Review Article

Multifunctional role of pancreatic stellate cells in pancreatic cancer

Alpha R. Mekapogu, Srinivasa P. Pothula, Romano C. Pirola, Jeremy S. Wilson, Minoti V. Apte

Pancreatic Research Group, South Western Sydney Clinical School, Faculty of Medicine, The University of New South Wales, Ingham Institute for Applied Medical Research, Sydney, Australia

Contributions: (I) Conception and design: All authors; (II) Administrative support: None; (III) Provision of study materials or patients: None; (IV) Collection and assembly of data: None; (V) Data analysis and interpretation: None; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

Correspondence to: Prof. Minoti V. Apte. Pancreatic Research Group, South Western Sydney Clinical School, Faculty of Medicine, University of New South Wales, Level 4, Ingham Institute for Applied Medical Research, Liverpool, NSW 2170, Australia. Email: m.apte@unsw.edu.au.

Abstract: Pancreatic ductal adenocarcinoma (PDAC) is the fourth leading cause of cancer related deaths with a dismal 5-year survival rate of only 8%. PDAC is characterised by extensive desmoplasia constituting about 50–80% of the tumour volume. Activated pancreatic stellate cells (PSC) are the major cellular source for stromal collagen; these cells drive pancreatic fibrosis and progression of PDAC. PSC are known to be activated by paracrine signals from several sources including injured epithelium, cancer cells, extracellular matrix, immune cells and nerve cells. Stromal-tumour interactions are now recognised as key processes in the development and progression of PDAC. Improved understanding of the mechanisms underlying stromal-tumour interactions may be the key for the discovery of new therapeutic targets in PDAC. This review summarises current knowledge regarding the role of PSCs in cancer biology and discusses the potential for development of novel therapeutic approaches targeting factors such as dysregulated signalling pathways, the stromal reaction itself and epigenetic changes in stromal and/or cancer cells.

Keywords: Pancreatic stellate cell (PSC); pancreatic cancer; stromal-tumour interactions; desmoplasia; tumour microenvironment

Received: 31 January 2019; Accepted: 24 May 2019; Published: 12 June 2019.
doi: 10.21037/apc.2019.05.02
View this article at: http://dx.doi.org/10.21037/apc.2019.05.02

Epidemiology of pancreatic cancer

Pancreatic ductal adenocarcinoma (PDAC) constitutes 90% of all pancreatic malignancies and is characterised by extensive desmoplasia and early metastasis. The risk factors for PDAC include a family history of the disease, cigarette smoking, chronic pancreatitis, obesity and diabetes mellitus (1,2). PDAC most commonly occurs in the 60 to 80 year age group, and its incidence is 50% higher in men than in women and (1) genetic mutations such as *BRCA2*, *CDKN2A*, *STK11*, *PRSS1*, *SPINK1*, *CFTR*, *PALB2* and ATM increase the risk for PDAC (3), patients with chronic pancreatitis have a 15 fold increased risk of developing PDAC (4), cigarette smoking increases the risk of PDAC by 75% even up to 10 years after cessation of smoking and (5) diabetes mellitus increases the risk by 30% up to 20 years after diagnosis (6). Obesity and high body mass index are positively correlated with the risk of developing PDAC (7). One meta-analysis showed a 19% increase in risk of developing PDAC in obese individuals (BMI ≥30 kg/m^2) compared to normal participants (BMI 22 kg/m^2) (8).

The genetic mutational landscape of PDAC involves the alteration of 69 genes which affect 12 core signalling pathways (9). Both oncogenes and tumour suppressor genes are mutated of which the major genes involved in PDAC are the oncogene Kristen Rat Sarcoma Virus (KRAS), and three tumour suppressor genes—*TP53*, *CDKN2A* and *SMAD4*. KRAS is the earliest mutation seen in low-grade pancreatic

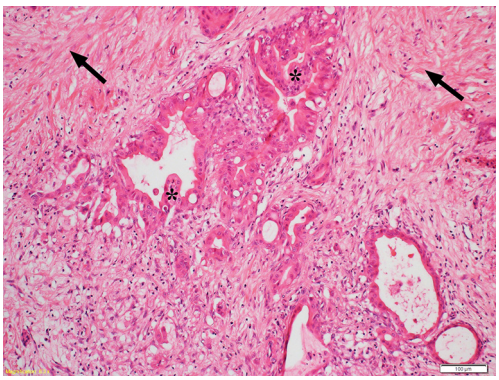

Figure 1 Desmoplasia in pancreatic cancer. A representative photomicrograph of a haematoxylin and eosin stained human pancreatic cancer section showing malignant elements (duct-like and tubular structures- indicated by asterisks) embedded in highly fibrotic stroma (indicated by arrows). Reprinted with permission from Elsevier (17).

intraepithelial neoplasia (PanIN) lesions, the precursors of PDAC (10). CDKN2A is also found early in neoplasia while SMAD4 and TP53 alterations occur late in tumorigenesis corresponding to grade 3 PanINs and invasive stages (11).

Despite its low incidence, PDAC is the fourth leading cause of cancer related deaths and, alarmingly is projected to become the second leading cause of cancer related deaths by 2030 (12). The high mortality in PDAC can be attributed to the presence of metastases even at the time of first diagnosis and the lack of effective treatments. Even with advances in current chemotherapy such as Folfirinox, and Gemcitabine-Abraxane, in addition to surgery and radiotherapy, the 5-year survival rate of PDAC has only marginally improved from 4% a decade ago to 8% (13,14). Surgical resection is the mainstay of treatment for prolonged survival (15) but unfortunately only 15–20% of cases are suitable for surgical resection because the majority of patients have visible metastatic disease at the time of diagnosis (16).

