Pancreatic stellate cell: Pandora’s box for pancreatic disease biology

Pancreatic stellate cells (PSCs) were identified in the early 1980s, but received much attention after 1998 when the methods to isolate and culture them from murine and human sources were developed. PSCs contribute to a small proportion of all pancreatic cells under physiological condition, but are essential for maintaining the normal pancreatic architecture. Quiescent PSCs are characterized by the presence of vitamin A laden lipid droplets. Upon PSC activation, these perinuclear lipid droplets disappear from the cytosol, attain a myofibroblast like phenotype and expresses the activation marker, alpha smooth muscle actin. PSCs maintain their activated phenotype via an autocrine loop involving different cytokines and contribute to progressive fibrosis in chronic pancreatitis (CP) and pancreatic ductal adenocarcinoma (PDAC). Several pathways (e.g., JAK-STAT, Smad, Wnt signaling, Hedgehog etc.), transcription factors and miRNAs have been implicated in the inflammatory and profibrogenic function of PSCs. The role of PSCs goes much beyond fibrosis/desmoplasia in PDAC. It is now shown that PSCs are involved in significant crosstalk between the pancreatic cancer cells and the cancer stroma. These interactions result in tumour progression, metastasis, tumour hypoxia, immune evasion and drug resistance. This is the rationale for therapeutic preclinical and clinical trials that have targeted PSCs and the cancer stroma.

Key words: Pancreatic stellate cells; Pancreatic fibrosis;
Pancreatic cancer stroma; Physiological functions; Pancreatic stellate cells-cancer-stromal interactions; Therapeutic targets

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Core tip: Pancreatic stellate cells (PSCs) have emerged as one of the major effector cells in chronic pancreatitis and pancreatic ductal adenocarcinoma. In this review, we discuss the physiological function of PSCs and the profibrogenic mechanisms. We also discuss various pathways, transcription factors and miRNAs implicated in the inflammatory and profibrogenic functions mediated by PSCs. We further discuss the crosstalk among PSCs, pancreatic cancer cells and pancreatic cancer stroma and mechanisms that lead to cancer progression, metastasis, tumour hypoxia, immune evasion and drug resistance. We conclude with recent preclinical and clinical studies that have targeted PSCs and cancer stroma.

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HISTORICAL PERSPECTIVES

Stellate cells were described for the first time in the perisinusoidal spaces of the liver by Karl Wilhelm Von Kupffer in 1876 and were called "Sternzellen" (meaning star shaped cells). Later in 1951, Ito described the presence of lipid droplet containing cells in the perisinusoidal spaces of the liver and named them "Ito cells"[1]. The Ito cells were shown to emit blue-green fluorescence due to the presence of vitamin A in the lipid droplets[2]. Later in 1971, the usage of multiple techniques provided unequivocal evidence that the "sternzellen" reported by Kupffer and "Ito cells" identified by Ito were the same cell type: the hepatic stellate cells (HSCs)[3,4]. In 1982, a cell type carrying vitamin A containing lipid droplets and exhibiting a transient blue-green fluorescence were described in mouse pancreas[5]. In 1991, the cells exhibiting the vitamin A autofluorescence were identified in the healthy pancreatic sections from humans and rats[6]. These cells are now identified as pancreatic stellate cells (PSCs), which localize the periacinar regions, with long cytoplasmic projections extending towards the basolateral aspects of the acinar cells. Later in 1998, the development of in vitro tools to isolate and culture the PSCs laid a strong foundation to characterize their basic biology[7,8]. These cells also surround the perivascular and periductal regions. Sustained PSC cultures have helped to decipher the crucial factors that act in the inflammatory mechanisms and their mechanistic role in the pancreatic fibrosis in chronic pancreatitis (CP) and pancreatic ductal adenocarcinoma (PDAC). However, in view of the challenges of limited viability of the PSCs in primary cultures, there had been several attempts to modify isolation and culture techniques. In this regard, techniques were developed to immortalize the normal and tumour associated PSCs. However, further validation studies will be required prior to their routine use in PSC research[9-12]. Interestingly, even though PSCs were associated primarily with the exocrine pancreas, a recent study has reported isolation of PSCs from rat and human pancreatic islets too. These cells demonstrated certain morphologic and functional differences from the conventional PSCs in terms of fewer lipid droplets, lower rates of proliferation, migration and easier activation[13,14].

BASIC BIOLOGY OF PANCREATIC STELLATE CELLS

Origin

The origin of PSCs is still being debated. Till date no direct studies have been executed to identify the origin of PSCs. However, the studies on the origin of HSCs have helped in gaining some insight into this aspect. Even though initially a neuroectodermal origin of PSCS was proposed, it was eventually negated in genetic cell lineage mapping studies[15]. A recent study forwarded refreshing evidence supporting a mesodermal origin of HSCs by using the conditional lineage analysis approach[16,17]. Since most of the characteristic features and functions that sketched the biology of PSCs are similar to HSCs, it is believed that even PSCs might have evolved from a mesodermal origin. Employing such similar tracer techniques might help in ascertaining the origin of PSCs.

In the context of CP and PDAC, even though most of the proliferating PSCs are derived from the resident PSCs within the pancreas, a proportion of PSCs are thought to originate in the bone marrow. This was proposed in a novel sex mismatched study, which evidenced that even bone marrow (BM) derived cells may also contribute to PSC population in CP and PDAC apart from the resident cells of pancreas[18,19]. The speculation that bone marrow is another potential source of PSC was further supported by a recent study involving dibutylin chloride induced CP wherein a model of stable hematopoietic chimerism by grafting enhanced green fluorescence protein (eGFP)-expressing BM cells was used. In this study, 18% of the PSCs in the pancreas was found to originate in the bone marrow[20]. A recent study that used enhanced green fluorescent protein (EGFP)(+)-CD45(-) cells transplanted from EGFP-transgenic mice in a carbon tetrachloride (CCL4) model suggested that infiltrating monocytes could also differentiate into stellate cells within the pancreas and liver under the influence of
monocyte chemoattractant protein-1 (MCP-1)\textsuperscript{[21]}. 

**Morphologic characteristics**

Most of the characteristic features exhibited by quiescent as well as activated PSCs have been determined based on *in vitro* studies using rat and human PSC isolates. Cultured PSCs display prominent vitamin A containing lipid droplets with perinuclear localization in the cytoplasm. These lipid droplets elicit a fugacious blue-green autofluorescence when exposed to UV light at 328 nm or 350 nm wavelength. The expression of glial fibrillary acidic protein (GFAP) is specific to PSCs in the pancreas and presence of lipid droplets in the cytoplasm define the quiescent phenotype of PSCs\textsuperscript{[5-8]}. The underlying mechanisms involved in the accumulation and disappearance of lipid droplets are still not elaborately elucidated. It was demonstrated in a few studies that albumin colocalizes with the lipid droplets within quiescent PSCs. Activated PSCs, which are characterized by disappearance of lipid droplets, re-developed the lipid droplets and showed resistance against the activating effects of transforming growth factor-\(\beta\) (TGF-\(\beta\)) when transfected with the plasmids expressing albumin, thereby confirming the contribution of albumin in lipid droplet formation. The albumin was reported to be a downstream effector of peroxisome proliferator activated receptor-\(\gamma\) (PPAR-\(\gamma\)), a nuclear receptor that is known to inhibit PSC activation\textsuperscript{[22,23]}. 

