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REVIEW ARTICLE

Pancreatic cancer: the role of pancreatic stellate cells in tumor progression

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Abstract

Pancreatic ductal adenocarcinoma is an aggressive and highly lethal disease frequently characterized by a dense stromal or desmoplastic response. Accumulating evidence exist that tumor desmoplasia plays a central role in disease progression and that e.g. activated pancreatic stellate cells (PSCs) are responsible for the excess matrix production. The mechanisms underlying the tumor versus stroma interplay are complex. Pancreatic cancer cells release mitogenic and fibrogenic stimulants, such as transforming growth factor β 1, platelet-derived growth factor (PDGF), sonic hedgehog, galectin 3, endothelin 1 and serine protease inhibitor nexin 2, all of which may promote the activated PSC phenotype. Stellate cells in turn secrete various factors, including PDGF, stromal-derived factor 1, epidermal growth factor, insulin-like growth factor 1, fibroblast growth factor, secreted protein acidic and rich in cysteine, matric metalloproteinases, small leucine-rich proteoglycans, periostin and collagen type I that mediate effects on tumor growth, invasion, metastasis and resistance to chemotherapy. This review intends to shed light on the mechanisms by which PSCs in the stroma influence pancreatic cancer development. The increased understanding of this interaction will be of potential value in designing new modalities of targeted therapy.

Key words: pancreatic cancer, treatment, stellate cells, desmoplasia, signal transduction

Introduction

Pancreatic ductal adenocarcinoma (PDAC) is a devastating disorder for most people who are afflicted, with a reported 5-year survival of less than 1% [1]. In Western countries, pancreatic adenocarcinoma comprises the fourth most common cause of malignancy-related death, and the annual incidence has been estimated to be approximately 10 cases per 100,000 population [2]. Cigarette smoking, advanced age and genetic disorders (e.g. hereditary pancreatitis, familial breast cancer, and hereditary non-polyposis colon cancer) are recognized as established risk factors.

The current model of progression of normal ductal epithelium, via pancreatic intraepithelial neoplasias, to invasive ductal adenocarcinoma, includes activating point mutations in K-ras, and loss of P53, p16 and SMAD4/DPC4 tumor suppressors. More recently, the microenvironment surrounding the pancreatic cancer cells has received increased attention.

The cancer microenvironment is characterized by a desmoplastic reaction with the stromal part often being greater than the epithelial component of the tumor itself [3]. The stroma is a dynamic milieu, where fibroblasts, pancreatic stellate cells (PSCs), extracellular matrix (ECM), matrix metalloproteinases (MMPs), tissue inhibitors of MMP, inflammatory cells, macrophages, nerve fibers, stem cells, endothelial cells as well as different growth factors and cytokines can interact with cancer cells and alter their behavior. Although it has been postulated that the cancer-associated stroma may represent a host defense against malignant spread [4], most lines of evidence indicate that the desmoplasia of pancreatic cancer is paramount to tumor promotion and progression [5-10].

This review will summarize recent advances in the understanding of the mechanisms involved in tumor-stroma interactions, with particular focus on PSCs and future directions in stroma-targeted therapies.

