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Review

CAF Subpopulations: A New Reservoir of Stromal Targets in Pancreatic Cancer

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Cancer-associated fibroblasts (CAFs) are one of the most significant components in the tumour microenvironment (TME), where they can perform several protumourigenic functions. Several studies have recently reported that CAFs are more heterogenous and plastic than was previously thought. As such, there has been a shift in the field to study CAF subpopulations and the emergent functions of these subsets in tumourigenesis. In this review, we explore how different aspects of CAF heterogeneity are defined and how these manifest in multiple cancers, with a focus on pancreatic ductal adenocarcinoma (PDAC). We also discuss therapeutic approaches to selectively target protumourigenic CAF functions, while avoiding normal fibroblasts, providing insight into the future of stromal targeting for the treatment of PDAC and other solid tumours.

Cancer-Associated Fibroblasts: Major Players in Pancreatic Tumourigenesis

PDAC is one of the most lethal solid malignancies, with a 5-year survival rate of ~9% [1]. Widespread fibrotic desmoplasia is one of the cornerstones of PDAC development, progression, metastasis, and treatment resistance, where stromal components of the TME can have fundamental and integrated roles in promoting tumourigenesis [2–6]. This extensive desmoplastic reaction is characterised by the recruitment and activation of CAFs, aberrant extracellular matrix (ECM) deposition and remodelling, tumour angiogenesis, altered blood supply, as well as increased inflammation coupled with altered (and often impaired) innate and adaptive immune responses [4,5,7–9].

CAFs are one of the most prominent and active components in the pancreatic TME [4,5]. During early tumour initiation, reciprocal tumour–stroma signalling drives the reprogramming of mesenchymal cells, such as pancreatic stellate cells (PSCs), into CAFs [6], after which they can perform numerous anti- and protumourigenic functions in the TME. For instance, PDAC CAFs are the chief source of fibrotic matrisomal components, such as collagens, hyaluronic acid (HA), and fibronectin, among others, as recently described by Tian et al. [10]. This fibrosis has downstream biomechanical and biochemical effects in the TME [11], including impaired drug efficacy due to reduced interfibrillar spacing in the interstitium, which leads to reduced vascular patency and poor immune cell infiltration [12–17]. Moreover, CAFs produce several chemokines, cytokines, growth factors, miRNAs, exosomes, and metabolites that can instruct cancer cells and other TME components to promote malignant biology [4,5].

Given the central role of CAFs in the TME, there have been several attempts to target them in combination with other therapeutic approaches, such as chemotherapy or immunotherapy, for the treatment of PDAC. Thus far, this treatment approach has been largely unsuccessful and, in some preclinical studies, harmful. This is best exemplified through the studies of Özdemir et al. [18] and Rhim et al. [19], which both found that depletion of CAFs in genetically engineered mouse models (GEMMs) of PDAC resulted in poorly differentiated and aggressive tumours and, therefore, worse survival. These findings were also reflected in the clinic, where the therapeutic targeting of stromal fibrosis via Hedgehog (Hh) pathway inhibition in several clinical trials with patients with PDAC added no benefit or were more harmful than standard-of-care gemcitabine chemotherapy and/or neoadjuvant treatment with the cytotoxic drug combination FOLFIRINOX alone [20–22][i,ii]. Conversely, other preclinical studies have reported that targeting the TME in this way may sensitise the tumours to immunotherapy, which we discuss later in this review. Furthermore, targeting stromal Hh signalling has proved beneficial in other cancers, such as breast cancer [23][iii], indicating that the therapeutic benefits of this approach may be tissue or cancer type specific. In parallel, virtual microdissection of a

Highlights

Tumour heterogeneity drives a diverse and plastic spectrum of cancer-associated fibroblasts (CAF) subpopulations in pancreatic ductal adenocarcinoma (PDAC).

CAF heterogeneity is spatially regulated by signals derived from genetically diverse cancer cells and distinct microenvironment conditions, resulting in a range of tumour-promoting and/or tumour-suppressive functionalities.

The recent appreciation of CAF diversity has resulted in innovative approaches to targeting their tumour-promoting functions.

Normalisation of pathogenic CAF subtypes, or the blockade of their tumour-promoting functions, can increase tumour cell sensitivity to cytotoxics or immunotherapy, highlighting the potential for dual treatment approaches in PDAC.