The presence of lipid droplets together with expression of GFAP, desmin, nestin and vimentin is used to differentiate the PSCs from pancreatic fibroblasts\textsuperscript{[24]}. Using GFAP-LacZ transgenic mice model, it was proven that GFAP promoter activity was unique to PSCs alone in the pancreas\textsuperscript{[25]}. 

Autotransformation of quiescent PSCs to activated phenotype is observed *in vitro*. The basic phenotypic differences that were observed when the PSCs switch to activated phenotype include the disappearance of lipid droplets and transformation into a myofibroblast-like phenotype. The expression of \(\alpha\)-smooth muscle actin (\(\alpha\)-SMA) marks the transdifferentiation of the quiescent PSC to an activated phenotype. Figure 1 shows the morphology of PSCs in culture at different time points.

**PSC functions**

The physiological and pathological functions of PSCs have been summarized in Table 1. Under physiological conditions, PSCs are believed to contribute to the exocrine cell structure and function *via* maintenance of the normal basement membrane\textsuperscript{[26,27]} and carry out normal ductal and vascular regulation by virtue of their localization\textsuperscript{[28]}. Quiescent PSCs have a low mitotic index and bear the capability to synthesize matrix proteins and maintain the physiological extracellular matrix. The expression of matrix metalloproteinases (MMPs) and tissue inhibitors of matrix metalloproteinases (TIMPs) are complementary to each other and is a prerequisite to poise the ECM turnover. Increased production of the ECM proteins fibronectin, peristostin, MMPs and TIMPs are the most common features exhibited by the activated PSC phenotype\textsuperscript{[29]} and hence described as the effector cells contributing to the stroma associated with CP and PDAC. Besides laying and maintaining the ECM, PSCs have also been demonstrated to secrete acetylcholine that might function as an intermediate regulator for cholecystokinin mediated pancreatic exocrine secretion\textsuperscript{[30]}. 

Until recently, much attention was paid to unveil the functions of activated PSCs as a multiple cytokine producing profibrogenic cell type, which promotes self-proliferation, migration and fibrogenesis. However, recent advances have even demonstrated certain non-fibrogenic functions of PSCs, which projected PSCs as immune cells\textsuperscript{[31]}, intermediary cell\textsuperscript{[30,32]} and also as a progenitor cell\textsuperscript{[33-35]}. An earlier study showed that PSCs could phagocytize senescent neutrophils in experimental acute pancreatitis (AP) and this was reduced by the presence of cytokines while augmented by presence of PPAR-\(\gamma\) ligand\textsuperscript{[31]}. The same group subsequently demonstrated that PSCs could also phagocytize necrotic acinar cells and themselves undergo cell death. No change in TGF-\(\beta\) concentration was detected in the PSC media and medium with PSC and acinar cells, thereby indicating that the death of PSCs could result in inhibition of fibrogenesis in the setting of AP\textsuperscript{[36]}. This role in innate immunity was further supported by the capacity of PSCs to recognize pathogen-associated molecular patterns *via* Toll-like receptors (TLRs) that are expressed on their surface\textsuperscript{[37]}. 

**Table 1  Function of pancreatic stellate cells in the quiescent state and after activation**

| Physiological functions | Pathological functions |
|-------------------------|------------------------|
| Store fat and retinoids in perinuclear droplets, expressing GFAP, desmin and vimentin | Exhibit cell proliferation and migration |
| Secretes MMPs and TIMPs | Deranged ECM turnover due to loss of balance between MMPs and TIMPs |
| Maintains ECM turnover | Secrete various cytokines, chemokines and growth factors and thereby contribute to inflammatory milieu |
| Involved in maintenance of pancreatic tissue architecture | Stimulate cancer cell proliferation and migration and inhibit their apoptosis |
| No or limited secretion of cytokines, chemokines and growth factors | Mediate invasion and metastasis of carcinoma cells |
| Function as an immune, progenitor and intermediary cell | Mediate chemoattractant and radioresistance thereby promoting cancer cell survival |
| Possible role in exocrine and endocrine secretions | Contribute to the hypovascular and hypoxic tumour microenvironment |
| GFAP: Glial fibrillary acidic protein; MMPs: Matrix metalloproteinases; TIMPs: Tissue inhibitors of matrix metalloproteinases; NGF: Nerve growth factor. | Promote angiogenesis, neural invasion and epithelial-mesenchymal transition |

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Studies have now also proposed a regenerative role especially in the context of AP, where the interaction between extracellular matrix laid by PSCs and acinar surface integrin receptors could result in a scaffold for acinar regeneration. Excess matrix deposition could also potentially be ameliorated by matrix degrading enzymes and apoptosis/cytolysis of activated PSCs [38].

In addition to the above-mentioned functions of PSCs, it is now becoming more evident that these multifunctional cells also affect endocrine secretion in CP. This speculation surfaced from experiments that demonstrated increased numbers of PSCs in rat pancreas in a Type 2 diabetic model [39]. Extension of this study in vitro showed that PSCs could reduce insulin secretion and induce β-cell apoptosis [40-42]. On the contrary, another study showed that PSCs increase insulin secretion from mouse islets [43]. Interestingly, INS-1 cell culture supernatants reduced the secretion of proinflammatory cytokines (that mediate β-cell dysfunction) and ECM proteins from PSCs [44]. Moreover, the expression of regenerating islet-derived protein-1 was high in islet stellate cells (ISCs) isolated from the diabetic mice, which inhibited the viability, migration, synthesis and secretion of ECM proteins in ISCs in vitro [45]. As the in vitro results are more divergent, meticulous studies need to be designed and executed to understand the precise role played by these cells during their reciprocal interaction.

Figure 1  Morphological changes observed in cultured rat pancreatic stellate cells at different time points after isolation. A: Quiescent pancreatic stellate cells (PSCs) in culture exhibiting a flattened shape with lipid droplets, 6 h after isolation (× 20); B, C: PSCs showing flattened angular appearance and exhibiting cytoplasmic extensions with lipid droplets after 24 and 48 h respectively in cultures (× 20); D: PSCs exhibiting dense lipid droplets (lipid droplets are indicated with black arrows) in the cytoplasm (× 40); E: Activated PSCs showing long cytoplasmic processes with no lipid droplets in the cytoplasm after 72 h in cultures (× 20); F: Passage 2 rat PSCs in culture, immunostained for α-smooth muscle actin (α-SMA), a cytoskeletal marker for activated PSCs. Green striations indicate α-SMA and blue spots indicate nuclei, stained with DAPI (× 20).
The fate of activated PSCs is an important question that remains unresolved. Figure 2 depicts a schematic representation of the fate of PSCs. One of the two possible explanations that were proposed is that sustained inflammation may perpetuate PSC activation, leading to fibrosis; while the other explanation proposed that the activated PSCs may undergo apoptosis or may revert back to the native phenotype if the inflammation or injury is ceased. Recently, Fitzner et al.[46] proposed that activated PSCs could undergo senescence as evidenced by increased senescence-associated β-galactosidase, higher expression of CDKN1A/p21, mdm2 and interleukin (IL)-6. On the contrary, there was lower expression of α-smooth muscle actin. The authors also observed that senescence increased the susceptibility of PSCs to cytolysis and concluded that inflammation, PSC activation and cellular senescence were coupled processes that took place in the same inflammatory microenvironment of CP[46]. Figure 3 depicts the autocrine and paracrine mechanisms of PSC activation and the resulting fibrosis.