The pancreatic stellate cell

In 1998, the star-shaped cells in the pancreas were identified and characterized, termed PSCs [11, 12]. These cells are considered to be critical for the development of the desmoplastic reaction associated with chronic pancreatitis, as well as pancreatic cancer. In the normal pancreas, PSCs represent approximately 4% of the resident cells and are located in the periacinar and interlobular space. A central feature of the desmoplastic response is the transformation of PSCs from quiescent vitamin A-containing cells into activated myofibroblast-like cells. Characteristic features of this transition include an increase in the production of ECM, including type I and III collagens, laminin, fibronectin, as well as matrix metalloproteinases (MMPs), and tissue inhibitors of metalloproteinases. Other important features of activation include loss of vitamin A lipid droplets, proliferation, enhanced α -smooth muscle actin expression, and upregulation of various cytokines and growth factors. Included in this latter group are transforming growth factor- β 1 (TGF- β 1), platelet-derived growth factor (PDGF), and vascular endothelial growth factor [13]. During pancreatic injury or cancer, a variety of factors, such as epidermal growth factor (EGF), PDGF, interleukin 1, tumor necrosis factor- α , fibroblast growth factor (FGF) and TGF- β 1, participate in the induction of PSC activation. PSCs may also be activated by ethanol and its metabolites, oxidant stress and endotoxin (Figure 1). Sustained activation of PSCs is further maintained by autocrine signaling via e.g. TGF- β 1, PDGF, connective tissue growth factor, interleukins 1 β and 15, and endothelin 1 (ET-1). Moreover, activin A, a member of the TGF- β family, also has autocrine properties, increasing the secretion and expression of collagen and TGF- β [14]. Several signal transduction molecules involved in PSC activation have successfully been characterized, including mitogen-activated protein (MAP) kinase, peroxisome proliferator-activated receptor γ , phosphatidylinositol 3-kinase/Akt, ρ -kinase, NF- κ β , JAK/signal

transduction and activation of transcription factor (STAT), TGF- β /SMADs, and reactive oxygen species [15].

Cancer cells stimulate PSCs

Pancreatic cancer cells can specifically activate surrounding PSCs. This may occur through cancer cell-induced release of mitogenic and fibrogenic factors, such as PDGF, FGF2 and TGF- β 1. PDGF induce proliferation of PSCs through Src-dependent activation of the JAK2-STAT3 pathway [16] and the MAP kinase pathway extracellular signal-regulated kinases (ERK) 1/2 [17, 18] and p38 [19]. Pancreatic cancer cells also possess an attracting effect on PSCs. PDGF can induce migration of PSCs through activation of the phosphatidylinositol 3-kinase/Akt pathway [18, 20]. TGF- β 1 exerts its effects on PSCs through SMADs 2, 3 and 4 as well as SMAD-independent pathways such as MAP kinases [21]. Moreover, cancer cells express a surface glycoprotein known as ECM metalloproteinase inducer, which has been demonstrated to induce MMP-2 synthesis in PSCs [13, 22]. MMPs are associated with the development of tumor desmoplasia, as well as cancer cell invasiveness through the degradation of the basement membrane. Other mechanisms of cancer cell-induced PSC activation have recently been elucidated and include the action of sonic hedgehog protein, galectin 3, cyclooxygenase (COX), ET-1 and serine protease inhibitor nexin 2 (serpine 2).

Sonic hedgehog protein

There are three known human hedgehog family members: sonic (SHH), Indian, and desert. SHH has recently been detected in precursor lesions and tumors from patients with PDAC. An experimental model aiming at understanding the contribution of SHH to pancreatic cancer revealed an increase in PSCs, collagen I and fibronectin production, and increased tumor weight using a transformed primary pancreatic epithelial cell line in which SHH was

overexpressed [23]. SHH may stimulate PSCs and desmoplasia directly or via TGF- β signaling. In a transgenic mouse model of pancreatic cancer, inhibition of hedgehog signalling was found to reduce tumor-associated stromal tissue and ameliorate gemcitabine uptake in tumor cells [24]. In another experimental study, inhibition of SHH was associated with prolonged survival of 6 days [25]. Clinical trials using pharmacological SHH inhibitors would be of potential future interest when investigating pancreatic cancer.

Galectin 3

This is a member of the β -galactoside-binding protein family, which has been strongly implicated in inflammation and cancer. Galectin-3 has been shown to stimulate proliferation of PSCs in in vitro experiments using the pancreatic cancer cell line SSW1990 [9]. This finding further implies that ECM and polysaccharides might play a role in the progress of pancreatic cancer, given that galectin 3 is a lectin that senses various glycoconjugates.

