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Review

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Desmoplastic Reaction in Pancreatic Ductal Adenocarcinoma

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ABSTRACT

Despite significant effort and research funds, Pancreatic Ductal Adenocarcinoma (PDAC) remains one of the deadliest diseases. This cancer is characterized by a distinct desmoplastic reaction that constitutes 80% of the tumor volume. Accumulating evidences suggests that the stromal compartment in which the cancer cells are embedded contributes to many clinical characteristics of pancreatic cancer. The stromal compartment is comprised of abundant extracellular matrix (ECM), fibroblasts, stellate cells, immune cells, nerve cells, growth factors and cytokines. To date, desmoplastic reaction components have been shown not only to contribute to the growth and metastasis of pancreatic cancer but also to chemotherapy resistance. Therefore, further assessment of stroma-targeted therapies and their translation into clinical situation may open a new era in pancreatic cancer management.

KEYWORDS: Pancreatic cancer; Pancreatic ductal adenocarcinoma; Desmoplastic/stromal reaction; Tumor-stroma interactions.

INTRODUCTION

Pancreatic Ductal Adenocarcinoma (PDAC) is the fourth leading cause of cancer related death in the USA and Europe. Despite substantial progress of understanding the molecular biology of PDAC, the prognosis remains still poor with a combined overall 5-year-survival rate of less than 7%.[1] This high death rate is due to late diagnosis with no effective screening tools for detection of early tumors and the lack of curative treatment methods.[2] Accumulating evidence suggests that the aggressive phenotype of PDAC is not only due to epithelial cancer cells, but also to the stromal compartment in which cancer cells are embedded.[3,4]

The dense desmoplastic reaction is one of the histological hallmarks of PDAC which can often constitute 50-80% of the tumor volume and is comprised of abundant extracellular matrix (ECM), fibroblasts, stellate cells, immune cells, nerve cells, growth factors and cytokines.[5] The ECM itself is composed of structural proteins, enzymes and proteins involved in cell communication.[5]

ACELLULAR COMPONENTS OF THE STROMAL COMPARTMENT

As mentioned above, one of the most important features of PDAC is the development of the desmoplastic reaction around tumor cells which is mainly due to excess ECM production produced by Pancreatic Stellate Cells (PSCs).[6] The ECM is composed of variety of fibrous proteins (*i.e.*, collagens), glycoproteins (*i.e.*, fibronectin), proteinases (*i.e.*, matrix metalloproteinase 9) and glycosaminoglycans (*i.e.*, hyaluron).[6] Additionally, modulators of the cell matrix interaction such as periostin, tenascin C, SPARC and thrombospondin are found in the PDAC desmoplastic reaction as well.[7] (Figures 1A and 1B)

One of the predominant proteins within the ECM is type I collagen which has been

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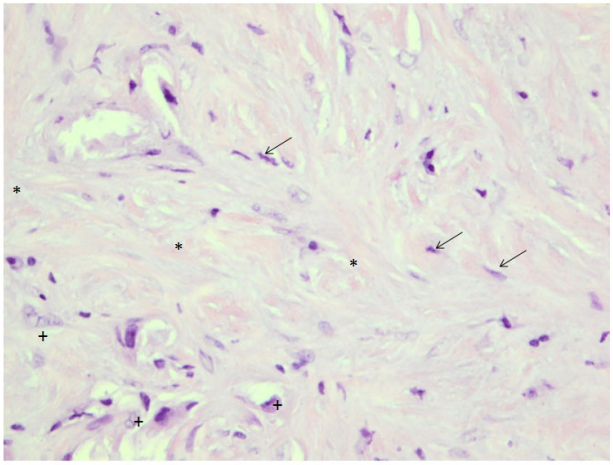

Figure 1A: Distinct desomplastic reaction in pancreatic cancer (*=Desmoplastic reaction; ⟵ =Pancreatic stellate cells; +=Single tumor cells) (H&E, 400x).

