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MINI-REVIEW

Targeted stroma in Pancreatic Cancer: Promises and Failures of Target Therapies†

Afsane Bahrami[1], Majid Khazaei[2], Fariba Bagherieh[1], Majid Ghayour-Mobarhan[3], Mina Maftouh[3], Seyed Mahdi Hassanian[3,4,#], Amir Avan[3,5,#]

1) Molecular Medicine group, Department of Modern Sciences and Technologies; Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.
2) Neurogenic Inflammatory Research Center and Department of Physiology, Mashhad University of Medical Sciences, Mashhad, Iran
3) Metabolic syndrome Research center, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
4) Department of Medical Biochemistry, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.
5) Cancer Research Center, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.

[#]Corresponding authors:
1. Amir Avan, PhD; Metabolic syndrome Research center, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran; Fax: +985138002287; Tell: +985138002298; Email: avana@mums.ac.ir & amir_avan@yahoo.com
2. Mahdi Hassanian, PhD; Department of Medical Biochemistry, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran. Fax: +985138002287; Tell: +985138002298; Email: Hassaniansm@mums.ac.ir

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Abstract

Desmoplasia or abundant fibrotic stroma is a typical property of most malignancies, which has a great effect on tumorigenesis, angiogenesis, and resistance to therapy. The activated stroma cells comprises several cell types including endothelial cells, nerve cells , inflammatory/macrophages cells , stellate cells, and extracellular matrix. In other word, the interactions of cancer–stroma modulate tumorigenesis, therapy resistance and poor delivery of drugs. Therefore, targeting the tumor stroma in combination with conventional chemotherapeutic agents could provide a promising approach in the treatment of pancreatic cancer. This review summarizes the current knowledge about pancreatic stellate cells, targeting stroma compartments with particular emphasis on preclinical and clinical trials on targeting of stroma as an option in pancreatic cancer treatment. This article is protected by copyright. All rights reserved

Keywords: Pancreatic cancer; stroma; pancreatic stellate cells; desmoplastic reaction

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Introduction

Pancreatic cancer is the fourth cause of cancer death (1). Pancreatic cancer has an extremely poor prognosis, with a five year survival rate at the best below 7% (2).Pancreatic ductal adenocarcinoma (PDAC) mainly includes more than 90% of pancreatic cancers. In spite of extensive scientific and clinical researches, the remarkably poor prognosis of this cancer has not improved over the last decades (3).The main causes for the ineffective therapeutic strategies include aggressive behavior of PDAC and its natural resistance to chemotherapy regimens(3).

Gemcitabine as the most common chemotherapeutic agent used in the treatment of PDAC, however, only less than 12% response rate in these patients (4). Only, Erlotinib-gemcitabine was the treatment that was approved, but this combination provided one month of additional life. However, a recent phase III trial reported that the combination of fluorouracil, leucovorin, irinotecan, and oxaliplatin is a good option for the treatment of metastatic patients (5). However, these therapeutic programs are related with high toxicity and thus, there is an essential need to develop innovative anticancer agents that both improve gemcitabine activity, within novel combinatorial regimens, or with a better efficacy compared to gemcitabine.

Pancreatic cancer stellate cells and fibroblasts

Histologically, PDAC is typified by an extensive and dense desmoplastic or fibrotic stroma that consist a large amount of pancreatic stellate cells (PSCs) or carcinoma-associated fibroblasts (CAFs) in activated form, extracellular matrix (ECM) proteins(6) and other cells including endothelial cells, immune cells, pericytes, and nerve fibers. The presence of desmoplastic reaction in primary tumors has been directly related with worse clinical outcomes (7, 8). The desmoplastic reaction in pancreatic adenocarcinoma is contributed to the invasive phenotype of tumor and increasing drug resistance.

The transition from normal to invasive cancer is earlier or accompanied by the local host stroma activation (9). ECM is another component of the tumor microenvironment. The different type of

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growth factors and proteins are found in the ECM of PDAC. ECM was a mesh of fibrous molecules helping the surrounding tissues and plays a role in development, differentiation, remodeling homeostasis and etc. in healthy organs (10).

This acellular part of pancreatic tumor stroma is consisting of variety of firous proteins (*i.e.*, collagen), glycoproteins (*i.e.*, fironectin) and polysaccharides (*i.e.*, hyaluronan). In several studies, collagen type I was linked to gemcitabine resistance, a standard cytotoxic drug applied for pancreatic cancer treatment (11, 12).An atypical glycosaminoglycan with upper capacity of water retention, Hyaluronan (HA), has identified as a potential target for pancreatic cancer therapy. HA is high-expressed in PDAC and its abundance has been associated to barrier to perfusion causing elevated intra-tumoral fluid pressure lead to vascular collapse(13, 14).