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**PSGS AND FIBROSIS**
A pathological hallmark of CP and PDAC is progressive fibrosis that is mediated by the PSCs. One of the earliest cellular events at the initiation of fibrosis is activation of PSCs, which can be mediated concomitantly by a variety of factors, such as oxidative stress, cytokines, growth factors, activin-A, angiotensin, hyperglycemia and pressure, to name a few. Interestingly, activation of PSCs can occur by both autocrine and paracrine mechanisms, which imply that the effects of PSC activation, primarily inflammation and resultant fibrosis can progress, even after removing the primary source. The distinctive sources of exogenous factors that activate the PSC include activated macrophages, monocytes, pancreatic acinar cells, endothelial cells, pancreatic cancer cells and platelets in inflamed pancreas.[47-50]. Figure 3 depicts the autocrine and paracrine mechanisms of PSC activation and the resulting fibrosis.

**Alcohol, smoking and PSC activation**
Alcohol and smoking are now recognized as independent risk factors for the development of CP. It is known that pancreatic acinar cells can metabolize alcohol to form toxic metabolites that results in oxidative stress. This results in inflammation and PSC activation.[51-53]. Furthermore, PSCs themselves can metabolize ethanol to acetaldehyde and generate oxidative stress, thus promoting their own activation and lipid peroxidation. The above findings have been confirmed by immunostaining for 4-hydroxy-nonenal (4-HNE), a reactive product of lipid peroxidation, that demonstrated localization of 4-HNE stained PSCs in fibrotic areas adjacent to acinar cells.[54-56]. Ethanol activated PSCs
showed increased proliferation by enhancing the activation of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase system stimulated by platelet derived growth factor (PDGF)\(^5\). Also shown was expression of connective tissue growth factor (CCN2/CTGF) that was attributed to production of acetaldehyde and oxidant stress in ethanol stimulated PSCs, which rendered the properties of cell adhesion, migration and collagen synthesis when stimulated with profibrogenic molecules\(^5\).

Recently, CCN2 was also shown to increase miR-21 expression that in turn enhanced collagen \(\alpha_1\) expression in a murine alcoholic CP model. CCN2 and miR-21 were shown to be colocalized in PSC derived exosomes that were positive for cluster of differentiation (CD) 9. In vitro studies revealed that these exosomes serve as molecular cargos to activate and transfer fibrogenic signals to the adjacent PSCs\(^5\).

Lee et al\(^6\) has recently demonstrated that PSCs express nicotinic acetylcholine nAChRs (isoforms \(\alpha_3, \alpha_7, \beta_1, \epsilon\)). Furthermore, nicotine and nicotine-derived nitrosamine ketone and cigarette smoke extracts were shown to activate PSCs both in the presence and absence of alcohol. This reiterates the clinical observation of role of smoking as an independent risk factor in the initiation and progression of CP\(^6\).

**Pressure and PSC activation**

Ductal hypertension resulting from obstructing pancreatic ductal calculi or stricture has been long believed to be a major contributor of pain in CP. This formed the rationale for ductal clearance of stone/stricture by endotherapy and/or surgery in an attempt to ameliorate pain in CP. Experimental evidence to support this concept came forward from studies by Asaumi et al\(^6\) where externally applied pressure of 80 mmHg induced activation of PSCs and generation of reactive oxygen species (ROS) within the activated PSCs\(^6\). ROS generation was observed as early as 30 min after application of pressure and reached peak by 1 h.

**Hyperglycemia and PSC activation**

In a study by Ko et al\(^6\), exposure of PSCs to high glucose concentration resulted in stimulation of \(\alpha\)-SMA expression, proliferation and expression of extracellular matrix proteins such as CTGF and collagen type IV. PSC activation by hyperglycemia was also confirmed.
by subsequent studies by Nomiyama et al. and Hong et al. and the latter study also suggested an additive effect of hyperglycemia and hyperinsulinemia in inducing PSC activation and islet fibrosis in the context of Type 2 diabetes. Observations from these studies have provided an insight into the role of hyperglycemia in preserving the activated phenotype and also in the context of secondary diabetes in patients with CP. A more recent study has indicated that hyperglycemia could result in induction of Cysteine-X-Cysteine ligand (CXCL) 12 production by the PSCs and its receptor, CXCR4 on cancer cells.

Cytokines and other activation factors that mediate proinflammatory function of PSCs

Fibrous tissue in CP and PDAC abounds in type I collagen. Among the cytokines that can cause PSC activation, TGF-β stands among the most important. Studies have shown increased collagen synthesis and upregulation of MMP1 in PSCs that were stimulated with TGF-β1 and TGF-β2. Other activators of PSCs include interleukin-8 (IL-8), MCP-1 and RANTES (Regulated on Activation, Normal T-cell Expressed and Secreted), which promote PSC activation via autocrine pathways. Activin-A and angiotensin II have also been identified as the autocrine activators of PSCs, contributing to further TGF-β1 expression and PSC proliferation. Expression of TGF-β1 and collagen secretion, has also been shown to result from application of external pressure and with hyperglycemia.

Migration and proliferation of PSCs are other important properties that go parallel along with the proinflammatory and profibrotic cascade. Proliferation and migration of PSCs is mediated by PDGFαββ(79,80), (which is expressed after TGF-β1 mediated activation) and endothelin-1. A proinflammatory chemokine, CX3CL1 (fractalkine), reported to circulate in the serum of patients with alcoholic chronic pancreatitis, was demonstrated as an activation and proliferation factor for PSCs and PSCs were shown to express the receptor (CX3CR1) for this chemokine. Recently, another new activation factor, namely parathyroid hormone related protein (PTHRP) was demonstrated to be expressed by acinar cells during experimental pancreatitis using an acinar cell specific PTHR gene knockout model. Receptor for this factor (PTHR1) has been shown to be expressed in PSCs and receptor-ligand interaction between the two proteins resulted in fibrogenesis. Of note, IL-6 has been shown to inhibit both PSCs proliferation and collagen synthesis. Recently it was also demonstrated that IL-4 and IL-13 secreted by PSCs mediate macrophage activation, which in turn participate in promoting the pancreatic fibrosis.

To summarize the effect of the above experimental evidence, different paracrine factors released during the injury will result in activation, proliferation and migration of PSCs and the activated phenotype is further retained by an autocrine loop, even in the absence of paracrine triggers.

