedly increased invasive and metastatic potential observed in cancer cells. Based on these factors, inhibition of MMPs was considered a potential therapeutic strategy to target PDAC [25,26]. Marimastat, an MMP inhibitor showed single-agent activity and safety in PDAC patients but the further therapy in combination with gemcitabine present no benefit compared to gemcitabine alone [27]. Additionally, another MMP inhibitor, tanomastat (BAY 12-956), a specific inhibitor of MMP2, -3, -9, and -13, made a similar result when compared to single-agent therapy of gemcitabine, with 1-year survival rates of 10% respectively [28]. These frustrated results urge us to further study the mechanism of MMPs in tumor invasion and migration to explore more effective therapy. In vivo, targeting MT1-MMP has been proposed to sensitize pancreatic tumors to gemcitabine, and attempts at developing selective MT1-MMP inhibitors have been utilized [25,29]. Recent pre-clinical study using anti-MMP9 antibody in combination with nab-paclitaxel-based for chemotherapy has been observed a potential benefit of antitumor and anti-stromal effect, besides, it was certified to promote antitumor immunity by activating T cells in another solid tumor microenvironment [30]. Other explored approaches using miRNA to regulate multiple MMPs expression could serve as an attractive therapeutic target [31]. Future efforts will be oriented on exploring more effective therapeutic strategy through targeting metalloproteinases [32].

ROCK Rho kinase inhibitor: The Rho/ROCK signal pathway play an essential role in regulating cell shape, cell adhesion, mobility and interaction with surrounding ECM [33]. In pancreatic cancer, Rho interacts with tumor cells and tumor stroma to mediated the drug resistance mechanism. ROCK is the key mediator of RhoA activity, and ROCK is overexpressed in PDAC and exerts its effect on metastases via collagen remodeling in the ECM [34]. Given that following characteristics, an emerging therapeutic strategy using Rho kinase inhibitor may be potentially beneficial. Short priming with ROCK inhibitors has shown promising effects with chemotherapy in pre-clinical models. The administration of Fasudil, an Rho kinase inhibitor (priming) improved the response to gemcitabine/nab-paclitaxel chemotherapy in pancreatic cancer cell line and patient-derived tumors [35,36].

Pirfenidone: Pirfenidone is a known antibiotic agent that mitigates the expression several mediators of fibrosis such as TGF-β and collagen [37]. In pancreatic cancer, Pirfenidone modulate PSC mediated-stromal accumulation and enhance delivery of chemotherapy [38]. Combination of pirfenidone with gemcitabine efficiently inhibits tumor growth compared to either drug independently. In preclinical studies pirfenidone has demonstrated some promising effects in combination with gemcitabine, with β-cyclodextrin MMP2 responsive liposome and with the antioxidant N-acetyl cysteine [39]. Besides, Pirfenidone could induce G0/G1 cell cycle arrest to disrupt the proliferation of pancreatic cancer cells [40]. This novel therapeutic strategy has shown potential value on targeting not only fibroblasts but also tumor cells in the tumor microenvironment, also it might be further evaluated and studied.

Targeting cellular components

Pancreatic stellate cell: PSCs are star-shaped stromal cells located at the basolateral aspect of acinar cells or the surrounding perivascular and periductal regions in the pancreas. Under homeostatic conditions, most of the PSCs stay quiescent and have the function of reserving vitamin A- rich lipid droplets, exo/endocrine secretion, and maintenance of pancreatic stromal architecture, stimulation of anylase secretion, phagocytosis, and immunity [41]. While in the PDAC tumor microenvironment, cancer cells secrete variable amounts of pro-inflammatory growth factors/cytokines, including TGF-β1, PDGF, TNF-α, and IL-1/6 and activate PSCs. Activated PSCs (aPSCs) lose cytoplasmic vitamin A- storing lipid droplets and express Alpha-Smooth Muscle Actin (α-SMA), transform into a myofibroblast-like phenotype and secrete large amounts of Extracellular Matrix (ECM) proteins, comprised of collagens, fibronectins, and laminins in periacinar regions to form desmoplastic
Conventional therapeutic strategies with chemotherapy for PDAC are able to reduce desmoplasia via hampering the PSCs mediated tumor microenvironment remodeling, fail to gain significant survival benefits because of impaired drug delivery by the stiff stroma. In addition, some investigations indicated that therapeutic strategies aiming to deplete PSCs and myofibroblast contribute to immunosuppression, enhanced tumor hypoxia, EMT program and enhanced aggressiveness of PDAC [47]. Hedgehog (Hh) signaling pathway is currently known to activate PSCs via paracrine effects and to regulate stromal abundance. Therapeutic effects using hedgehog inhibitor, IPI-926 and gemicitabine as a combination therapy had been explored, however, the outcome turn out to more aggressive disease and conversed survival benefit [48,49]. Other attempts via stroma-depleting therapeutic strategies contribute to similar results to IPI-926 [50]. Considering that dissatisfaction, more emphasis should be focus on inhibiting the activation of quiescent PSCs or reversing aPSCs or CAFs into quiescent PSCs. Aforementioned aPSCs are often in deficiency of cytoplasmic vitamin A - storing lipid droplets, and patients with PDAC also lack of vitamin A and D. In vitro, All-Trans Retinoic Acid (ATRA) were observed to reverse aPSCs into quiescent PSCs and contribute to inhibition on aPSC migration and collagen synthesis [51]. Current phase Ib study is under way investigating ATRA along with gemicitabine and nab-paclitaxel in PDAC [52]. Besides, aPSCs express high levels of the Vitamin D Receptor and its related pathway is essential factor to regulate the conversion of activated state. In contrast to gemicitabine treatment alone, treatment using VDR ligand in combination with gemicitabine result in promising survival benefit and shrunken tumor volume in KPC mice model [53-54]. Recent study revealed that miRNAs are potentially valuable for targeting PSCs [55]. miRNA-29 were maintained in decreased level in aPSCs and restore miR-29 expression reduced stroma accumulation and tumor growth. Other miRNA sequence, for example, miRNA-21, miRNA-199a and miRNA-214 were found overexpressed in CAFs and aPSCs may be future explored for target therapy [56,57].