Role of stroma in pancreatic cancer

Desmoplasia is a striking feature of PDAC accounting for 50–80% of the tumour volume (*Figure 1*) (17). This stromal reaction consists of cellular components such as pancreatic stellate cells (PSCs), which are the main source of the stromal collagen (*Figure 2*) (18), fibroblasts, immune cells, vascular and neural elements and non-cellular components such as collagens, fibronectin, glycoproteins, proteoglycans, hyaluronic acid, cytokines, growth factors, and secreted protein acidic and rich in cysteine (SPARC) (19). The immune cells mostly comprise immunosuppressive leukocytes such as myeloid derived suppressor cells (MDSC) and macrophages (20).

Several studies have examined the influence of the stroma on the progression and outcome of PDAC (21,22). In a cohort of 233 patients who underwent surgical resection, Erkan *et al.* (23) demonstrated that overt stromal activity, as assessed by the expression of alpha smooth muscle actin (αSMA, a marker of activated PSCs) was associated with a poor prognosis. Similarly, Fujita *et al.* (24) observed that in 109 patients with PDAC, higher αSMA mRNA levels were correlated with poorer outcome. A stage-dependent influence of stroma on PDAC outcome was observed by Wang *et al.* (25) in a study involving 145 PDAC patients who underwent resection followed by gemcitabine-chemotherapy. The authors reported that increased expression of αSMA was significantly associated with poor outcome only during the early stages of the tumour development. Higher expression of other stromal factors such as annexin II, stromal tenascin C (26), interleukin-1 receptor-associated kinase 4 (IRAK4) expression (27), SPARC (28) and periostin (29) have also been reported to correlate with poor prognosis. Thus, these studies support the concept that stromal activity correlates with the progression of PDAC and a poor outcome.

In contrast to the above findings, Ozdemir *et al.* (30) observed that low expression of αSMA correlated with worse outcome, albeit in a relatively small cohort of 53 PDAC patients. In addition, Bever *et al.* (31) observed that high stroma density was associated with longer survival but that stronger expression of αSMA was not associated with differences in clinical outcome.

The apparent contradictory findings on the influence of stroma on PDAC progression in the above studies could be due to the differences in the methodology of assessment of stromal activity (stromal density *vs.* αSMA expression), differences in patient cohorts, and stage of the tumour (early *vs.* late) and a possible biphasic influence of stroma on tumour progression. Another factor that may be relevant to the disparate findings is the presence of functionally different subsets of PSCs within the stroma of pancreatic tumours. In this regard Yuzawa *et al.* (32) have reported the presence of fibroblasts with varying expression of αSMA and PDGFRβ, with higher expression of PDGFRβ being associated with a worse prognosis, but αSMA being unrelated to outcome. In another study, Ohlund *et al.* (33) showed that the PSC-derived cancer-associated fibroblasts

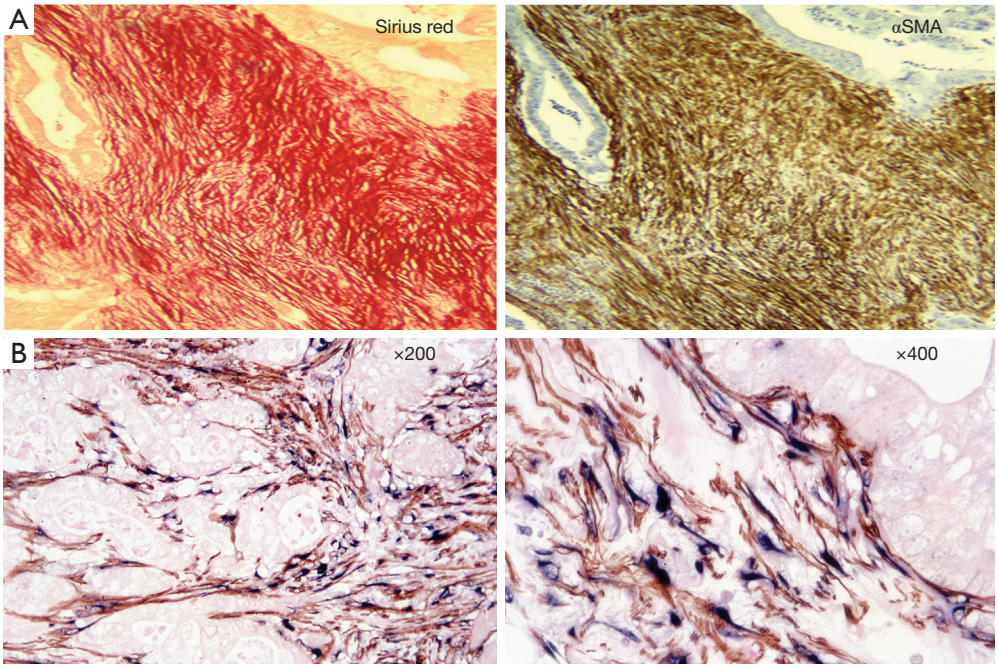

Figure 2 Pancreatic stellate cells are the major source of collagen in the stroma of pancreatic cancer. (A) A representative pair of serial sections of human pancreatic cancer showing stromal areas with strong positive staining for collagen as well as for αSMA, indicating the presence of activated PSCs in the fibrotic reaction in pancreatic cancer. Original magnification, ×100; (B) Colocalisation of α-SMA (brown), a marker for activated PSCs and procollagen α1 mRNA (blue, *in situ* hybridisation), restricted to stromal areas of human pancreatic cancer tissue (low and high power views) indicating that activated PSCs are the major source for collagen in the stroma of pancreatic cancer. Original magnification, ×200 and ×400 respectively. Reprinted with permission from Wolters Kluwer Health (18).