Molecular pathways, microRNAs, transcription factors and proteomics in PSC mediated pancreatic fibrosis

Studies conducted over the past decade have implicated the involvement of several proteins and molecular pathways (Figure 4) in perpetuating the profibrogenic role of PSCs.

MAPK, JAK-STAT and PI3K signaling pathways

Mitogen activated protein kinases (MAPKs) are serine/threonine protein kinases with three families: extracellular signal regulated kinase (ERKs), c-Jun N-terminal kinase (JNK) and p38, and all the three MAPKs have been studied extensively for their role in PSCs activation. In vitro studies demonstrated that the activation of ERK1/2 is the initial pathway that precedes the transformation of PSCs into activated phenotype and PDGF was shown to mediate ERK1/2 and Activator protein-1 (AP-1) dependent proliferation and migration of PSCs. Studies have also demonstrated involvement of the Janus-activated kinase-signal transducers and activators of transcription (JAK2-STAT3) pathway in PDGF-BB induced PSC proliferation. All the 3 MAPKs were described in human PSCs to express IL-32α and IL-33 when treated with proinflammatory cytokines. IL-33 was shown to activate PSCs. HNE was reported to activate all the 3 classes of MAPKs and AP-1. PSCs treated with HNE showed increased production of type I collagen with no significant effect on proliferation and transformation, implicating oxidative stress mediated pathogenesis of pancreatic inflammation and fibrosis.

The all the three MAPKs including AP-1 were triggered in PSCs when stimulated with ethanol and acetaldehyde. Inhibition of p38, JNK and Rho associated protein kinase (ROCK) pathways demonstrated the inhibition of PSC activation, supporting the involvement of above mentioned pathways in the pathogenesis of alcohol induced pancreatic injury.

Smad signaling pathway

TGF-β1, which is a proven profibrogenic cytokine, is required in the regulation of PSC activation. Smads are the signaling effectors of TGF-β1 mediated functions and have also been ascribed a regulatory role in PSC functions. Results from co-expression of Smad2/3 with dominant negative Smad2/3 mutants and inhibition of ERK showed that the activation, proliferation and TGF-β1 mRNA expression are mediated through the Smad2/3 and ERK dependent pathways in PSCs. The autocrine loop between IL-1β and TGF-β1 and the one existing between the IL-6 and TGF-β1 were
mediated by Smad3/ERK dependent and Smad2/3 and ERK dependent pathways. Further investigations had confirmed the existence of a TGF-β autocrine loop and supported the role of PSCs in preserving the activated phenotype and collagen synthesis\[92,93\]. TGF-β1 induced expression of cyclooxygenase-2 (COX-2) by PSCs also followed Smad2/3 dependent pathway in response to proinflammatory cytokines\[94\]. This pathway has been suggested to be protective against the inhibitory activity of reversion-inducing-cysteine-rich protein with kazal motifs (RECK), a membrane anchored MMP inhibitor in the activated PSCs\[95\]. The stimulation of activated PSCs with TGF-β unveiled the possible role of Ras-ERK and PI3/Akt pathways in the expression of MMP-1\[68\].

Wnt signaling and β-catenin pathway

Yet another signaling pathway aberration that could result in PSC activation, proliferation and transformation into a profibrotic phenotype is that of Wnt signaling. This observation came from an experimental CP model by Hu et al\[96\] where the authors have shown that there was increased expression of Wnt and its second messenger β-catenin and that this imbalance could result in persistent activation of PSCs. Yet another study by Xu et al\[97\] showed that cancer cell invasion and migration are promoted by Wnt2 protein secreted by the PSCs.

Hedgehog signaling pathway

Indian hedgehog (IHH) and sonic hedgehog (SHH) are the other important pathways in PSCs. Receptors, namely smoothened and patched-1, for the IHH protein are expressed on the surface of PSCs and the receptor-ligand binding results in localization of the membrane-type 1 matrix metalloproteinase on PSC plasma membrane, which in turn could mediate PSC migration\[98\]. SHH was shown to influence the PSC mobility and differentiation\[99\] and also perineural invasion, metastasis, tumour growth and pain in pancreatic cancer\[100,101\].

microRNAs

Implications on the involvement of microRNAs (miRs) has recently being reported frequently in the context of CP and PSCs. A recent study reported upregulation and downregulation of 42 miRs each in activated PSCs\[102\]. miR-15b and 16 have been shown to induce apoptosis of rat PSCs via influencing the anti-apoptotic Bcl-2 protein\[103\]. An even more recent study demonstrated a paracrine pathway wherein CCN2 mRNA and miR-21 containing exosomes liberated by PSCs were engulfed by surrounding PSCs. This results in further expression of the CCN2 and miR-21 by the activated PSCs\[99\].

Figure 4  Signaling pathways mediating pancreatic stellate cell activation. Expression of α-SMA, proliferation, migration and deposition of matrix proteins are the important properties attained by activated pancreatic stellate cells (PSCs) when stimulated with various growth factors and proinflammatory cytokines. Proliferation and migration is mediated through the MAP kinase and PI3K pathways when PSCs are stimulated with HNE, alcohol, PDGF and IL-33 and other cytokines. TGF-β1 induces the Smad proteins and stimulates the proliferation and collagen secretion by PSCs. Activation of Indian Hedgehog (IHH) signaling in PSCs promotes their migration, proliferation and collagen deposition. PSC mediated Sonic Hedgehog (SHH) signaling promotes cancer cell invasion and migration. Wnt signaling can cause collagen deposition and cancer progression. PDGF: Platelet derived growth factor; HNE: Hydroxy-nonenal; ERK: Extracellular signal-regulated kinases; JNK: c-Jun N-terminal kinase; TGF-β: Transforming growth factor-β; α-SMA: α-smooth muscle actin; COX-2: Cyclooxygenase-2; IL: Interleukin.
Transcription factors and interactions with cytokines

Different cytokines exert their effect by inducing various transcription factors such as nuclear factor-κB (NF-κB), Activator protein-1 (AP-1), STAT proteins and Gli, to name a few. NF-κB is stimulated by various cytokines associated with different cellular functions[104]. Activated PSCs showed NF-κB mediated expression of intracellular adhesion molecule when stimulated with IL-1β and tumor necrosis factor (TNF-α), which was not observed in the quiescent phenotype[105]. Expression of MCP-1, cytokine inducing neutrophil chemoattractant-1, IL-6, IL-8 and RANTES was observed via NF-κB activation when induced with galectin-1, various ligands of TLR and cytokines, substantiating the role of PSCs in mediating the infiltration and accumulation of inflammatory cells[106-108].

Proteomics

Proteomic studies using the immortalized PSC lines from Mus musculus and Rattus norvegicus showed the expression of cytoskeletal and ribosomal proteins by activated PSCs. The studies also demonstrated proteins involved in protein degradation, MAPK 3 and Ras related proteins by pseudo-quiescent PSCs[109,110]. Proteomic profiling of mild and severe CP by label free quantitative proteomic approach displayed varied expression of proteins with a relative change in the proteins related to ECM and PSC activation which includes perioisin, fibrillin 1, transgelin and a group of collagen. An accompanying study showed increased expression of transgelin in stromal and periaclinar regions of CP, confirming its role in PSC activation[111,112].