**Cancer-associated fibroblasts:** Cancer-Associated Fibroblasts (CAFs) are activated fibroblasts which originated from resident fibroblasts and Pancreatic Stellate Cells (PSCs), the recruitment and proliferation of Pancreatic Stellate Cells (PSCs) and contribute to inhibition on aPSC migration and collagen synthesis [51]. Current phase Ib study is under way investigating ATRA along with gemicitabine and nab-paclitaxel in PDAC [52]. Besides, aPSCs express high levels of the Vitamin D Receptor and its related pathway is essential factor to regulate the conversion of activated state. In contrast to gemicitabine treatment alone, treatment using VDR ligand in combination with gemicitabine result in promising survival benefit and shrunken tumor volume in KPC mice model [53-54]. Recent study revealed that miRNAs are potentially valuable for targeting PSCs [55]. miRNA-29 were maintained in decreased level in aPSCs and restore miR-29 expression reduced stroma accumulation and tumor growth. Other miRNA sequence, for example, miRNA-21, miRNA-199a and miRNA-214 were found overexpressed in CAFs and aPSCs may be future explored for target therapy [56,57].

**Tumor associated macrophages (TAMs):** Tumor Associated Macrophages (TAMs) reside within the TME in PDAC and are thought to serve as a potential therapeutic target in several solid tumors. In fact, Tumor-Associated Macrophages (TAMs) have been identified as one of the most abundant immune infiltrated cells in PDAC [71,72]. M1-like polarized macrophages, capable of pro-inflammatory reaction and phagocytosis, are recognized as anti-tumor macrophages. And different from M1-like macrophages, M2-like polarized macrophages contribute to development of an immunosuppressive microenvironment and promotion of tumor growth by secreting anti-inflammatory cytokines, proteases and growth factors [73]. Therapeutic strategies aiming to reduce the rate of M2 polarization TAMs and reprogram M2 TAMs into an effective anti-tumor activity have started to emerge. CL2-CCRC2 pathway has been shown to play a critical role in recruitment of TAMs [74,75]. In an orthotropic model of mouse PDAC, CCR2 blockade using a small molecule inhibitor PF-04136309 depleted inflammatory monocytes and macrophages in the TME and decreased tumor growth and metastatic potential [76]. In a phase I ongoing study, combined with PF-04136309, Gemicitabine plus Nab-Paclitaxel severed a result that 4-month disease control rate was 70%. Besides, low dose irradiation in xenotransplant mouse models reprogrammed M2-like to M1-like macrophages, which resulted in effector T-cell recruitment [77].

Modulated macrophage polarization towards M1-like phenotype by adjuvant gemicitabine-based chemotherapy could reach a similar result [78]. CD40, as a macrophage cell surface marker, can inhibit cytotoxic functions. Using CD40 agonist antibodies induce high expression of M1 markers contribute to a significant antitumor effect and enhancement of chemotherapy [79]. It is noteworthy that the crosstalk between macrophages, tumor cells, and stromal cells have a complicated influential on the polarization and function of macrophages [80]. In addition, previous study revealed that several signal pathways are engaged in macrophage polarization such as JNK, PI3K/Akt, Notch and JAK/STAT signals pathway [81]. Further investigation of novel agents targeting TAM in human pancreatic cancer is warranted to with the hope of improving the outcome of this fatal disease.