(CAFs) close to cancer cells exhibited increased αSMA expression, but those at the periphery of the tumour, (away from the tumour cells), lacked αSMA expression, secreted inflammatory mediators including IL-6 and were correlated with poor outcome. Overall, the weight of evidence supports the view that the stroma exerts significant influence on the progression of PDAC, suggesting a need for selectively targeting and reprogramming the stroma to help improve patient outcomes.

PSCs in the healthy pancreas

The finding by Apte *et al.* (18) that the collagenous stroma of the pancreas is produced by PSCs has led to numerous studies of the functions of this cell. We now know that in the normal pancreas, PSCs are stellate-shaped cells located around pancreatic acini and account for 4–7% of all parenchymal cells in the gland (34). In health, PSCs manifest a quiescent phenotype characterised by abundant vitamin A-containing lipid droplets in the cytoplasm. PSCs are identified by immunostaining for selective markers, such as desmin, glial fibrillary acidic protein (GFAP), nestin, neural cell adhesion molecule, nerve growth factor and synemin (35-37). Although the origin of PSCs is not clear, it was demonstrated that a proportion of PSCs originate from bone marrow and replenish PSCs in the pancreas (38). In addition, Ino *et al.* (39) showed that chemokine receptor type 2 ($CCR2^+$) monocytes migrate from the bone marrow and transform into PSCs through the monocyte chemoattractant protein-1 (MCP-1)/CCR2 pathway. The monocyte lineage origin of PSC is also supported by the expression of monocyte specific marker α-naphthyl butyrate esterase (ANBE) (40).

PSCs play key roles in both the healthy and diseased pancreas. In normal pancreas, PSCs express Toll-like receptors (TLRs) 2, 3, 4 and 5 (41) and have the ability for phagocytosis (42) and play a part in innate immunity as first-line defense against early injury. PSCs also secrete matrix metalloproteinases such as MMP2, MMP9 and MMP13 and metalloproteinase inhibitors such as TIMP1

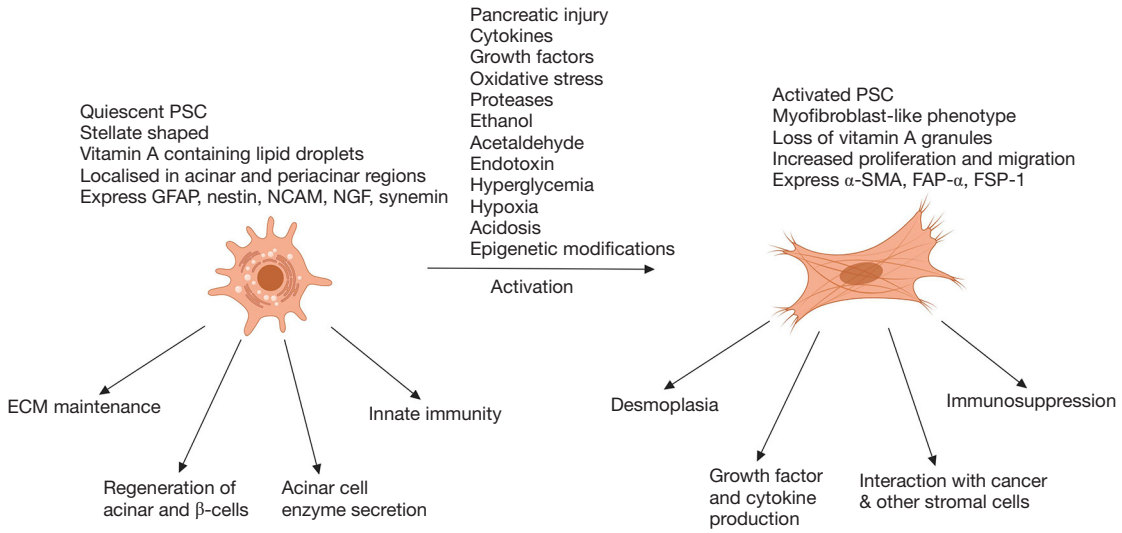

Figure 3 Biology and activation of pancreatic stellate cells. Quiescent PSCs exhibiting vitamin A-containing lipid droplets are localised in around pancreatic acini and islets and express selective markers such as GFAP, nestin, NCAM, NGF and synemin. During health, quiescent PSCs are involved in ECM maintenance and may also play a role in regeneration of β-cells, innate immunity and acinar secretion. During pancreatic injury, quiescent PSCs are activated by several factors and acquire a myofibroblast-like phenotype, lose vitamin A and express α-SMA, FAP-α and FSP-1 markers. Activated PSCs produce excess ECM leading to desmoplasia, interact with cancer and other cells in the stroma through increased cytokine production, and also cause immunosuppression. FAP-α, fibroblast activation protein alpha; FSP-1, fibroblast-specific protein-1; GFAP, glial fibrillary acidic protein; NCAM, neural cell adhesion molecule; NGF, nerve growth factor; PSC, pancreatic stellate cell; α-SMA, alpha-smooth muscle actin.

and TIMP2 to maintain the balance of extracellular matrix, which is usually disrupted after activation in pancreatitis and PDAC. Several *in vitro* and *in vivo* studies suggest that PSCs may have progenitor-like capabilities, possessing ATP-binding cassette G2 (ABCG2) and they transform into insulin-secreting cells upon exposure to the relevant culture medium (40,43-46).