Cyclooxygenase

Cyclooxygenase enzymes are rate-limiting enzymes in the conversion of arachidonic acid to prostaglandins. Two isoforms of the enzyme, COX-1 and COX-2, have been recognized. COX-1 is constitutively expressed in most tissues under normal conditions, whereas COX-2 is inducible in response to inflammation. The conditioned medium from pancreatic cancer cells has been found to upregulate the COX-2 protein in PSCs [26]. Inhibition of COX-2 decreased growth of PSCs in response to pancreatic cancer-conditioned media, implying a central role for COX-2 in pancreatic-cancer-cell-stimulated PSC proliferation. The induction of COX-2 by pancreatic cancer cells was most likely mediated by ERK 1/2.

Endothelin 1

Endothelins have been suggested to play an important role in fibrogenesis in several organs, with ET-1 and its receptor being expressed in pancreatic cancer cell as well as PSCs, suggesting the presence of autocrine signaling. Bosentan, a combined endothelin A and B receptor antagonist, has been demonstrated to inhibit the growth of both cell types in vitro [27].

Serpine 2

Serpine 2 is an extracellular serine protease inhibitor that is upregulated in pancreatic cancer. Xenografts models demonstrated that tumors that express serpine 2 show a higher degree of invasiveness and desmoplasia than those negative for serpine 2 [28, 29]. Type I collagen, vimentin and fibronectin are all upregulated in tumors expressing serpine 2, and these are proposed as possible mediators for the effects of serpine 2, together with an altered MMP production [29].

Details of ways in which pancreatic cancer cells influence PSCs are thus slowly emerging (Figure 2). In conclusion, current data indicate that cancer cells promote proliferation, migration and ECM production of PSCs.

PSCs promote tumor progression

The integral role of PSCs in pancreatic tumor progression is becoming increasingly clear. Conditioned medium of PSCs has been shown to induce proliferation, migration and invasion of pancreatic cancer cells in a dose-dependent manner [8, 30]. The proliferation of pancreatic cancer cells is partly mediated by PDGF secretion from PSCs. Other factors that are suspected

to promote proliferation of cancer cells are stromal-derived factor-1, EGF, insulin-like growth factor 1 and FGF [8].

An in vitro approach revealed that cancer cell proliferation significantly increased in direct culture with PSCs as compared to the indirect coculture system [31]. In addition, the direct coculture of PSCs and cancer cells enhanced Notch signaling, suggesting the presence of direct cell-cell contact regulatory mechanisms between PSCs and cancer cells.

Studies with xenograft and orthotopic models in nude mice, in which human PSCs and cancer cells have been implanted simultaneously, directly link PSCs with increased tumor frequency, volume, proliferation and stromal production [7, 8, 22, 30]. In addition, transferase-mediated uridine nick end labeling staining shows that PSCs are capable of reducing cancer cell apoptosis [8].

The role of the ECM

The ECM is composed of collagens, noncollagen glycoproteins, glycosaminoglycans, growth factors, and proteoglycans. However, there exists also another group of ECM proteins, termed matricellular proteins, which lack structural roles but function as modulators of cell–matrix interactions and cell function [32]. Examples of these proteins include periostin, connective tissue growth factor, tenascin C, SPARC (secreted protein acidic and rich in cysteine) and thrombospondin (TSP).

The ECM is an important component in regulating the development and progression of pancreatic cancer. PSCs secrete type I collagen, which has been associated with increased integrin mediated cell-cell adhesion, proliferation and migration of pancreatic cancer cells [33]. SPARC is a 32 kDa calcium-binding matricellular glycoprotein with antiproliferative and de-adhesive functions [34]. During cancer development, SPARC may function as a tumor promoter or tumor suppressor depending on the cancer type [35]. Recent studies have

revealed high levels of SPARC expression in stromal tissue from patients with pancreatic cancer, with SPARC being frequently absent in cancer cells [36, 37]. High SPARC expression in the stroma was correlated with a less favourable prognosis [37, 38]. Recent data by Mantoni et al. [38] and Chen et al. [39] have demonstrated that PSCs express higher levels of SPARC than pancreatic cancer cells, and that SPARC could be detected in the conditioned medium of PSCs [39]. However, the precise function of SPARC in PDAC progression remains conjectural. An increased invasiveness of pancreatic carcinoma cells in the presence of exogenous SPARC has been noted, which could explain the decreased survival rates of patients with stromal SPARC overexpression [38]. However, exogenous SPARC inhibits cell migration and proliferation, and inhibition of endogenous SPARC in cultured cancer cells by shRNA augments growth and migration, suggesting that endogenous SPARC may act as a tumour suppressor in pancreatic cancer [39]. Additional studies are needed to clarify the exact role of SPARC in the progression of pancreatic cancer.