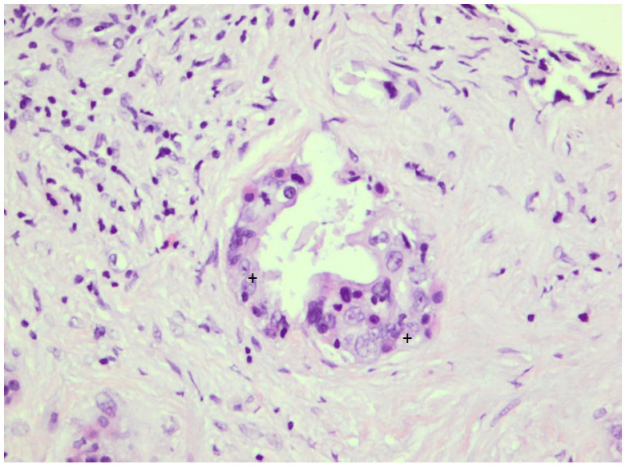

Figure 1B: Picture of pancreatic cancer with stromal reaction (+=Tumor cells) (H&E, 400x).

Figure 1: H/E Staining of pancreatic cancer with desmoplastic reaction (1A) and tumor cells (1B) (H&E, 400 ×). Pictures from Prof. Dr. med. Timo Gaiser, Pathology Institute, Medical Faculty Mannheim, University of Heidelberg, Germany.

shown to contribute to the generation of the malignant phenotype.[8] Specifically, it was demonstrated *in vitro* that type I collagen promotes cell adhesion, proliferation and maximal hypokinetic cell migration of cancer cell lines.[8] Moreover, 3 dimensional collagen I has been reported to contribute to gemcitabine resistance through MT1-MMP-mediated expression of HMGA2 in pancreatic cancer.[9]

Increased resistance towards anti-cancer drugs can also be achieved by attachment of PDAC cells to collagen I, IV and fibronectin. This decreases cytotoxicity of anti-cancer drugs such as 5-fluorouracil, cisplatin and doxorubicin. The exact molecular mechanism, however, must still be determined.[10]

Contact to collagen I was also associated with higher expression of N-Cadherin and activation of c-Jun-NH-2-terminal-kinase-1 in cancer cells, indicating its contribution to metastasis.[11] Other studies also showed that collagen I modulates transcription factors of epithelial to mesenchymal transition (EMT), namely Snail and E-Cadherin. This results in increased invasiveness of PDAC cells.[12,13]

Other ECM proteins believed to participate in tumor cell invasion and metastasis are matrix metalloproteinases (MMPs).[14,15] MMPs are a group of zinc-dependent endopeptidases with different substrate specificities.[16] Gelatinase subgroups of MMPs that include MMP-2 and MMP-9 are known to degrade type IV collagen. By this, they seem to promote cancer invasion.[17] Strong expression of MMP-2 has been observed in the stromal compartment of PDAC.[18] A high level of MMP-2 correlated with poor prognosis in pancreatic cancer patients.[19] Additionally, suppression of MMP-2 *via* RNA interference decreased PDAC cell invasiveness and adhesion.[20] It is noteworthy that overexpression of MMP-7, another important member of MMP family is involved in subsequent invasion of PDAC cells and PDAC metastasis.[21,22] It was shown that MMP-7 can induce cell dissociation and subsequent invasion of cancer cells in pancreatic cancer through activating EGFR mediated MEK-ERK signaling.[21]

Furthermore Hyaluronic acid (HA), a matrix glycosaminoglycan, is known to be synthesized and secreted by several PDAC cell lines. It is also presented in huge amounts in pancreatic cancer stroma.[23] Moreover, enzymatic degradation of HAlet to increased survival in a mouse model of PDAC.[24] Therefore HA might also be an interesting therapeutic target in pancreatic cancer.