Response of PSCs to injury

During pancreatic injury, PSCs are activated, a process associated with loss of the vitamin A-containing lipid droplets and transformation to a myofibroblast-like phenotype with expression of the activation marker alpha smooth muscle actin (αSMA), fibroblast activation protein-α (FAP-α), fibroblast-specific protein-1 (FSP-1), and fibrinogen (35). Apte *et al.* (17,37,47) were the first to demonstrate that the collagenous stroma in pancreatic injury was produced by PSCs. Platelet derived growth factor (PDGF) induces differentiation of PSCs into the myofibroblast phenotype (*Figure 3*) (32,48). In addition, activated PSCs express intercellular adhesion molecule (ICAM)-1, produce cytokines including interleukins 6, 7, and 8 and monocyte chemoattractant protein (MCP), and induce angiogenesis (34,49-51).

PSCs can be activated by several mediators such as pro-inflammatory cytokines (52), oxidant stress (53,54), ethanol and its metabolites, acetaldehyde and fatty acid ethyl esters (55,56), fatty acids (oleate) (57) and endotoxins (58). Other PSC activating factors of particular relevance to PDAC include acidosis (59), hypoxia (60), increased interstitial pressure (61), and hyperglycaemia (62). Recently, epigenetic modifications (increased acetylation of histones) have been shown to play an important role in the activation of PSCs and collagen synthesis (63). Bromodomain extra terminal domain (BET) proteins bind to acetylated motifs on histones leading to gene activation via recruitment of transcription factors and other chromatin regulators (64). Kumar *et al.* (63) demonstrated that BET proteins are expressed in PSCs and that pan inhibition of BET proteins (subtypes BRD 2, 3, and 4) using JQ1, a BET inhibitor leads to PSC quiescence and decreased collagen I synthesis. Furthermore, using specific siRNA for the BRD subtypes, the authors found that collagen synthesis was positively regulated by BRD4 but negatively regulated by BRD 2 and 3.

Mechanical properties of the local environment have been shown to play a role in activating PSCs. Using a physiomimetic system to recapitulate the mechanical microenvironment, Lachowski *et al.* (65) demonstrated that matrix stiffness can regulate the activation and migration of PSCs. These findings implicate the role of mechanical stimulation by the microenvironment in the progression PDAC through activation of PSC and fibrosis.

Interactions of PSCs and cancer cells

Apte *et al.* (18) established that activated PSCs are primarily responsible for the characteristic desmoplasia in PDAC. Activation of PSCs is considered an early event in PDAC due to the presence of activated PSCs expressing periostin—a cell adhesion protein, galectin-1 (a glycan binding protein) and αSMA (an activation marker) in precursor lesions such as PanINs (66) and intra-ductal papillary mucinous neoplasms (IPMN) (67). It is now established that PSCs and tumour cells have bidirectional effects on each other. On one hand, pancreatic cancer cells (PCCs) promote PSC proliferation, migration and extracellular matrix synthesis (68,69) while on the other hand PSCs promote PCC progression, migration, and tumour cell survival (68,70).

The complementary bidirectional influence of PCCs and PSCs has been well demonstrated by both *in vitro* and *in vivo* studies. In co-culture studies, PCCs increased PSC proliferation and ECM synthesis mediated by PDGF, FGF2, and TGFβ1 (71). PCCs also promoted the secretion of matrix metalloproteinases by PSCs (72) through ECM metalloproteinase inducer (EMMPRIN) secretion (73) and TGFβ1 signalling (74). It is interesting to note that cancer cells induce autophagy in PSCs resulting in the degradation of proteins and release of amino acids such as alanine which then acts as an alternative carbon source for the TCA cycle and lipid synthesis in cancer cells. As a result, cancer cells have decreased dependency on glucose and glutamine in the nutrient-poor, and hypoxic environment of PDAC (75). The process of autophagy also activates PSCs leading to increased stromal synthesis. Endo *et al.* (76) demonstrated that knockdown of autophagy proteins in PSCs led to a decrease in ECM and IL-6 production *in vitro* and that co-administration of these PSCs with PCCs led to smaller tumours and fewer metastases in nude mice. The authors also observed that in 133 patient-derived PDAC samples, autophagy significantly co-related with tumour growth, invasion, metastases and poor outcome.

PSCs influence several critical steps in PDAC progression. PSCs induced epithelial mesenchymal transition (EMT) leading to increased invasion and migration of PCCs (77,78), and formation of a cancer stem cell (CSC) phenotype (79) (*Figure 4*). Although PSCs exhibit functionally diverse subsets with differential influence on PDAC progression (22), stromal signalling appears to be indispensable for PDAC progression. Indeed, Sherman *et al.* (80) clearly demonstrated that Kras mutation-induced signalling (the major gene mutation involved in PDAC) alone was insufficient to drive oncogene transcription and required permissive signalling from the stroma for the development of PDAC.

In animal models, co-injection of PCC with PSCs resulted in larger tumours with significant desmoplasia compared to injection of PCCs alone in both subcutaneous xenograft models (71) and orthotopic models (68,70,81); this increase in tumour size was due in part to PSC-induced PCC proliferation. The overexpression of the serine protease inhibitor SERPINE2 by PCCs was reported to be responsible for the increased tumour growth *in vivo* in the presence of PSCs (82).