Stroma contains nerve fibers that produce nerve growth factors encouraging growth and migration of PDAC cell (15),also bone marrow-derived stem cells that may have the differentiate into different stromal cells (16). Stroma proliferation is further promoted by the recruitment of enormous of inflammatory immune cells and the activation of endothelial cells and pericytes that over expressing level of profibrotic and proangiogenic factors to the pancreatic tumors peripheral(17, 18).

The main cellular parts of desmoplastic reaction in PDAC are PSCs and fibroblasts.

The origin of PSCs is unclear, but they are suggested to derived from endodermal, mesenchymal, and neuro-ectodermal origins. They can be identified in fibroblasts according to the expression of desmin , glial fibrillary acidic protein (GFAP), vimentin, nestin (intermediate filament proteins) , and neuroectodermal markers such as nerve growth factor (NGF) as well as neural cell adhesion molecule(19).

PSCs are accounted to play a considarable role in the pathobiology of pancreas disorders including pancreatitis and PDAC. In which disorders PSCs change to an activated form and obtain property of myofibroblasts and high express α-smooth muscle actin (αSMA). Activated PSCs in diseased organ,

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are postulated to come up from quiescent PSCs, fibroblasts, epithelial- mesenchymal transition (EMT), or bone marrow-derived cells. Whatever their origins, PSCs are activated against pancreatic injury and inflammation and play a crucial role in the progression of carcinoma(20).

PSCs secrete to respond abundance type of cytokines and have been shown to actively proliferate, propagate, and produce ECM proteins, including fibronectin and collagen type I.

A number of profibrogenic mediators that regulate PSCs have been known, such as platelet-derived growth factor (PDGF) (21), fibroblast growth factor (FGF) (21), activin A (22), transforming growth factor β (TGF- β) (21), tumor necrosis factor α (TNF- α), reactive oxygen (21, 23), interleukin (IL)-1 and IL-6 (23). Among them, PDGF is identified to be the most putative mediator (21).In regard to these profibrogenic mediators, PSCs endure an activation process that contributes development, proliferation, display a myofibroblastic-like phenotype and elevated secretion of excessive components of the ECM through activating the mitogen-activated protein kinase (MAPK) family enzymes (24). Actually, activated PSCs synthesized cytokines such as cyclooxygenase-2 (COX-2) (25) TGF- β (26), matrix metalloproteinases (MMPs), activin A (22), and tissue inhibitor of metalloproteinases (TIMPs) (27) which may further potentiate an aggressive phenotype. These autocrine loops involved in stroma modulation and provoke fibrosis.

One of the signaling pathways mediate PSC activation is Hedgehog which is necessary for embryonic development, but in natural not detectable in human healthy pancreas(28).This pathway has been also involved in stem cell regulation and neoplasia (29). Binding of the Hedgehog ligand to its receptor Patched releases the co-receptor Smoothened from suppression and lead to translocation of the transcription factor Gli-1 from the cytoplasm to the nucleus where it regulates genes contributed in cell development, proliferation, apoptosis, adhesion, and metastasis. Recent studies examining Hedgehog signaling in PDAC have disclosed that sonic hedgehog ligand secreted from malignant epithelial cells acts on fibroblasts and PSCs, promoting desmoplasia and increased metastasis in an orthotopic

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model(28, 30) .Furthermore, Infante *et al*. illustrated that intra-tumoral SPARC expression was linked with poor prognosis in resected PDAC patients (31).

Global gene expression analysis of differentially expressed genes in fibroblasts cultured in with or without of pancreatic cancer cell lines reported 43 high-expressed and 31 down-expressed transcripts (32). Among the most high up-regulated genes were CXC/CC chemokine family members including MCP-1 (CCL2), GRO1 (CXCL1), interleukin- (IL)-8 (CXCL8), and GRO2 (CXCL2); all of which have been indicated in invasion and angiogenesis of tumor. PSCs, fibroblasts, and epithelial cells all participate to regulating the composition of the ECM within proteolytic enzymes, or matrix metalloproteinases (MMPs), that are commonly over-expressed in pancreatic cancer cells (33, 34). The regulation of EMMPRIN, an extracellular matrix metalloproteinase inducer, through cancer cells stimulates the PSCs to produce MMP-2 led to increased tumor cells invasion (35). Furthermore, tumor cells expressed serine protease inhibitor nexin 1 (SERPINE2) that promotes invasion and tumor growth just in presence of PSCs (36). Several lines of evidence have also recognized inhibitors of the extracellular proteinases, tissue inhibitors of metalloproteinases (TIMPs) which are high-expressed in PDAC(37-39).

Endothelial cells

Angiogenesis in pancreatic cancer is regulated by a complex contraction between different cell types in the tumour stroma. Under hypoxic, compared to normoxic conditions, cultured PSCs enhance production of collagen type Ⅰ and the proangiogenic vascular endothelial growth factor (VEGF)(40, 41).