Recently, periostin, an osteoblast-specific secretory protein has been suggested to promote invasiveness and resistance of PDAC cells.[25] This protein is produced by stromal cells rather than PDAC cells.[26] Although periostin suppressed the malignant phenotype of tumor cells at low concentrations, it increases migration when expressed at high levels.[26]

Tenascin-C (TN-C) is an ECM glycoprotein synthesized by PSCs and strongly expressed in pancreatic cancer.[27] Although its expression is low in normal adult tissue, the protein level rises dramatically under various physiological and pathological conditions, such as tissue remodeling, neovascularization and inflammation.[28] It has also been reported that TNC expression correlates with pancreatic cancer cell differentiation.[29] Taking into account that TNC promotes proliferation, migration and adhesion of poorly differentiated pancreatic cancer cell lines, it might play a role in PDAC infiltration and metastasis *in vivo*.[30]

Another important ECM glycoprotein, SPARC, is produced by both cancer cells and stromal cells and was shown to increase invasiveness of PDAC cells lines. It also correlates with patient survival. Therefore it seems to have an important pathophysiological role.[31,32]

One of the most notable extracellular macromolecules involved in PDAC invasion is thrombospondin-1 (TSP-1) which

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is mainly produced by stromal cells.[33,34] PDAC cells might contribute to TSP-1 secretion in relatively low amount. Its expression in cells can be regulated by Transforming growth factor-β1(TGF-β1).[34] It was reported that TSP-1 modulates PDAC cell invasiveness by upregulating MMP-1 and TIMP-1.[34] Therefore, it would be promising approach to further elucidate contribution of TSP-1 to malignant phenotype of pancreatic cancer.

CELLULAR COMPARTMENTS OF TUMOR STROMA

Fibroblasts

The most prominent cells of the stromal compartment of PDAC are activated fibroblasts, also known as Pancreatic Stellate Cells (PSCs).[6] PSCs are also part of the physiological stroma of the pancreas.[6] Their homeostatic role is still poorly understood, however they have been shown to contain fat droplets in their cytoplasm; indicating potential role in lipid metabolism.[35] In PDAC these cells become activated, obtain myofibroblastic phenotype, express α-smooth muscle actin (SMA) and gain the capacity to produce ECM components collagen I, III and fibronectin in significant amounts.[36] These phenotypic changes are induced by several cytokines and growth factors such as TGF-β1, activin A, IL-1, IL-6, platelet-derived growth factor (PDGF) and VEGF.[6] In addition, mitogenic factors like PDGF induce cell proliferation of PSCs. On the other hand, fibrogenic mediators like TGF-β1 and connective tissue growth factor (CTGF) induce the synthesis of collagens and fibronectin in activated PSCs.[37]

It was shown that direct co-culture of PSCs and PDAC cells promote PDAC cell proliferation through activation of Notch signaling pathway.[38] Moreover, co-injection of PSCs with PDAC cells has been shown to promote metastasis and angiogenesis in a mouse model of PDAC.[39] It was observed that palpable tumors developed earlier in mice injected with PDAC cell lines and PSCs compared to mice injected with cancer cell lines alone. In addition, tumors in mice injected with PDAC cell lines and PSCs together became significantly larger than those in controls.[39] But not only cancer growth is affected by PSCs. PSCs produce many factors such as collagen I, laminin, and fibronectin that are thought to promote acquired resistance to anticancer drugs such as 5-fluorouracil, cisplatin and doxorubicin. Therefore PSCs might also take part in chemotherapy resistance.[10]

Immune cells

It is now widely accepted that PDAC tissue is infiltrated with immune cells, such as T-cells, B-cells, NK cells, neutrophils, and macrophages as well as myeloid-derived suppressor cells (MDSCs).[40,41] Higher levels of CD8 positive T-cell infiltration have been shown to correlate with a better survival,[40,41] while macrophage and neutrophil infiltration as well as high levels of MDSCs have been reported to be associated with poor survival.[40] The infiltration of MDSCs in PDAC tissue leads to the establishment of antigen-specific T-cell tolerance, which might enable cancer cells to escape from immune surveillance.[42]

Evasion of the immune system is also a well-recognized feature of pancreatic cancer.[43] PDAC cells have been shown to evade host immune response by producing granulocte-macrophage colony-stimulating factor to suppress anti-tumor T-cell immunity.[43]

Recent studies suggest that PSCs may contribute immune evasion as well.[44] It has been reported that PSCs negatively modulate immune responses *via* reducing the migration of CD8 positive T-cells to cancer cells in human PDAC and the KPC mouse model of pancreatic cancer.[41] Additionally, PSCs have been shown to activate mast cells *in vitro* promoting tryptase and IL13 release from the latter. These mast cell-derived factors have been shown to stimulate cancer cell proliferation. Mast cells also induce PSC proliferation which is mediated by IL1.[45]