PSCs also have a facilitatory role in metastasis of tumour cells. In a gender mismatch study, Xu *et al.* (69) demonstrated the presence of PSCs in metastatic nodules suggesting dissemination of PSCs via the circulation to distant organs, possibly facilitating the seeding and growth of cancer cells at these sites. Similar observations were made by Suetsugu *et al.* (83) who observed that both PSCs and PCCs co-migrated from the spleen (the site of injection), to the metastatic nodules. Storck *et al.* (84) demonstrated that calcium-gated potassium 3.1 channels are crucial for the process of migration in PSCs and blocking these channels abolished both migration and chemotaxis of PSCs. In addition to migrating PSCs, local hepatic stellate cells in the liver also aid the formation of a metastatic niche for migrating PCCs (85).

Recently, exosomes have emerged as important mediators of the bidirectional interactions between stellate cells and tumour cells. Takikawa *et al.* (86) demonstrated that exosomes derived from PSCs contained a variety of microRNAs and an abundance of miR-21-5p and miR-451a and mediated proliferation and migration of PCCs. Similarly, exosomes derived from PCCs stimulated the activation, proliferation, and migration of PSCs through upregulation of transforming growth factor β1 (TGFβ1) and tumour necrosis factor (TNF) (86). The signalling pathways involved in the PSC and PCC interactions are

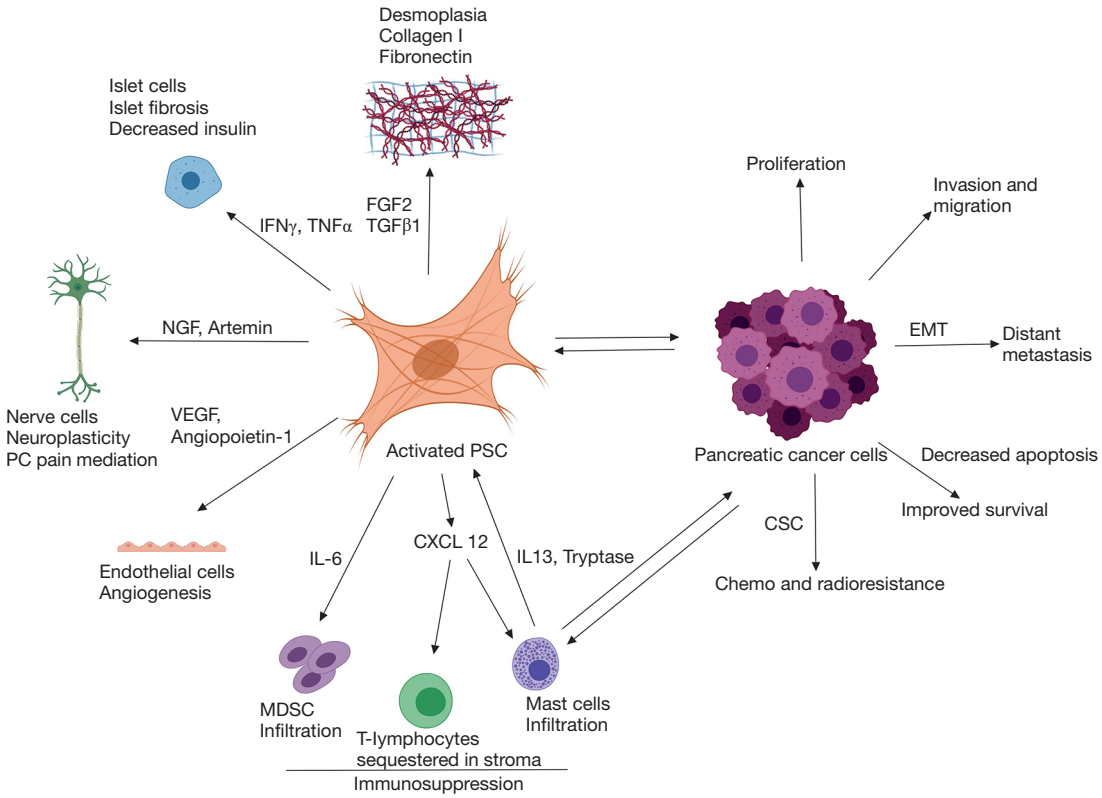

Figure 4 Interactions of activated pancreatic stellate cells with tumour and stromal cells. Activated PSCs are involved in crosstalk with tumour cells that is mediated by several cytokines, leading to proliferation, invasion, metastasis, improved survival, and chemoresistance as well as radio-resistance of cancer cells. In turn, tumour cells promote ECM synthesis, proliferation, and migration of PSCs. Activated PSCs are primarily involved in the synthesis of collagen I and fibronectin leading to desmoplasia. Activated PSCs also lead to fibrosis of islets and decrease insulin production from β-cells, thereby possibly playing a role in diabetes mellitus. During pancreatic cancer, neurotrophic factors secreted by PSCs are involved in nerve cell plasticity and neurogenic pain. Angiogenic factors secreted by PSCs cause increased angiogenesis. Activated PSCs play a crucial role in creating an immunosuppressive environment by interacting with immune cells leading to T-cell sequestration and MDSC and mast cell infiltration. CSC, cancer stem cells; CXCL, chemotactic chemokine ligand; CXCR, chemotactic chemokine receptor; EMT, epithelial mesenchymal transition; FGF, fibroblast growth factor; IFNγ, interferon gamma; IL, interleukin; NGF, nerve growth factor; MDSC, myeloid derived suppressor cells; PSC, pancreatic stellate cells; TGFβ, transforming growth factor beta; TNFα, tumour necrosis factor alpha; VEGF, vascular endothelial growth factor.

summarised in *Table 1*.