A comparative proteomic profiling of human HSC and PSC lines LX-2 and RLT-PSCs identified 1223 different proteins. Among 1223 proteins 1222 were found to be commonly expressed in both cell lines and a single protein (amintransferase) was found expressed in HSCs alone. The proteins in abundance from human PSC lines in this study were implicated for their role in maintaining the cellular structure[113]. The proteomic analysis of nicotine treated human, mouse and rat PSCs by GeLC-MS/MS approach demonstrated the differential expression of proteins and signaling pathways, while the expression of integral protein 2B, procollagen type VI alpha, toll interacting protein and amyloid interacting proteins was found to be common[114]. Expression of lysosomal proteins, indicators of pancreatic disease, proteins involved in defense mechanism and alteration in the phosphorylation sites were observed in another study[115]. Few other proteomic studies of similar kind have reported the mechanism and alteration in the phosphorylation sites were observed in another study[116].

PSC-PANCREATIC CANCER CELLS-CANCER STROMAL INTERACTIONS

It has now been conclusively demonstrated that majority (50%-80%) of PDAC volume is composed of a fibrous stroma, amidst which lay the islands of cancer cells[118]. There has been increasingly accumulating evidence that supports substantial two-way interactions between the stromal components and cancer cells and the association between the cancer cells and cancer associated PSCs have received several monikers such as “dangerous liaisons”[119], “friend or foe”[120] and “unholy alliance”[121]. The stroma in pancreatic cancer consists predominantly of collagenous fibres laid down by the PSCs, along with other cellular [mast cells, macrophages, lymphocytes, myeloid derived suppressor cells (MDSC) and endothelial cells][122-131] and non-cellular (ECM proteins such as collagen, laminin, fibronectin, glycoproteins, proteoglycans and glycosaminoglycans; non-ECM proteins such as growth factors, osteopontin, perioisin and serine protein-acidic and rich in cysteine] components[122,131]. These stromal components can mediate the interaction between the PSCs and cancer cells and eventually influence the biological behavior and clinical outcomes of PDAC. Apart from vascular endothelial growth factor (VEGF) and angiopoietin-1, PSCs also secrete hepatocyte growth factor (HGF) and mediators responsible for endothelial cell proliferation and tube formation. This appears to operate through the HGF/c-MET pathway via induction of the downstream PI3K and p38 signaling pathways[134]. Of note, upon neutralizing the HGF activity, proliferation and migration of cancer cells could be inhibited and apoptosis could be induced[135].

Even though fibrosis that was observed early in development of PDAC led to the belief that PSC produced stroma is protective, this eventually shifted towards the concept of the stroma having a tumour permissive effect. However, the current opinion holds that PSC-stroma-cancer cell interaction is dynamic and stage-dependent, with protective effect in the earliest stage and harmful effect in later stages[38]. The mechanism of PSC induced fibrosis in PDAC is similar to that seen in CP. Therefore, in the next section we will discuss only the cancer specific interactions and phenotypic effects of stroma-cancer cell interactions. While the PSC-pancreatic cancer cell interactions result in cancer growth and PSC activation, interaction between PSCs and stromal cells may be instrumental in metastasis, immune evasion, tumour hypoxia and resistance to chemoradiotherapy.

PSC-PDAC crosstalk

Pancreatic intraepithelial neoplasia (PanINs) are the precursor lesions of PDAC. It is now well known that PSCs get activated even at the early PanIN stages of
PDAC and initiates fibrosis around these precursor lesions. Several in vitro and in vivo studies have provided insight into the bipolar interactions between the PSCs and PDAC. In vitro co-culturing of PSCs with pancreatic cancer cells accelerated the proliferation and increased survival by inhibiting apoptosis\[^{139}\]. Furthermore, co-culturing also resulted in epithelial-mesenchymal transition (EMT) as evidenced by increased expression of vimentin and snail (mesenchymal marker) with corresponding reduction in E-cadherin and cytokeratin (both epithelial markers)\[^{137}\]. This was associated with migration of the cancer cells, which indicates the capability of PSCs to trigger the metastasis of pancreatic cancer cells\[^{138}\].

Recurrent of PDAC after therapy has been postulated to be an effect of persistence of a treatment resistant cancer stem cell niche. PSCs have been shown to regulate the genesis of a cancer stem cell niche as marked by increased expression of stem cell markers such as ABCG-2, Lin28 and nestin, while also attaining capability to form spheroids in vitro\[^{139}\]. Interestingly, it has been shown that the same PDAC can contain a heterogeneous population of PSCs based on the expression of CD10, which is a cell-membrane associated MMP. CD10(+) cells are associated with a higher propensity to proliferate and invade, thereby indicating that the relative proportion of PSC subtypes could also determine the disease biology and prognosis\[^{140}\].

While the foregoing paragraphs discussed the effect of PSCs on pancreatic cancer cells, the cancer cells also induce profound effects on the PSCs. Pancreatic cancer cells produce factors such as PDGF, trefoil factor 1\[^{141}\] and COX-2, which could induce PSC proliferation. COX-2 expression is upregulated not only in the cancer cells, but also in the PanINs and PSCs exposed to conditioned medium from cancer cell lines\[^{142-145}\]. Galectin-1 and Galectin-3, members of galectin family of β-galactoside binding proteins, are also important drivers of the PSC-PDAC crosstalk. Galectin-3 expression by pancreatic cancer cell lines was found to promote its own proliferation along with PSCs\[^{146,147}\].

Figure 5 outlines the overall crosstalk between PSCs and pancreatic cancer cells.

**Role of PSCs on invasion and metastasis**

Galectin-3\[^{147}\], thrombospondin-2\[^{148}\], stromal cell derived factor\[^{149}\] and nerve growth factor (NGF)\[^{150}\] expressed by PSCs are shown to drive the invasion of PDACs. Studies on xenograft models showed that PSCs exert a modulatory function and potentiate the invasiveness of SUIT2 pancreatic cancer cells expressing serine protease inhibitor nexin2 (SERPINE2)\[^{151,152}\]. Pancreatic cancer cells and PSCs express fibroblast growth factor (FGF) and fibroblast growth factor receptor (FGFR) that have been shown to mediate interaction between the tumour and stromal cells resulting in development of an invasive phenotype\[^{153}\].

A recent study confirmed that pro-invasive character results from nuclear localization of FGFR1 and FGFR2 in PSCs\[^{154}\]. Perhaps the most convincing and concept changing data on the role of PSCs in metastasis was reported in the study by Xu et al\[^{155}\] which showed that the PSCs could rapidly acquire a tumour inductive property even after a short exposure of pancreatic cancer cells, thereby facilitating tumour growth and metastasis. The authors used a gender mismatch approach in which they injected a combination of female pancreatic cancer cells and male human PSCs into the pancreas of female nude mice. Interestingly, they could demonstrate Y-chromosome positive (i.e.,...
the injected male human PSCs) in metastatic liver nodules. This implied that the PSCs from the liver could intravasate blood vessels, transport in circulation and extravasate into metastatic nodules alongside the metastatic cancer cells. The findings also suggest that the metastatic PSCs could mount an active stromal reaction even in the metastatic nodule. The property of transendothelial migration of the PSCs was further supported by in vitro studies and was found to be mediated by PDGF.