**Myeloid-derived suppressor cells (MDSCs):** Myeloid-Derived Suppressor Cells are a population of cells defined by their immature state, myeloid origin and capacity to suppress the immune response [82]. They are strongly immunosuppressive by their ability to inhibit T-cell proliferation, IFN-γ production, effector T-cell function and macrophage conversion into M2 phenotypes, and to favor Treg generation through the secretion of ROS, Arg1, and iNOS [83]. Besides, upregulation of the Programmed Death-Ligand 1 (PD-L1) on MDSCs is responsible for immunosuppression in the TME by rendering the inactivation of lymphocytes [84]. In PDAC, MDSCs promote tumor growth by VEGF and MMP9 secretions. Pancreatic cancer cells can induce mobilization of MDSCs out of the bone marrow...
and into systemic circulation before they get recruited into the TME, which is associated with high concentration of MDSCs in the peripheral blood. This is a potential therapeutic target in PDAC. Targeted depletion of Gr-MDSC (Gr-MDSC) has been successful in other tumor types, clinical trials in PDAC did not demonstrate significant disease response and survival advantages [105]. In future, we should focus more on the exploration of other potential immune checkpoint and multiple targeted approaches for PDAC.

**Conclusion**

The treatment of pancreatic adenocarcinoma remains a challenge because of limited effective treatment options in spite of several targets in PDAC. Recent fundamental advances in the understanding of tumor microenvironment have created opportunities for the development of more effective and personalized approaches for pancreatic cancer patients. Nevertheless, results from some preclinical and clinical studies targeting the tumor microenvironment were still controversial, suggesting a comprehensive and in-depth research on the basic science of the tumor microenvironment. Future work focusing on combinatorial approach of targeting tumor cells, targeting TME and immunotherapy is needed to explore the more ideal treatment strategy for PDAC.

**References**

13. Hingorani SR, HarrisWP, Beck JT. Phase Ib Study of PEGylated antitumor immune response of survival after surgery in patients with (stage IIB/III) pancreatic cancer [91]. T-cell dependent antitumor immunity is thought to be in part via activation of the TNF receptor superfamily member CD40 [92]. Thus, through an indirect effect, CD40 activating monoclonal antibodies activate CD8+ T cells and potentially can reverse the immunosuppressive environment observed in pancreatic cancer. In a phase I study, CP-8970,893, a CD40 agonist antibody, was given in combination with gemcitabine in patients with treatment naive unresectable PDAC. Four of the 22 enrolled patients experienced a partial response, which demonstrated the potential of CD40 agonists as a treatment modality in pancreatic cancer. In addition, there is a clinical study investigating the efficiency of gemcitabine/nab-paclitaxel chemotherapy combined with CD40 agonistic monoclonal antibody and anti-PD-1 monoclonal antibody in pancreatic cancer patients [93].

**Immune checkpoint**: The PDAC tumor microenvironment has been characterized by few tumor cells with a robust infiltrate of immune cells, usually dominated by Myeloid Derived Suppressor Cells (MDSCs). Tumor-Associated Macrophages (TAMs) and neutrophils, with TILs present but in smaller number [94,95]. The massive accumulation of infiltrated MDSCs, TAMs, TILs develop the immunosuppressive tumor microenvironment by hampering effective T cell cytotoxicity and inducing immune escape of tumor cell. In this process, the immune checkpoint plays an essential role [96]. Targeting immune checkpoints is that blockade of the inhibitors harnesses the endogenous anti-tumor response of the immune system to combat the disease. PD-1 binds to PD-1 ligands PD-L1 and PD-L2, resulting in activation and suppression of T-cell activity and allowing for immune escape of tumor cells in the TME [97]. Emerging immunotherapy strategies with the application of PD-1/PD-L1 checkpoint inhibitor are investigated. As a monotherapy, PD-1/PD-L1 inhibitor didn’t reach a satisfied disease response in clinical trials for PDAC [97,98], but current combinatorial strategies with chemoradiotherapy and other immunotherapy like cancer vaccine, targeting immune cell or stroma therapy provides a favorable data of survival and safety. Advanced study demonstrated that checkpoint inhibitors are likely to enhance other immunotherapy by disruption of crosstalk between tumor cells and immune cells [99-103]. CTLA-4 is another protein receptor that acts as an immune checkpoint expressed in PDAC, in pre-clinical animal trials CTLA-4 inhibitor showed promise with prolonged survival, but failed to gain survival benefit in phase II trial [104]. Up to now, although targeting immune checkpoint has been successful in other tumor types, clinical trials in PDAC did not demonstrate significant disease response and survival advantages [105]. In future, we should focus more on the exploration of other potential immune checkpoint and multiple targeted approaches for PDAC.


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