Interactions of PSCs with other cells in the stroma

PSCs interact with other stromal cells such as endothelial cells, immune cells, neural cells and islet cells in the stroma. PSCs interact with endothelial cells by producing proangiogenic factors such as vascular endothelial growth factor (VEGF) (69), periostin (29), angiopoietin-1 (60), and Hepatocyte growth factor (HGF) (103) which are shown to induce endothelial proliferation *in vitro*. On the other hand, PSCs also display antiangiogenic features such as (I) secretion of collagenous stroma (104,105), (II) expression of vasohibin-1, which acts as negative feedback for VEGF-induced gene expression (60,106), and (III) induction of PCCs to secrete endostatin (104). Taken together, the bi-functional role of PSCs in angiogenesis may play a role in the selective vascularisation in PDAC, wherein tumours are usually better perfused at the periphery (leading edge) and less perfused towards the core (107). While pro-angiogenic factors expressed by PSCs are responsible for the hypervascularity, cancer cell-secreted endostatin is implicated in the hypo-vascularity of the juxta-tumoral stroma (108). Furthermore, targeting PSCs has been

Table 1 Signalling pathways reported to be involved in the interaction between PSCs and PCCs

Signalling pathway	Mediator	Functional role	Inhibitor/activator used	Reference
Rho-ROCK pathway	Rho-associated protein kinase	α-SMA expression, proliferation, chemotaxis, type I collagen, fibrosis	Y-27632: a specific ROCK inhibitor	(87)
Mitogen-activated protein kinase (MAPK) signalling pathway	MAPK	PSC proliferation, TIMP-1 production, α-SMA	U0216: a specific MAP kinase (MEK) inhibitor	(88)
PI3-Kinase pathway	PDGF	PSC migration and proliferation	Wortmannin: a specific PI3-kinase inhibitor	(89)
SMADS	SMAD-2,3	PSC activation, proliferation, ECM deposition, transdifferentiation, TGF-β1 expression	PD98059: a specific inhibitor of mitogen-activated protein kinase (MEK1)	(90)
Protein kinase C	Hyperglycaemia	PSC proliferation, α-SMA, collagen-I production, angiogenesis	Calphostin C: a protein kinase C (PKC)-specific inhibitor	(91)
Peroxisome proliferator-activated gamma (PPARγ)	PPAR-γ ligands	Inhibition of PSC activation, proliferation, and collagen synthesis; increased phagocytosis	siRNA	(42)
Hedgehog pathway	Smo, Gli	PSC activation, ECM synthesis, migration, desmoplasia, angiogenesis; PCC proliferation, migration and chemoresistance	NVP-LDE225: a Hh pathway inhibitor	(92)
Wnt/β-catenin signalling	β-catenin	PSCs promote invasion of PCCs	Retinoic acid	(93)
Vitamin D receptor	Vitamin D	Inhibition of PSC activation; decreased chemoresistance	Calcipotriol: a vitamin D receptor activator	(94)
Toll-like receptor (TLR) signalling	TLR9	Expression of PSC-derived cytokines (e.g., CCL3, CCL11) immunosuppression	ODN1826: a TLR9 ligand	(95)
Periostin pathway	Periostin	Periostin secreted by PSCs promotes PCC proliferation, EMT and resistance to nutrient deprivation and hypoxia	Erlotinib: an EGFR inhibitor and SCH772984: an Erk inhibitor	(96)
Hypoxia inducible factor 1 (HIF-1)	CCL2	PSC activation, macrophage recruitment	HIF-1α siRNA	(97)
HGF/c-MET pathway	HGF	PSC promote proliferation and metastasis of tumour cells	AMG 102: monoclonal antibody for HGF; compound A: small molecule inhibitor of c-MET	(81)
IL6/JAK/STAT	IL-6	PSC activation and proliferation	Ruxolitinib: a Jak1/2 inhibitor; MEK162: a MAPK inhibitor	(98)
Integrin	Kindlin-2	Increased cytokines production in PSCs facilitating progression and migration of PCC	Kindlin-2-knockdown	(99)
Galectin-1	PSC-derived SDF-1	Proliferation of PSC and chemokine secretion facilitating PCC metastasis	BAY 11-7082: a NF-κB inhibitor; AMD3100: a CXCR4 blocker	(100)
Reactive oxygen species (ROS)	Suppression of miRNA-21	PSC activation and induction of glycolysis	Resveratrol	(101)
CXCL12 (SDF-1) signalling	PSCs-derived CXCL12 (SDF-1)	Immunosuppression by preventing $CD8^+$ T cells	AMD3100: an SDF-1/CXCL12 inhibitor	(102)

α-SMA, alpha smooth muscle actin; c-MET, tyrosine-protein kinase of Met; CCL, chemokine ligand; CXCL, chemotactic cytokine ligand; ECM, extracellular matrix; JAK/STAT, Janus kinase/signal transducers and activators of transcription; HGF, hepatocyte growth factor; PSC, pancreatic stellate cells; PCC, pancreatic cancer cells; SDF, stromal derived factor; PDGF, platelet-derived growth factor; SMAD, small worm mothers against decapentaplegic.

demonstrated to reduce neo-vascularisation *in vivo* (81).