Neural cells

In contrast to immune cells, little is known about the neural elements of the desmoplastic reaction. Generally, perineural invasion (PNI) is the process of the cancer cell invasion of nerves and correlates with the extent of desmoplastic reaction in PDAC.[46] PNI is a common but not specific feature of PDAC and believed to correlate with poorer prognosis.[47] Most probably, it is a result of tumor cells migrating into the adjacent neural tissue where they cannot be removed during routine tumor resection, leading to tumor recurrence.[48] Moreover, nerve growth factor has been reported to enhance PDAC cell growth and invasion *in vitro* and *in vivo*.[49] Although cross-talk between PSCs and neural elements has not yet been widely studied, recently the effects of cancer cells on neurite outgrowth in the presence and absence of PSCs was assessed.[50] It was found that nerve invasion index and the Dorsal Root Ganglion (DRG) outgrowth index were significantly increased in the presence of PSCs compared with the absence of PSCs. Additionally, the authors further showed that the interaction between cancer cells and neural cells was likely mediated *via* a paracrine effect of Sonic Hedgehog ligand binding to the receptor Smo on PSCs with subsequent activation of PSCs. These data indicate that PNI might have a role in pancreatic aggressiveness and metastasis. Thus, further studies on PNI could improve survival prognosis of pancreatic cancer patients and also might lead new therapeutic approaches in PDAC treatment.

Accumulating evidence suggest that several neurotrophins including nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3) might promote tumor cell invasion and might be key mediators in the PNI pathogenesis.[51,52] For instance, NGF may stimulate cancer cell growth and may mediate nerve invasion through its interaction with TrkA, an NGF-specific receptor.[53] This may lead to the activation of the p44/42 MAPK signaling pathway and the upregulation of MMP-2, a pro-invasive mediator. It is also im-

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portant to note that NGF and its receptor TrkA are overexpressed in pancreas cancer cell lines as well as the perineurium of peripheral.[53]

Members of the glial cell-derived neurotrophic factor (GDNF) family promote pancreatic cancer invasion into peripheral nerves as wel.[54] In another study, GDNF up-regulated expression and enzymatic activity of MMP-9, thus promoting pancreatic cancer invasion.[55]

SIGNALING PATHWAYS IN PANCREATIC DESMOPLASTIC REACTION

Sonic Hedgehog

One of the signaling pathways known to mediate pancreatic desmoplastic reaction is Hedgehog signaling pathway.[56] Binding of the Hedgehog ligand (Sonic, Indian, and Desert Hedgehog) to its receptor Patched releases the co-receptor Smoothened from repression and results in translocation of the transcription factor Gli-1 from the cytoplasm to the nucleus where it regulates genes involved in cell differentiation, proliferation, apoptosis, adhesion, and migration.[57] In pancreatic cancer Smoothened is highly expressed by PSC, whereas Sonic Hedgehog ligand is expressed only by cancer cells.[58] Administration of cyclopamine, a Smoothened antagonist, in genetically engineered pancreatic cancer mouse model resulted in an extension of the overall median survival.[59] Moreover, IPI-926, a semisynthetic derivative of cyclopamine, has been shown to improve the delivery of chemotherapeutic agent to the tumours and an extension of the median survival.[60] However, the lack of translation of these encouraging preclinical results to the clinical setting arises questions about effectiveness of these models.

TGF-β

Another important signaling pathway in PDAC desmoplastic reaction is TGF-β signaling pathway.[61] In short, TGF-β binding to its type II-receptor (TβRII) results in recruitment and phosphorylation of type I receptor (TβRI), followed by activation of SMAD2 and SMAD3. SMAD2 and SMAD3 then bind to SMAD4 and translocate to the nucleus and regulate expression of TGF-β associated genes.[62] SMAD4 mutation is seen in an estimated 50% of PDAC tumors[63] and its deficiency combined with activated K-ras mutation has been shown to accelerate the activation of PSC and production of ECM.[64] In mouse models, overexpression of SMAD7, which negatively regulates TGF-β signaling, has been reported to reduce fibrosis and diminish activation of PSCs.[65] Taken together, these finding indicate that TGF-β and SMAD family proteins play an important role in PDAC desmoplastic reaction and might be interesting therapeutic targets.