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large cohort of primary and patient-derived PDAC specimens using a bioinformatics approach found two subpopulations of PDAC fibroblasts that had either a 'normal' or 'activated' genomic signature, with the activated status resulting in significantly poorer patient outcomes [24]. These studies have prompted the field to re-evaluate the function and biology of stromal populations, such as CAFs, with a view that these cells are more heterogeneous and plastic than was previously thought. Since then, several groups have identified distinct subpopulations of pancreatic CAFs, which have multiple, and in some cases differential, functions in the TME [25,26]. Furthermore, this functional diversity in CAFs and other stromal populations is mirrored in other malignancies, such as breast [27], lung [28], and colon cancer [29]. Importantly, all these studies reveal the dynamic complexity of CAFs and hint that normalisation, or targeted depletion, of certain subsets rather than widespread ablation may be a more promising approach to stromal targeting in PDAC. Evidently, new work should focus on how CAF phenotype and function are spatiotemporally regulated throughout pancreatic tumourigenesis, and how this heterogeneity in stromal populations drives tumour progression. We argue that understanding this diversity will uncover novel and specific therapeutic targets, presenting an opportunity to improve the dismal outcomes currently observed in patients with PDAC.
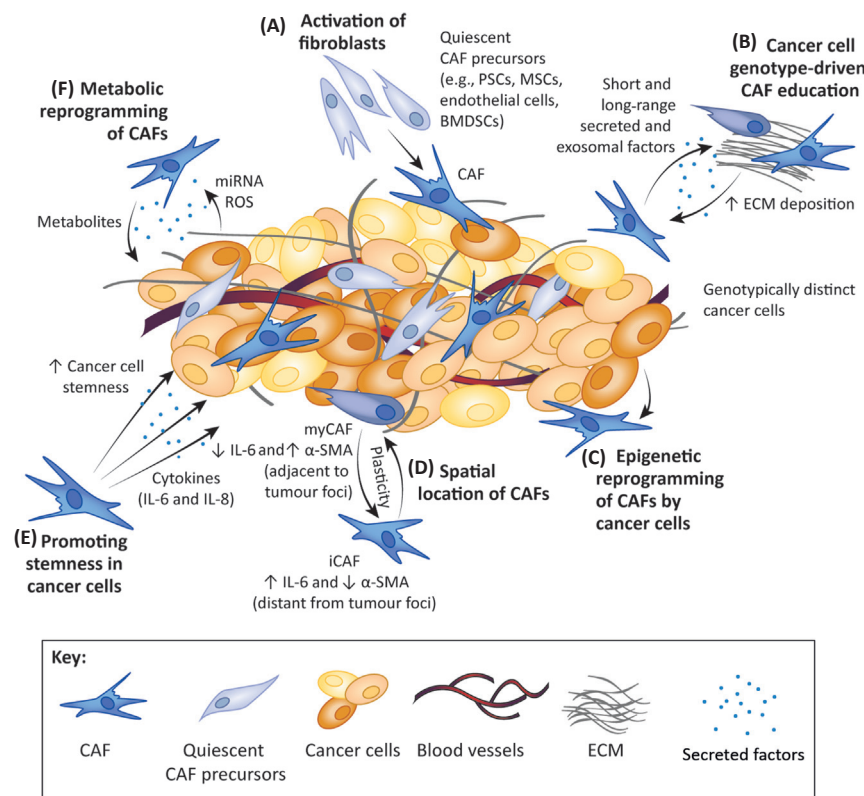
Defining CAF Heterogeneity

Heterogeneity of CAF Biomarkers

Generally speaking, the term 'cancer-associated fibroblast' is used to describe all activated fibroblastic cells in the TME of solid cancers that have a phenotype, function, or location distinct from normal, quiescent fibroblasts. It is thought that the most frequent cellular precursor for pancreatic CAFs are PSCs [30,31]. PSCs can be identified by the expression of desmin and glial fibrillary acidic protein (GFAP), as well as acetylcholine receptors and vitamin A-containing lipid droplets, all of which are distinct from other fibroblast populations [32–34], but share similarities with other stellate cells throughout the body, such as hepatic stellate cells [34,35]. Besides PSCs, there is evidence that PDAC CAFs can also arise from mesenchymal stem cells (MSCs), bone marrow-derived stem cells, and/or endothelial cells (Figure 1A) [36–38]. In other solid tumours, there is evidence to suggest that CAFs also derive from local resident fibroblasts [39], adipocytes [40], adipose-derived MSCs [41,42], hematopoietic stem cells [43], and pericytes [44]. However, most of these studies were performed using cell culture or transplantation approaches, highlighting the lack of lineage-tracing studies that adequately address the origin of CAFs (see Outstanding uestions). Evidently, more research is required to elucidate these transitions further. Also, future studies should investigate whether the developmental origin of a CAF correlates with its functions and ability to be targeted (see Outstanding Questions). Interestingly, some populations of MSCs can also influence pancreatic tumourigenesis. For example, in 2016, Waghray et al. identified and characterised a novel population of cancer-associated MSCs (CA-MSCs) in PDAC that controls tumour growth and progression via granulocyte-macrophage colony-stimulating factor (GM-CSF) [36].