Decorin, lumican and versican are strongly expressed in pancreatic cancer [40]. PSCs are the major source of these proteins. Decorin and lumican are small leucine-rich proteoglycans that may possess antitumor properties, while versican (a large proteoglycan) seems to facilitate cancer invasiveness and metastasis. Conditioned medium from pancreatic cancer cell lines suppressed the expression of decorin and lumican, but stimulated the expression of versican in cultured PSCs [40]. In this manner, tumor cells can alter the ECM, creating a less tumor hostile environment.

The matricellular protein tenascin C is expressed in several contexts of tissue remodeling, including the desmoplastic reaction of pancreatic cancer. Increased expression of tenascin C and its receptor, annexin II, was observed in the progression from pancreatic intraepithelial neoplasia 1 lesions to pancreatic cancer [41]. Tumor necrosis factor α and TGF- β 1 were shown to induce the tenascin expression of PSCs.

Periostin is another matricellular protein that is expressed by PSCs. Coculture studies have demonstrated that periostin expression in PSCs is induced by pancreatic cancer cells. Low concentrations of periostin have suppressive effects on malignant behavior of pancreatic cancer cells, while higher concentrations induce phosphorylation of Akt and promote cell migration [42]. The level of periostin is suggested to regulate whether the desmoplastic reaction represents an advantage or disadvantage for tumor progression.

MMPs are proteolytic enzymes that promote matrix degradation and cancer invasion. Both MMP-2 and MMP-9 have been associated with the development of pancreatic cancer. It has been shown that PSCs secrete MMP-2 and its inhibitors, i.e. tissue inhibitors of metalloproteinases 1 and 2 [43, 44]. Additionally, factors secreted by PSCs can promote MMP production in pancreatic cancer cells, e.g. TSP that is expressed in stromal cells and increase cancer cell production of MMP-9 [45]. In this manner, PSCs are able to facilitate the local spread of the tumor.

The stromal component of pancreatic cancer has been suggested to cause resistance to chemotherapy and radiation in pancreatic cancer. In vitro, PSCs induce cancer cell resistance to both gemcitabine and radiation [30]. This effect may partly be mediated by PSC secretion of e.g. the ECM proteins laminin and fibronectin, which have been shown to have anti-apoptotic effects [8].

Tumor angiogenesis and metastasis

In pancreatic cancer, a hypoxic microenvironment exists within the tumor mass [46], with microvessel density being significantly reduced in PDAC compared to normal pancreatic tissue [47]. This is perplexing, given that both cancer cell and PSCs can produce hypoxia-inducible factor 1a [48] and that PSCs can secrete proangiogenic substances such as vascular endothelial growth factor, FGF, and periostin. However, the dichotomous role of PSCs as

both an angiogenesis stimulator and inhibitor must be considered. It has been shown that PSCs modulate the production of the antiangiogenic protein endostatin of pancreatic cancer cells. Supernatants from cocultured cancer cells and PSCs significantly increased the amount of endostatin ($210 \pm 14\%$, $P < 0.001$) [47]. During hypoxia, activated PSCs increase their profibrogenic response through the secretion of type I collagen and fibronectin [47,49]. Taken together, these studies indicate that PSCs may create a locally proangiogenic microenvironment at the invasive front of cancer cells (early event), while contributing to tissue hypoxia via antiangiogenic effects on cancer cells and fibrotic compression of vessels (later event).