Besides contributing to distant metastasis, PSCs have also been implicated in neural invasion. This notion has been supported by studies that reported expression of neurotrophic factors such as glial derived neurotrophic factor and brain derived neurotrophic factor and stimulation of neurite formation towards pancreatic cancer cells by activated PSCs. These effects appear to be mediated by SHH paracrine signaling pathway[100,101].

**Tumour hypoxia and resistance**

Similar to CP, PDAC is also characterized by hypoxic microenvironment. Tumour hypoxia arising from fibro-inflammatory environment is shown to induce the expression of hypoxia-inducible factor-1α (HIF-1α) and stimulate the secretion of SHH ligand by cancer cells, leading to stromal deposition by tumour associated fibroblasts. Organotypic culture of thick pancreatic sections under hypoxic conditions depicted the activation (α-SMA) and proliferation (Ki67) of PSCs along with higher expression of HIF-1α, mediating the activation of hypoxic pathways[102]. In vitro studies on the role of hypoxic milieu on the interactions between PSCs and PDACs led to interesting observations. The hypoxia exposed PSCs expressed type I collagen and VEGF, showed increased migration and also promoted the endothelial cell proliferation, migration and angiogenesis[157]. Another study also yielded similar results where the hypoxia induced PSCs showed increased expression of periostin, collagen type I, VEGF and fibronectin. In co-cultures, the hypoxia treated PSCs enhanced the endostatin production by cancer cells and increased the endothelial cell growth[158]. A similar kind of study using 3D matrices also reported that the hypoxia induced procollagen-lysine, 2-oxoglutarate 5-dioxygenase 2 (PLOD2) in PSCs promotes cancer cell migration[159]. Periostin, a matrix protein, with its persevered autocrine loop was shown to promote ECM synthesis and cancer cell growth under hypoxia and starvation during chemotherapy by maintaining the activated phenotype of PSCs[160]. PSCs were also shown to express miR-21 and miR-210 under hypoxic conditions, where miR-20 was reported to regulate the interactions between PSCs and PDACs via ERK and Akt pathways[161] and miR-21 contributing to cancer cell invasion and metastasis[162]. Apart from these 2 miRs, miR-199a and miR-214 expressed by PSCs shown to have a pro-tumoral effect and also promote their own proliferation and migration[163].

Overexpression of miR-29, the expression of which was lost during the transformation of PSCs into activated phenotype, resulted in the reduction of collagen deposition, cancer cell growth and viability[164].

The outcomes of the above studies not only confirmed the central role of PSCs in desmoplasia but also exhibited the proangiogenic functions mediated by them in tumour hypoxia.

**Immune escape of PDAC**

Emerging data over the recent years have strongly suggested that pancreatic cancer cells could evade host immune surveillance. One of the major factors that mediate immune evasion of pancreatic cancer cells is by sequestration of CD8(+) cells within the stroma, thereby preventing them from invading peritumoral areas where they could mediate immune mediated injury to the cancer cells. This appears to be mediated by the chemokine CXCL12[145]. The other important mediator that sustain an immunosuppressive milieu is the β-galactoside binding lectin galectin-1, which is overexpressed by PSCs in pancreatic cancer. Using siRNA induced knockout and overexpression studies, it was shown that galectin-1 could induce T-cell apoptosis and reduced Th-1 response (with concomitant increase in Th-2 responses) and thereby reducing the immune mediated injury to the cancer cells. This was further reconfirmed and was shown that the effects were significantly higher in poorly differentiated tumours compared to the well-differentiated ones[166].

Other PSC mediated mechanisms that has been proposed to result in disruption of anti-tumour immunity are cytokine secretion by macrophages[167] and expression of the fibroblast activation protein-α[168], migration of MDSC[169], mast cell degradation leading to release of tryptase and IL-13[124].

**THERAPEUTIC IMPLICATIONS**

Given the background of substantial understanding of the mechanisms of PSC involvement in pancreatic fibrosis and pancreatic cancer, several experimental, preclinical and early phase clinical studies on CP and pancreatic cancer have appeared in the literature over the recent years. Experimental studies (both in vivo and in vitro) that have targeted the profibrogenic function of PSCs have shown favorable results; however these results have not yet been satisfactorily reproduced in human CP. Table 2 summarizes the drugs and their effects in experimental studies of CP[169-218].

In the context of pancreatic cancer, where conventional chemotherapy has shown dismal results, the current concept is to target the stroma along with conventional chemotherapy. Since the pancreatic cancer stroma has been shown to be associated with tumour hypoxia, metastasis, drug resistance, it is expected that prior stromal degradation could result in chemosensitivity of the tumour even with the conventional chemotherapeutic drugs. Table 3 shows the
Amino sulfonic acid
Ethanol stimulated culture activated rat PSCs
Rofecoxib
Wistar Bonn/Kobori rats
Agent
In vivo
Outcome of the study
Trinitrobenzene sulfonic acid (TNBS)
Mutant MCP-1
Ascorbic acid
Increased pancreatic weight and decreased pancreatic MPO and serum ACE activity was observed
Camostat mesilate
Candesartan
Antifibrotic cytokine
DBTC induced CP in Sprague-Dawley rats
Decreased malondialdehyde (MDA), hyaluronic acid, laminin concentrations and pancreatic injury
DBTC induced pancreatic fibrosis in Wistar rats
Allopurinol
Oral protease inhibitor
AdTb-ExR
Lisinopril and
Increased pancreatic weight and expression of angiotensinogen and angiotensin II receptor type 2 mRNA
Thioredoxin-1 (TRX-1)
Class/type of agent
Suppressed the expression of IL-6 and CINC and pancreatic acute phase proteins (PAP and p8)
Inhibited Caerulein induced experimental CP in female
PPAR-α/γ agonist
Carboxamide derivative
Antioxidant
Adenoviral vector system
Increased pancreatic weight and expression of angiotensinogen and angiotensin II receptor type 2 mRNA
Decreased: pancreatic MPO and serum ACE activity and hydroxyl proline content