PSCs are involved in immune modulation in PDAC. PSCs express TLRs 2, 3, 4 and 5 and take part in innate immunity (41). Notably, PSCs play an important role in creating an immunosuppressive environment via the secretion of proinflammatory cytokines which mediate a number of effects on immune cells including:

(I) Sequestration of cytotoxic $CD8^+$ T-cells (thereby preventing them from acting on cancer cells), mediated by CXCL12 produced by PSCs;
(II) Infiltration of $CD4^+$ $Foxp3^+$ Tregs cells and reduced T-cell and NK-cell mediated cytolysis, associated with increased production of the chemokine IP-10;
(III) Elevated MDSC infiltration and differentiation of MDSCs to a $CD11b^+$ $CD33^+$ phenotype which suppress T-lymphocyte proliferation; the latter effect is induced by IL6 produced by PSCs;
(IV) T cell apoptosis and Th2 cytokine secretion—effects mediated by galectin-1 which is overexpressed in PSCs;
(V) Mast cell infiltration (109) resulting from PSC mediated activation of mast cells which in turn further activate PSCs through IL-13 and tryptase.

As noted earlier, PSCs express the neural marker GFAP and secrete several neurotrophic factors such as nerve growth factor (NGF) and brain derived neurotropic factor (BDNF) (110,111). PSCs are implicated in the perineural invasion and migration of tumour cells along nerve axons (112) and may contribute to the pain of PDAC through the production of the neurotropic factor TRPV1 (111). These effects are mediated by activation of the sonic hedgehog pathway in both PCCs and PSCs.

As mentioned earlier, diabetes is a major risk factor for PDAC. The role of PSCs in islet cell dysfunction is suggested by the identification of activated PSCs in and around fibrosed islets in a diabetic rat model and by the findings that pancreatic β-cells co-cultured with PSCs exhibit decreased insulin expression and increased apoptosis (113). The role of PSCs in new onset diabetes (which has been recently recognised as an important predictive factor for PDAC development) is as yet unknown. While adrenomedullin a 52-amino acid peptide produced by PDAC cells has been implicated in PDAC associated new onset diabetes due to the ability of the peptide to inhibit insulin secretion (114), the influence of adrenomedullin on PSC function remains to be investigated.

Recently, the role of PSCs in ECM remodelling is gaining attention. Using an *in vitro* 3D spheroid tumour model and advanced imaging techniques, Drifka *et al.* (115) demonstrated the permissive role of PSCs in fashioning the alignment of collagen fibres to facilitate the invasion and co-migration of cancer cells through the stroma. Similarly, Sada *et al.* (116) also using a 3D culture setting, have reported that PSCs, in response to hypoxia, reorganised collagen fibres to a parallel alignment facilitating cancer cell migration. These interactions between PSCs and ECM are shown to be dependent on cluster of differentiation 51 (CD51) an integrin expressed in PSCs (117) which is also associated with aggressive disease and poor outcome. Other stromal proteins that are involved in PSC-ECM interactions include lumican, which enhances PSC adhesion and mobility (118) and periostin, which promotes cancer cell proliferation and metastasis (119).

PSCs and chemo and radio resistance

PDAC is highly resistant to chemotherapy as well as radiotherapy. Extensive desmoplasia combined with fibrosis mediated hypoxia leads to impaired drug delivery and chemoresistance (60,120,121). PSCs play an important role in the development of chemoresistance through various mechanisms. Activated PSCs secrete cytokines and large amounts of ECM rich in laminin, desmin, and collagen I/III (34) leading to fibrosis and decreased vascularity which hampers the translocation of drugs to cancer cells. When co-injected with cancer cells into mouse pancreas, PSC promote proliferation, migration, and reduced apoptosis of cancer cells, leading to large tumour volumes contributing to chemoresistance (68,70,122). Furthermore, PSCs are known to induce stemness in cancer cells which may also contribute to resistance of the cancer cells to chemotherapy (79). Stromal-derived factor-1 acting through the CXCR4 receptor was hypothesised to help in the evasion of gemcitabine-induced apoptosis of tumour cells in PDAC (123). PSCs are shown to increase the resistance of tumour cells to gemcitabine through increased expression of hairy and enhancer of split-1 (Hes1) in the notch signalling pathway (124).

A more recent report indicates that PSCs in primary tumours can internalise and store the active form of gemcitabine within their cytoplasm (due to low expression of inactivating enzymes), consequently preventing the drug from reaching tumour cells (125).

PSCs are also implicated in resistance of PDAC to radiotherapy. Mantoni *et al.* (126) demonstrated a radioprotective effect of PSCs on PCCs through β1-integrin signalling. On the other hand, PSCs also enhance the stem

cell phenotype in cancer cells through EMT mediated by TGFβ leading to radiotherapy resistance (127).

Targeting stromal-tumour interactions

Despite the recent experimental advances in combinations of chemotherapeutic agents, the impact on the survival of patients with PDAC is disappointing. We assert that the failure of current treatments may be partly due to the fact that stromal-tumour interactions have largely been ignored in drug development efforts in the past. However, with the increasing recognition of the critical role of the stroma in PDAC progression, several studies targeting stromal-tumour interactions have recently been reported, with encouraging results in the pre-clinical phase, and with some being taken to early clinical trials. These are summarised as follows.

Hedgehog pathway

The hedgehog pathway in PDAC manifests as overexpression of the ligand sonic hedgehog (Shh) in tumour cells with overexpression of its receptor Smoothened (Smo) mainly in cancer-associated fibroblasts (128). In murine models, ormeloxifene (129) and IPI-926 (130) were successful in targeting the Hh pathway resulting in decreased stroma, reduced tumour volume and increased sensitivity to co-administered gemcitabine. Rucki *et al.* (92) used KPC mice to demonstrate that dual inhibition of sonic hedgehog (Hh) and HGF/c-MET pathway sensitises PDAC to gemcitabine. However, targeting the Hh pathway in a clinical trial GDC-0449 using the inhibitors vismodegib, saridegib and sonidegib failed to yield a significant survival benefit (131).