Early local and distant metastasis is one of the hallmarks of PDAC. The ability of PSCs to facilitate malignant cell spread has been studied in orthotopic models. Hwang et al. [30] found that coinjection of BxPC3 cancer cells with PSCs in an orthotopic murine model of pancreatic cancer resulted in increased tumor metastasis in a dose-dependent manner. In a study by Vonlaufen et al. [8], the incidence of regional and distant metastasis was significantly higher in mice injected with both MiaPaCa-2 tumor cells and PSCs, compared with MiaPaCa-2 alone.

In summary, PSCs exert their influence on the proliferation, migration and invasion of pancreatic cancer cells by paracrine factors (Figure 2), direct cell-cell contact and by altering the ECM surrounding the cancer cells. Further, PSCs may contribute to angiogenesis as well as the propensity for distant metastasis associated with pancreatic cancer. The development of PDAC is generally assumed to be a multistep process that involves a progressive accumulation of genetic alterations driving malignant transformation. Although PSCs can promote pancreatic cancer, it remains unknown whether PSCs also play a role in the initiation of tumor development, i.e. carcinogenesis.

Targeting PSCs

As mentioned previously, several signaling pathways involved in PSC activation have recently been identified. Because activated PSCs are key players in PDAC promotion and progression, therapeutic targeting of these pathways may provide new avenues for anti-fibrotic and anti-neoplastic therapies. Hitherto, most studies have focused on modulation of stellate cell function in the context of chronic pancreatitis, and studies targeting PSC activation in the setting of pancreatic tumorigenesis are few [50]. In the following, we describe some potential ways of targeting PSCs.

Signaling by PDGF, the most potent mitogenic stimulus for PSC, is one therapeutic approach that has been studied. Administration of the PDGF inhibitor, trapidil, was found to suppress PDGF-induced ERK activation, leading to decreased PSC proliferation [51]. Similarly, treatment with curcumin (deferuloylmethane), a polyphenol compound found in turmeric, resulted in decreased PDGF-induced proliferation of PSC in vitro, along with reduction in α -smooth muscle actin and collagen type 1 expression as well as secretion of monocyte chemoattractant protein 1 [52]. The mechanism of action of curcumin is to some extent mediated by inhibition of ERK 1/2 activation through the induction of hemoxygenase 1 expression. Upregulation of hemoxygenase 1 inhibits ERK 1/2-mediated PDGF activation and may therefore have the potential to become a novel strategy in the prevention of pancreatic fibrosis [53]. Epigallocatechin-3-gallate, a natural antioxidant purified from green tea, has also shown beneficial effects with regard to PDGF-induced PSC proliferation. In a study on male Wistar rats, it was found that epigallocatechin-3-gallate inhibited PDGF-induced proliferation and migration of PSCs [54]. The antifibrotic effects of epigallocatechin-3-gallate were related to PDGF-induced phosphorylation of the PDGF- β receptor and the activation of the downstream signaling molecules ERK and phosphatidylinositol 3-kinase/Akt.

Another potential strategy is to target TGF- β , a major fibrogenic cytokine and activator of PSCs. A recent study examining the effects of SMAD7, an intracellular inhibitor of TGF- β signaling, using a transgenic mouse model reported antifibrotic activity and reduced PSC activation [55]. However, the role of TGF- β in pancreatic cancer is complex. In normal epithelial cells and early tumours, it may act as a tumour suppressor, while during tumour progression it may become an oncogenic factor that induces effects on proliferation, angiogenesis, invasion, and immune response [56]. Future potential therapies should therefore focus on selective inhibitors that do not involve the tumor-suppressive effects of TGF- β .

The inhibitory role of interferons on hepatic stellate cells [57], suggests that these cytokines may also be relevant to setting of pancreatic fibrogenesis. Interestingly, interferon (IFN) β and γ have shown inhibitory effects on PSC proliferation and collagen type I production [58]. Both IFN- β and IFN- γ were found to strongly induce the phosphorylation of the STAT1 and STAT3, resulting in growth inhibition of PSCs. In another experiment, IFN- γ decreased the expression of 2 autocrine mediators of PSC activation, connective tissue growth factor and ET-1 [59].