| Ref. | Agent | Class/type of agent | In vivo/in vitro (study) model | Outcome of the study |
|------|-------|---------------------|-------------------------------|----------------------|
| Nakamura et al.[159] | FOY-007 | Synthetic serine protease inhibitor | Cytokine stimulated human periacinar fibroblast like cells | Both attenuated proliferation and procollagen type I C-terminal peptide (PPI) | |
| Xie et al.[160] 2002 | FOY-305 | Carboxamide derivative | Wistar Bonn/Kobori rats | Suppressed the expression of IL-6 and CINC and pancreatic acute phase proteins (PAP and p8) |
| Kuno et al.[161] 2003 | IS-741 | Angiotensin-converting enzyme (ACE) inhibitor | Wistar Bonn/Kobori rats | Increased pancreatic weight and decreased pancreatic MPO and serum ACE activity was observed |
| Yamada et al.[162] 2003 | Lisinopril | Wistar Bonn/Kobori rats | Decrease in serum MCP-1 levels, intra-pancreatic hydroxyproline content was identified |
| Xie et al.[163] 2002 | Lisinopril | Wistar Bonn/Kobori rats | Suppressed TGF-β1 mRNA overexpression |
| Nagashio et al.[164] 2002 | FOY-007 | Synthetic serine protease inhibitor | FOY-007 also inhibited collagen synthesis |
| Masaumura et al.[165] 2003 | FOY-305 | Carboxamide derivative | DBTC induced CP in Lewis rats | Reduced the activated PSCs, number of apoptotic acinar cells and fibrosis |
| Zhao et al.[166] 2005 | Mutant MCP-1 | Wistar Bonn/Kobori rats | Decreased MCP-1, fibrosis and hydroxyl proline levels |
| Gibo et al.[167] 2004 | Camostat mesilate | Oral protease inhibitor | Wistar Bonn/Kobori rats | Reduced IL6, TGF-β1, IL-1β, MCP-1 and PDGF expression |
| Yamada et al.[168] 2005 | Lisinopril and candesartan | Culture activated PSCs | In vivo: DBTC induced CP in Lewis rat | In vivo: Inflammation, fibrosis and cytokines expression was inhibited |
| van Westerloo et al.[169] 2005 | Teglitazone | PPAR-γ ligand | Caerulein induced experimental CP in female C57BL/6 mice | Suppressed mRNA expression of TGF-β1, PDGF-β and TNF-α |
| Reding et al.[170] 2006 | Retinol and its metabolites | Vitamin of vitamin A | Ethanol stimulated culture activated rat PSCs | Suppressed mRNA expression of TGF-β1, PDGF-β and TNF-α |
| Asaumi et al.[171] 2006 | Taurine | Amino sulfonyl acid | DBTC induced pancreatic fibrosis in Wistar rats | Inhibited PSC activation, proliferation, expression of collagen I: All MAP kinases were activated |
| Rickmann et al.[172] 2007 | Tocopherols | Vitamin E family members | Culture activated PSCs from Wistar rats | Reduced viability of activated PSCs by apoptosis and autophagy |
| Reference | Compound | Description | Effect |
|-----------|----------|-------------|--------|
| Michalski CW et al. 2008 | Cannabinoid WIN 55,212-2 | Aminomethylindole derivative | Reduced fibronectin, collagen I and α-SMA levels |
| Weylandt et al. 2008 | Omega-3 polyunsaturated fatty acids (n-3 PUFA) | | Decreased IL-6, MCP-1 and MMP-2 secretion and invasiveness by PSCs |
| Karatas et al. 2008 | Halofuginone | Synthetic halogenated derivative of leftriugine | Increased n-3 PUFA tissue levels |
| Fitzner et al. 2008 | Bosentan | ET-1-receptor antagonist | Less pancreatic fibrosis and collagen content |
| Schwer et al. 2010 | Carbon monoxide-releasing molecules-2 (CORMs) | Metal carbonyl compounds delivering carbon monoxide | Inhibited PSC proliferation and collagen synthesis |
| Nathan et al. 2010 | Pancreatic secretory trypsin inhibitor (PSTI) | Culture activated PSCs isolated from Wistar rats | Reduced the expression of ET-1, α-SMA and CTGF |
| González et al. 2011 | Palm oil tocotrienol-rich fraction | Vitamin E family member | PSC proliferation was inhibited through p38/HO-1 pathway activation |
| Long et al. 2011 | Octreotide | Analog of somatostatin | Reduced MPO activity and inflammatory cell infiltration |
| Li et al. 2011 | Pancreatic stone protein/regenerating protein | Secretory stress proteins family | Reduced amylase, hydroxyproline and TGF-β1 levels were observed |
| Tang et al. 2011 | Sinisan | Chinese herb | Diminished α-SMA, fibroactin and collagen expression was identified |
| Wei et al. 2011 | Pravastatin | Competitive inhibitor of HMG-CoA reductase | Inhibited PSC proliferation, migration and reduced collagen I and fibronectin |
| Li et al. 2011 | α-Tocopherol | Vitamin E family member | Reduced serum amylase, mRNA expression of TNF-α, IL-1β and COX-2 were reduced and IL-10 was increased |
| Monteiro et al. 2012 | Vitamin E supplementation | Ethanol induced (alcoholic) CP in Wistar rats | Reduced fibrosis and enhanced survival rate |
| Matsushita et al. 2012 | Taurine | Amino sulfonic acid | Inhibited acinar cell apoptosis |
| Yang et al. 2012 | L-Cysteine | Amino acid | Decreased α-SMA, TIMP-1, IL-1β, TGF-β1 expression and hydroxyproline levels and increased MMP-2 levels |
| Bai et al. 2012 | Sulindac | Non-steroidal anti-inflammatory drug | Suppressed PSC proliferation and ECM synthesis |
| Lee et al. 2012 | Simvastatin and Troglitazone | HMG-CoA reductase inhibitor and PPAR agonists | Reduced fibrosis, acinar cell loss and inflammatory cell infiltration |
| | | | | Expression of TGF-β, PDGF-β, SHH and Gli was reduced |
| | | Culture activated PSCs isolated from Sprague-Dawley rats | PSC proliferation was inhibited synergistically |
Both drugs inhibited PSC proliferation.

Bone morphogeneic protein inhibits PSC proliferation.

In vivo studies showed that rats body weight was improved and reduced the fibrosis.

Apigenin inhibited the expression of TGF-β, IL-6, and other inflammatory cytokines.

PSCs isolated from C57BL6JOlaHsd mouse suppressed the expression of IL-1β and TNF-α.

Hydroxyflavone inhibited TGF-β and IL-6 expression.

Vitamin A metabolite, retinoid, and vitamin D3 inhibited PSC proliferation.

Immortalized human pancreatic stellate cell line was used to study the antifibrotic effects of compounds.

Collagen type I, fibronectin, and other extracellular matrix proteins were reduced in the treated samples.

Chemokine inhibitor, integrin inhibitor, and MEK inhibitor decreased the expression of TGF-β and IL-1β.

Pancreatic fibrosis, acinar cell atrophy and loss were reduced.

Increased HGF and decreased IL-1β, TGF-β, and collagen type I expression.

Decreased expression of TGF-β, IL-1β, and collagen type I in the treated samples.

Decreased the phosphorylation of ERK1/2 and NF-κB activation.

Decreased the expression of TGF-β and IL-1β.

Pancreatic fibrosis, acinar cell atrophy and loss were reduced.

Decreased the activation of NF-κB and PSC activation was inhibited.

Reduced the expression of TGF-β and IL-1β.

Decreased the phosphorylation of ERK1/2 and NF-κB activation.

Decreased the expression of TGF-β and IL-1β.

Decreased the activation of NF-κB and PSC activation was inhibited.

Reduced the expression of TGF-β and IL-1β.

Decreased the phosphorylation of ERK1/2 and NF-κB activation.

Decreased the expression of TGF-β and IL-1β.