Studies have linked hypoxia in patient tumors with worse clinical outcomes, including raised rates of tumor growth and metastasis. The hypoxic environment is predominantly considered as a result of PSCs generated fibrotic microenvironment and the expression of several antiangiogenic substances, including matrix metalloproteinase 12 and endostatin (41, 42).

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The hypoxic microenvironment in pancreatic cancer has been demonstrated to induce the over expression of hypoxia-inducible factor-1 (HIF1) in pancreatic tumors from patients. HIF1 expression has been connected with drug resistance and further invasion in pancreatic cancer, which may be mediated by Hedgehog signaling and c-Met (43, 44).

The stroma in PDAC contains large numbers of inflammatory cells such as macrophages, mast cells, neutrophilic granulocytes and T cells (45). Leukocytic infiltrates in pancreatic adenocarcinoma have immunosuppressive characteristic and linked to worse survival in humans (46). Also, proinflammatory markers, mainly IL-1 receptor antagonist, IL-6, IL-8, and IL-10 are elevated in the serum of patients with pancreatic cancer (47).

Moreover, normal pancreas has an ample nerve supply composing of ganglia, myelinated and unmyelinated nerve cells. The peri-neural invasion degree in the tumor has been relevant with worse survival after resection and has been found to be mediated by the chemokine receptor CX3CR1(48, 49). But the effect of these cells on tumor progression/ resistance is not clear understood.

Stromal composition and patient survival

In pancreatic cancer, although the ECM is a product of activated PSCs, the activity of PSCs evaluated by α-SMA expression does not always associate with the status of fibrosis or inflammatory cell infiltration(7, 50).

The cause of this discrepancy is that α-SMA expression is a marker for trans-differentiation of PSCs favorable than PSC activity(50).

The activated stroma index (ASI) is a novel index used to estimate stromal activity in PDAC(7). This index shows whether the stroma is dominantly fibrolytic, fibrogenic, inert, or dormant. Moreover, the somatic evolution of invasive cancer could be regarded as a sequence of phenotypical adaptations to overcome this barrier, hallmarking the importance role of the fibrotic tumour microenvironment in the pancreatic cancer behavior(50).

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Potential therapies for PDAC stroma

Pro-fibrotic growth factors, such as TGF- β, PDGF, EGFR and, IGF-1 can be recruited as potential therapeutic targets (51). TGF- β signaling plays a role in PDAC carcinogenesis and its stromal formation (52). EGFR is mostly overexpressed in PDAC and is correlated with poor prognosis and disease progression (53). EGFR signaling has also been found to affect pancreatic fibrogenesis by activating PSCs (54).

Besides, tumor cells and CAFs could attract IL-17 secreting CD4+ cells (Th17) by TGFβ secretion, involving to immune-suppression in the PDAC tumor microenvironment(55). McAllister *et al.* demonstrated that IL-17 induced infiltration of IL-17-expressing T cells lead to tumor progression and the prevention of IL-17 signaling disrupted PDAC formation in preclinical studies(56). Brodalumab and ixekizumab as antibodies against IL-17 signaling pathway are currently evaluated by clinical trials for psoriasis treatment and therapies using IL-17 inhibitors may hold swear in PDAC.

TNP-470 is a synthetic analogue of the fungus-derived bioactive agent fumagillin, which blocks proliferation of endothelial cell (57). TNP-470 significantly decreased tumour size and spread in orthotopic xenograft models of PDAC in mice (58). Administration of small doses of TNP-470 or gemcitabine alone had no significant effect, but a combination therapy declined tumor growth and migrations and improved median overall survival(59).

MMPs are a large family of zinc-proteolytic enzymes engaged in extracellular environment maintaining in physiological and pathological conditions (60). This family is expressed by PDAC cancer cells, activated PSCs, immunocytes, and fibroblasts (61). Moore and colleagues conducted a phase III trial with BAY-12-9566, a specific inhibitor of MMP-2, MMP-3, MMP-9 and MMP-13 in subjects with locally advanced or metastatic pancreatic cancer. The research was stopped because they found that the gemcitabine was significantly superior to new inhibitor (62). Because of the existence of a relation loop between PDAC and its stroma, eliminating the stroma may have great therapeutic effect.

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Shh signaling plays a casual role in pancreatic tumorigenesis by a paracrine signaling pathway received through tumor stromal cells (63). Olive *et al*. found that block the Shh signaling pathway resulted in a dramatic depletion of stromal components at the same by an increase in intratumoral vascular density in the mouse model of PDAC (43). Administration of gemcitabine plus IPI-926, another Shh signal pathway inhibitor, resulted in a significantly enhanced intratumoral volume of gemcitabine triphosphate, transient disease stability and a statistically significant survival prolongation. But, the noticeable stromal reaction and hypovascularity ultimately returned in the model, suggesting that the tumors can modify to chronic Shh suppression (43) .