Fitzner et al [27] have investigated the effects of ET receptor modulation in models of pancreatic fibrosis and cancer. The ET-1 receptor antagonist bosentan was able to inhibit proliferation of both PSCs and pancreatic cancer cells in vitro as well as collagen synthesis in PSCs. These findings indicate that bosentan has both antifibrogenic and antitumor effects, making it an intriguing potential therapeutic substance for patients with PDAC.

As described previously, decorin is a small leucine-rich proteoglycan, which not only functions as an ECM organizer, but also has antitumor properties. This latter effect possibly

involves the inhibition of various growth factors, such as TGF- β , EGF, PDGF, which can be released by cancer cells. Decorin is notably downregulated in several cancer forms. It has been demonstrated that decorin expression and production by PSCs are upregulated in pancreatic cancer, possibly representing a protective host reaction aimed at tumor growth inhibition [60]. With these results in mind, decorin surprisingly attenuated the chemotherapeutic effects of gemcitabine on 4 pancreatic cancer cell lines in vitro. Further studies should be undertaken regarding the potential attenuating effects of decorin on gemcitabine efficiency.

Downregulation of TSP-2 is yet another potential therapeutic approach because of the central role that TSP-2 has in migration and invasiveness of pancreatic cancer cells. TSP-2 is a matricellular protein which promotes adhesion to ECM proteins via integrins. PSCs are known to express TSP-2. Coculture of tumor-derived PSCs and pancreatic cancer cells resulted in the migration of cancer cells towards tumor-derived PSCs [61]. When TSP-2 expression was reduced with a selective siRNA, pancreatic cancer cell invasion mediated by tumor-derived PSCs decreased.

Very recently, adrenomedullin (AM), a hypotensive peptide originally isolated from human pheochromocytoma, has been found to be highly expressed in human pancreatic adenocarcinoma (43 of 48 samples) and in pancreatic cancer cell lines [62]. Exposure of pancreatic cancer cell lines to AM induces pancreatic cell proliferation and invasion in vitro. Systemic silencing of AM on both human MPan96 cancer cells and mouse cells reduced tumor growth with $88 \pm 0.4\%$ ($p < 0.05$) without having any deleterious effect on the animals. It has been suggested that the effects of AM are exerted via the AM receptor in an autocrine manner. Furthermore, recent data imply that the AM receptor exists not only in cancer cells, but also in PSCs and endothelial cells, suggesting that AM also mediates paracrine effects on PSCs and endothelial cells [63].

Finally, several other compounds appear to have inhibiting effects toward PSCs. Included in this group are peroxisome proliferator-activated receptor γ ligands, inhibitors of the renin-angiotensin system, antioxidants, protease inhibitors, and MAP kinase inhibitors [64].

Concluding remarks

In summary, a series of recent studies provides insight into mechanisms by which PSCs influence pancreatic cancer growth and progression. Pancreatic cancer cells produce mitogenic and fibrogenic factors such as TGF- β 1, PDGF, SHH, galectin 3, ET-1, and serpine 2, all of which may promote the activated PSC phenotype. In a positive feedback loop, activated stellate cells release a variety of stimuli, including PDGF, stromal-derived factor 1, EGF, insulin-like growth factor 1, FGF, SPARC, MMPs, small leucine-rich proteoglycans, periostin and collagen type I that mediate effects on tumor growth, invasion, metastasis and resistance to chemotherapy. Continued research will hopefully allow the potential development of novel therapeutic strategies targeted against the tumor microenvironment.

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Growth factors and cytokines (e.g. PDGF,
IL-1, TNF- α , TGF- β , activin A), ethanol,
oxidative stress, endotoxin



Figure 1. A central feature of the desmoplastic response is the transformation of PSCs from quiescent vitamin A-containing cells into activated myofibroblast-like cells. A variety of factors released during pancreatic injury or cancer, such as cytokines, growth factors, ethanol, oxidative stress, and endotoxin participate in the induction of PSC activation.