HGF/c-MET pathway

HGF and its receptor c-MET are increasingly recognised for their role in stromal-tumour interactions in PDAC. In PDAC, HGF is produced by PSC while its receptor is expressed on cancer cells and this pathway is involved in cancer cell proliferation, invasion and metastases (103,122). Recent studies report that inhibition of the HGF/c-Met pathway in an orthotopic mouse model developed by co-injection of cancer cells and PSC resulted in decreased tumour size and elimination of metastasis (81). Li *et al.* (132) observed that treatment with a combination of c-Met inhibitor XL184 and gemcitabine in a subcutaneous xenograft mouse model significantly decreased cancer growth, while individual treatments with either XL184 or gemcitabine were ineffective in decreasing the growth rate. Pothula *et al.* (81,122) showed that inhibition of both arms of HGF and c-Met pathway along with gemcitabine completely eliminated metastasis in an orthotopic mouse model strongly resembling human disease. The authors also found that gemcitabine alone stimulated stemness and aggressiveness of cancer cells suggesting the need for combination therapy. These findings have been corroborated by other studies which showed that gemcitabine treatment alone increased CSC numbers in PDAC (133,134).

A recent study suggests that c-MET downstream signalling is important for the development of an aggressive tumour phenotype. Lomberk *et al.* (135) examining epigenetic landscapes in PDAC showed that tumour populations can be grouped into two distinct epigenomic landscapes namely less aggressive "classical type" and more aggressive "basal type" tumours. Further, utilising super-enhancer mapping coupled with transcription factor binding motif and upstream regulatory analyses, the authors demonstrated that downstream MET signalling is involved in tumour proliferation and EMT in "basal type" tumours and that genetic inactivation of MET using siRNA, shifts the epigenotype towards a less aggressive "classical type" tumour.

The above studies suggest that combination treatments involving the targeting of the HGF/c-MET pathway plus a chemotherapeutic agent may be a promising approach to treat PDAC.

Vitamin A

Activated PSC lose cytoplasmic vitamin A (retinol) and transform into a myofibroblast phenotype leading to increased ECM synthesis and fibrosis (18). Han *et al.* (136) used a tumour microenvironment activated nano system to co-deliver all trans retinoic acid (a metabolite of retinol) and siRNA targeting heat shock protein 47 (HSP 47) to target both activated PSCs and ECM in an orthotopic pancreatic tumour mouse model developed by co-inoculation of luciferase-expressing Panc-1 cells and PSCs. The nanosystem induced quiescence of activated PSCs, inhibited ECM proliferation and improved the efficacy of concomitant gemcitabine treatment.

Vitamin D

Sherman *et al.* (94) found that activation of the vitamin D receptor (VDR) with calcipotriol in combination with gemcitabine induced quiescence of activated PSCs by

inducing Fabp4 (a PSC quiescence marker) and markedly reduced markers of inflammation and fibrosis in KPC mice (a transgenic PDAC model). On the other hand, vitamin D3 is reported to induce differentiation of immature $CD34^+$ myeloid cells into dendritic cells thereby promoting tumour immunity (137). Stemming from the studies reported by Sherman *et al.* (94) a vitamin D analogue paricalcitol, is currently under clinical trial in combination with gemcitabine, and nab-paclitaxel (NCT03520790).

Epigenetic targeting

Epigenetic modifications play an important role in the growth and progression of PDAC. As these modifications are reversible, targeting them offers new therapeutic opportunities in PDAC. Some studies have shown that targeting epigenetic pathways could help in reprogramming of the tumour microenvironment (138,139) to the detriment of the tumour. DNA methylation, histone modifications, bromodomain and extra-terminal domain (BET) family proteins are the common targets of therapy in PDAC. 5-Azacitidine, a DNA methyl transferase inhibitor is currently being evaluated in a Phase II clinical trial in PDAC resected patients with node positive disease and elevated CA 19-9 (NCT01845805).

Conclusions

In conclusion, there is growing consensus that PSCs have diverse roles in both health and disease and that PSC-cancer cell interactions are central to the progression of PDAC. Many studies are now targeting PSC activation and tumour-stromal interactions for developing better treatments for pancreatic cancer. Better understanding of PSC activation and their interaction with cancer and other stromal elements will aid in mitigating chemoresistance and developing novel therapies in the future for PDAC.

Acknowledgments

Figures 1,*2* are reprinted with permission from the publishers Elsevier and Wolters Kluwer Health. *Figures 3*,*4* were created using BioRender.
Funding: None.

Footnote

Provenance and Peer Review: This article was commissioned by the Guest Editor (Min Li) for the series "Science on Pancreatic Cancer" published in *Annals of Pancreatic Cancer*. The article has undergone external peer review.

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/apc.2019.05.02). The series "Science on Pancreatic Cancer" was commissioned by the editorial office without any funding or sponsorship. All authors report grants from Avner Foundation, grants from National Health and Medical Research Council of Australia, during the conduct of the study. The authors have no other conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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doi: 10.21037/apc.2019.05.02
Cite this article as: Mekapogu AR, Pothula SP, Pirola RC, Wilson JS, Apte MV. Multifunctional role of pancreatic stellate cells in pancreatic cancer. Ann Pancreat Cancer 2019;2:10.