Several preclinical studies have been investigated how stromal fibroblast cells can modify immune response against tumor. The elimination of fibroblast activation protein-α (FAP)-expressing stromal cells in PDAC led to an immune-mediated hypoxic necrosis of both tumor and stroma cells (64). Indeed, FAP-activated promelittin protoxin targeted cancer stroma firoblasts showed high tumor lysis and growth inhibition in xenograft mouse models of prostate and breast cancer (65). But, FAP positive stromal cells in patients with metastatic colorectal cancer targeted with humanized anti-FAP antibodies in phase Ⅱ clinical trials that did not report expecting results (66).Regard to consideration the outcomes from either preclinical or clinical studies, it is reasonable to propose that FAP-targeted stromal depletion shows immune activating effect, but requires further immune modulation to be efficient. It is convincing that FAP targeted stromal depletion and immune activation simultaneously, by both vaccination and immune checkpoint inhibition would result in increased advantages for PDAC patients (67).

Immunotherapy approaches are being evaluated in clinical trials for PDAC with a purpose to induce tumor infiltration and activation of effector cells (*i.e.*, CD8+ T cells) and consequently CD8+ T cell dependent tumor lysis. Several clinical trials of a fatally irradiated allogeneic GM-CSF secreting whole cell vaccine (GVAX) prescribed to patients with resected PDAC or metastatic PDAC reflected that

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increased response of interferon-γ secreting mesothelin-specific CD8+ T cells in the peripheral lymphocytes relates with improved survival(68, 69). A pilot study examining the GVAX plus ipilimumab (an anti-CTLA-4 antibody) compared to ipilimumab demonstrated a trend of increase in overall survival in metastatic PDAC patients that have been before treated with multiple lines of chemotherapy and so advocated the role of CTLA-4 inhibit in promoting anti-tumor response of GVAX (70). Whereas, it remains to be explored how vaccine-based immunotherapy activates anti-tumor effector cells by tumor microenvironment. Exploration of novel targets in tumor microenvironment may elevate development of immune modulatory therapies.

A successful immune modulatory target in microenvironment of tumor is CD40. Beatty et al. used a CD40 (a member of TNFα receptor family) agonist in KPC mouse model and expressed that macrophage activation possibly led to apoptosis of cancer cells and decreased stroma collagen (71). A Phase 1 study employed this CD40 agonist antibody plus gemcitabine in advanced PDAC with no before history of chemotherapy. Anti-tumour activity was seen with this approach but the results were very heterogeneous, in particular in metastatic disease (71). In a GEMM model ,inhibition of Smoothened within a cyclopamine derivative (IPI-926) had a hallmark, although transient, inhibitory effect on tumor growth and increased median overall survival (43). Another Smoothened inhibitor, AZD8542, has been found to decline tumor volume and metastasis in an orthotropic model of pancreatic cancer (72). Unfortunately, a phase 2 clinical trial with IPI-926 had discontinued due to declined survival of patients in treatment arm. Discrepancy between pre-clinical finding and clinical setting emphasizes the need for better assessment of any preclinical observations in a range of experimental settings so as to replicate the heterogeneity of pancreatic

A recent study reported that elimination of Sonic hedgehog decreased tumoural stroma; on the other hand, this increased vascularity and resulted in overall cancer invasiveness(73).

Preclinical studies of HIF blockage in PDAC have found tumors sensitization to radiation with or without treatment with 5- fluorouracil or gemcitabine (74)

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Another stromal target showing promising results in phase Ib clinical trials is hyaluronan. In mouse models of PDAC, enzymatic degradation of hyaluronan resulted in increased gemcitabine tumor cytotoxicity because of vascular collapse relief (14). Those confirm principle of experiments that lead to the development of pegylated recombinant human hyaluronidase (named PEGPH20) its enzyme that degrades hyaluronan. Administration of PEGPH20 combination with gemcitabine to PDAC patients with advanced stage revealed partial response in 43% of cancer patients and stable disease in addition 30% patients in phase I b clinical trials. More interestingly, the partial response rate was 64% in patients those PDACs over-expressed level of hyaluronan (75). This high response rate has led to further testing of PEGPH20 in complex with gemcitabine and nab-paclitaxel in a randomized phase II clinical trial (67).

Since, the current outcome of researches and clinical trials indicated that targeted pancreatic stroma does not show high efficiency. More importantly, recent published articles provide new scientific explanations for the failure of trials, suggesting elimination of the stroma may lead to poorly differentiated tumors and accelerate PDAC progression (58, 73, 76-78).

Conclusions

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