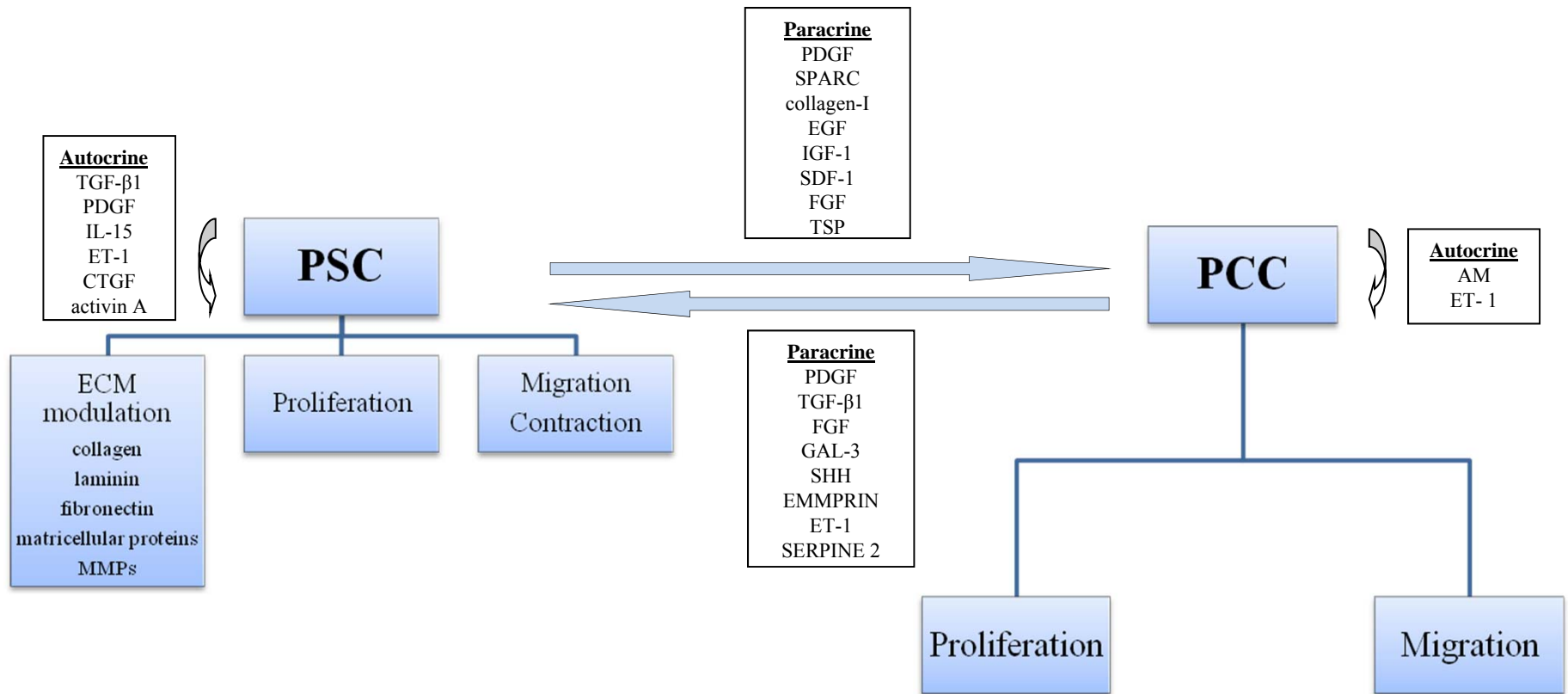


Figure 2. Signaling interactions between PSCs and tumor cells. Pancreatic cancer cells (PCC) produce a variety of mitogenic and fibrogenic stimulants acting on PSCs. Activated PSCs produce growth and survival factors that can mediate effects on cancer cells either directly or via altering the microenvironment. In this way, a positive stimulatory loop is maintained.