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Decreased the expression of TGF-β and IL-1β.

Decreased the activation of NF-κB and PSC activation was inhibited.

Reduced the expression of TGF-β and IL-1β.

Decreased the phosphorylation of ERK1/2 and NF-κB activation.

Decreased the expression of TGF-β and IL-1β.

Decreased the activation of NF-κB and PSC activation was inhibited.
### Table 3  Therapeutic agents that have been evaluated in experimental/pre-clinical studies targeting pancreatic stellate cells and cancer stroma in pancreatic ductal adenocarcinoma

| Ref. | Agent | Class/type of agent | In vivo/in vitro (study) model | Outcome of the study |
|------|-------|---------------------|--------------------------------|---------------------|
| Feldmann et al. 2007 | Cyclosporine | Steroidal alkaloid | Orthotopic xenograft model | Inhibited cancer cell invasion and metastasis by suppressing hedgehog |
| Diep et al. 2011 | Erlotinib | EGFR tyrosine kinase inhibitors | In vitro: Pancreatic cancer cell lines | Inhibited cancer cell proliferation, EGFR receptor signaling and induced apoptosis |
| | RDEA191 and AZD6244 | Tyrosine kinase inhibitors | In vivo: BxPC-3 and MIA PaCa-2 mice xenograft model | Suppressed tumour growth |
| Froeling et al. 2011 | ATRA, 9-cis-RA and 13-cis-RA | Metabolites of vitamin A | In vivo: LSL-KrasG12D/+;LSL-Trp53R172H/+;Pdx-1-Cre mice | Retinoic acid induced PSC quiescence and decreased migration |
| | | | In vitro: AsPC1 and Capan1 pancreatic cancer cell lines, PS1 and other PSC cell lines | Decreased and induced proliferation and apoptosis of cancer cells |
| Chauhan et al. 2013 | Losartan | Angiotensin inhibitor | Orthotopic mice model | Reduced stromal collagen production, expression of TGF-β, CCN2 and ET-1 |
| Sun et al. 2013 | Curcumin | Phenolic compound | TGF-β1 stimulated PANC-1 cell line | Improved drug and oxygen delivery to tumour |
| | | | Cancer associated fibroblasts | Inhibited proliferation and promoted apoptosis |
| Edderkaoui et al. 2013 | Ellagic acid | Polyphenolic and benzoquinone phytochemical | Pancreatic cancer cells and PSCs | Cancer cell invasion and migration was decreased |
| | Embelin | | | Induced apoptosis and inhibited proliferation |
| | | | | NF-κB activity was decreased |
| Macha et al. 2013 | Guggulsterone | Plant polyphenol | CD18/HPAF and Capan1 cell clones | Inhibited growth and colony formation |
| | | | | Induced apoptosis and arrested cell cycle |
| | | | | Decreased motility and invasion |
| Kozono et al. 2013 | Pirfenidone | Pyridone compound | Orthotopic tumour mice Model | Reduced tumour growth, PSC proliferation and the deposition of collagen type I and periostin in tumours was decreased |
| | | | In vitro: PSCs isolated from pancreatic tissue | In vitro: Proliferation, invasiveness and migration of PSCs was inhibited |
| Guan et al. 2014 | Retinoic acid | Vitamin A derivative | Panc-1 and Aspc-1 cell lines | Reduced α-SMA, FAP and IL-6 expression |
| | | | Cancer associated fibroblasts | Inhibited cancer cell migration and EMT |
| Gonzalez-Villasana et al. 2014 | Bisphosphonates and nab-paclitaxel | Monocyte-macrophage lineage inhibitors | Human PSCs and cancer cell line | In vitro: Inhibited PSC activation, proliferation MCP-1 release and collagen 1 expression and induced apoptosis |
| | | | | Proinflammatory cytokine and PSC proliferation was inhibited |
| Pominowska et al. 2014 | Prostaglandin E2 (PGE2) | Lipid compound | Human PSCs isolated from resected pancreatic tumour tissue | IL-1β and FGF induced COX-2 expression, TGF-β1 induced collagen synthesis and PDGF induced PSC proliferation was inhibited |
| Gong et al. 2014 | Nexrutine | Phytocerucial with COX-2 Inhibitor activity | In vitro: Pancreatic cancer cell lines | Suppressed COX-2 expression |
| | | | In vivo: BKS-COX-2 transgenic mice | In vivo: Improved drug and oxygen delivery to tumour |
| Yan et al. 2014 | Crizotinib | c-MET/HGF receptor and ALK tyrosine kinase inhibitors | In vitro: Human pancreatic cancer cell lines AsPC-1, PANC-1, MIA PaCa-2 and Capan-1 | In vitro: Growth and proliferation was inhibited |
| | | | In vivo: Mouse xenograft model | Induced apoptosis |
| | | | | In vitro: Inhibited angiogenesis, tumour growth and ALK activity |
| Zhang et al. 2014 | 5-Azacytidine | Cytidine analogue | Bxpc-3 cancer cell line | Inhibited cancer cell proliferation by suppressing Wnt/β-catenin signaling |
| Wang et al. 2014 | miR-216a | microRNA | Capan-2 and PANC-1 pancreatic cancer cell lines | In vitro: Inhibited cell growth and induced apoptosis |
| | | | In vivo: BALB/c nude mice | Down regulated survivin and XIAP expression |
| Kumar et al. 2015 | miR-let-7b and GDC-0449 | microRNA and Hedgehog inhibitor | Capan-1, HPAF-II, T3M4 and MIA PaCa-2 cell lines | In vitro: Decreased cell proliferation and induced apoptosis via Gli dependent mechanism |
| | | | In vivo: Reduced tumour cell proliferation with increased apoptosis and tumour growth was inhibited |
| Petrova et al. 2015 | RU-5KI 43 | Hedgehog acyltransferase inhibitor | Pancreatic cancer cell lines | Increased Akt and mTOR activity |
| | | | In vivo: Pancreatic cancer cell lines | In vitro: Tumour growth decreased |
| Masso-Valles et al. 2015 | Ibrutinib | Tyrosine kinase inhibitor | Pancreatic mouse and xenograft mice models | Reduced fibrosis and extended survival |
| Zhou et al. 2015 | Zileuton | 5-LOX inhibitor | Pancreatic cancer SW1990 cell line | Inhibited apoptosis, decreased proliferation and expression of 5-lipoxygenase |
recently tested drugs/biologics that targeted pancreatic cancer stroma in preclinical studies\textsuperscript{[219-242]}. Besides the preclinical studies, several SHH pathway inhibitors have also been tested in advanced or metastatic PDACs in phase I and II studies (both open labeled and randomized double-blind controlled trials). Few of these include Vismodegib (GDC-0449), Saridegib (IPI-926) and Erismodegib (LDE225), PDGFR inhibitor (TKI258), hyaluran (PEGPH20) and dasatinib, to name a few. These have been used along with gemcitabine and/or nab-Paclitaxel and FOLIRINOX. Discussion of details of the study designs and results of these trials can be obtained from recent high quality reviews\textsuperscript{[243,244]}

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