THERAPEUTIC TARGETING OF DESMOPLASTIC REACTION IN PANCREATIC CANCER

Since accumulating evidence supports the essential role of the desmoplastic reaction in pancreatic cancer, strategies need to be developed to target not only cancer cells, but also the stromal compartment of the tumor.

The Hedgehog signaling pathway is believed to have a crucial role in PSCs activation.[56] As mentioned above, inhibition of Smoothened by cyclopamine increased survival rate in mouse models.[59] Another Smoothened inhibitor, AZD8542 was reported to reduce tumor volume, metastasis, and Hedgehog downstream signaling activity in an orthotopic model of pancreatic cancer.[58] In a recent clinical study, depletion of Sonic Hedgehog has been shown to decrease tumor stroma. On the other hand it increased tumor vascularity, resulting in an overall increase in aggressiveness of the cancer.[66]

Administration of nab-paclitaxel (combination of nanoparticle albumin bound paclitaxel) alone or in combination with gemcitabine has been reported to deplete the stroma in a patient-tumor-derived subcutaneous xenograft model.[67] The exact mechanisms behind these effects are still unknown. However, it is postulated that albumin in nab-paclitaxel is bound by SPARC, leading to accumulation of nab-paclitaxel near tumor cells.[68]

In tumor bearing KPC mice the enzymatic degradation of HA using PEG-ylated human recombinant PH20 hyaluronidase (PEGPH20) increased gemcitabine delivery to cancer cells and improved median survival.[24] Although Phase Ib clinical trial showed only a partial response in patients with advanced pancreatic cancer, a Phase II multi-centre randomized trial has been started to evaluate PEGPH20 as a first line therapy in metastatic pancreatic cancer patients.

Using a subcutaneous mouse model, the effects of olmesartan, an angiotensin II receptor blocker, were analyzed.[69] It was shown that olmesartam inhibited the subcutaneous tumor growth in these co-injected mice but not in controls injected only with tumor cells. Moreover, olmesartan decreased expression of α-smooth muscle actin, a marker of activated PSCs, and collagen deposition.[69]

It is known that activation of CD40 (a member of the TNF-α receptor superfamily) leads to activation of macrophages in tumor stroma. Therefore, the antitumor activity of CP-870,893, an agonist CD40 antibody, was investigated in combination with gemcitabinein advanced pancreatic cancer patients. Its combination with gemcitabine was associated with higher anti-tumor activity.[70] However, the results were too heterogeneous, particularly in terms of metastatic disease.[70] (Table 1)

CONCLUSION

Until recently, targeting cancer cells has been the main approach in pancreatic cancer treatment. However, recent studies suggested that desmoplastic reaction also contributes to the aggressiveness and growth of tumor in pancreatic cancer. There-

Element	Mechanism	Effect	Model	References
Cyclopamine	Inhibition of Smoothened	Survival rate↑	In mouse models	59
AZD8542	Inhibition of Smoothened	Tumor volume↓ metastasis↓	Orthotopic model of pancreatic cancer	58
Nab-paclitaxel	Is bound by SPARC, leading to accumulation of nab-paclitaxel near tumor cells (postulated).	Stroma↓	Patient-tumor-derived subcutaneous xenograft model	67,68
PEGPH20	Enzymatic degradation of HA	Gemcitabine delivery↑ Median survival↑	Tumor bearing KPC mice	24
Olmesartan	An angiotensin II Receptorblocker	Subcutaneous tumor growth↓ α-smooth muscle actin↓	Subcutaneous mouse model	69
CP-870,893	An agonist CD40 antibody	Anti-tumor activity↑	Advanced pancreatic cancer patients	70

Table1: Therapeutic targeting of desmoplastic reaction in pancreatic cancer (PEGPH20, PEG-ylated human recombinant PH20 hyaluronidase).

fore, targeting not only cancer cells, but also desmoplastic reaction components may generate novel therapeutic strategies in pancreatic cancer treatment.

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CONFLICTS OF INTEREST

We have no conflicts of interest.

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