Perhaps unsurprisingly, there is no known unique biomarker to precisely identify the entire pancreatic CAF population [45,46]. Instead, there are several conventional and emerging biomarkers that can be used in concert to identify this diverse stromal population. The most commonly used PDAC CAF biomarkers are alpha-smooth muscle actin (α-SMA), fibroblast activation protein (FAP), vimentin, fibroblast-specific protein 1 (FSP1), podoplanin (PDPN/gp38), and platelet-derived growth factor receptor alpha and/or beta (PDGFRα/β) [23,47]; however, this list is neither all-inclusive nor entirely CAF specific [46]. In the pancreas specifically, PSC-derived CAFs lose lipid droplet expression once activated [30]. Clearly, there are difficulties in isolating CAFs from tissue, since even a biomarker panel approach will not identify all CAFs within a tumour. Therefore, it is important to consider the combination of markers utilised to isolate CAFs when comparing studies and interpreting results in this context; however, this could be circumvented with the discovery and development of novel and more specific CAF biomarkers in the future (see Outstanding Questions).

Cancer Cell Genotype-Driven CAF Heterogeneity

PDAC tumours are molecularly heterogeneous; different regions of the tumour contain molecularly distinct cancer cells, which results in distinct CAF subpopulations (Figure 1B) [24–26,31,48–51]. Laklai

Figure 1. Mechanisms of Cancer-Associated Fibroblast (CAF) Heterogeneity.
(A) CAFs can originate from several different cell types and, therefore, exhibit a range of activation states that can be further stimulated to alter cancer development. (B) The molecular heterogeneity of cancer cells drives differences in CAF subpopulations via direct, short-, and long-range paracrine signalling. (C) Cancer cells secrete factors that can reprogram the epigenome of CAFs, resulting in more aggressive phenotypes. (D) Varied localisation of CAFs within the tumour microenvironment (TME) leads to differences in the signals that CAFs receive, resulting in functionally and spatially distinct CAF subpopulations. (E) Specific CAF populations can promote a highly plastic, stem cell-like population of cancer cells that can contribute to chemoresistance. (F) Metabolic coupling between CAFs and tumour cells occurs via tumour cell manipulation of distinct CAF subpopulations, where CAFs produce energy-rich metabolites to feed the tumour cells. Abbreviations: α-SMA, alpha smooth muscle actin; BMDSC, bone marrow-derived stem cell; ECM, extracellular matrix; iCAF, inflammatory CAF; IL-6/8, interleukin 6/8; MSC, mesenchymal stem cell; myCAF, myofibroblastic CAF; PSC, pancreatic stellate cell; ROS, reactive oxygen species.

et al. (2016) revealed that the genetically regulated phenotype of pancreatic tumour cells tunes the function of the adjacent periductal stroma to promote tumourigenesis via integrin and Yes-associated protein (YAP) signalling [7]. In line with this, Wörmann and colleagues found that loss of tumour suppressor gene *p53* function in PDAC tumour cells resulted in a more fibrotic stroma compared with wild-type *p53* controls, which was conducive to tumour growth via activation of the Janus kinase 2-signal transducer and activator of transcription 3 (JAK2–STAT3) signalling pathway in cancer cells [52]. Furthermore, in a PDAC GEMM, pancreatic cancer cells with a gain-of-function (GoF) mutant *p53* (p53mut) genotype were found to induce fibroblast activation via altered exosomal secretion of podocalyxin (PODXL), a highly sialylated glycoprotein, compared with the exosomes from *p53*-null (p53null) cancer cells [53]. This p53mut-specific exosome signature also altered integrin signalling and increased ECM deposition by normal fibroblasts, pushing them towards a pro-invasive CAF-like phenotype [53]. The fibroblasts that were exposed to p53mut cancer cell-derived exosomes

produced an ECM with a similar stiffness to fibroblasts treated with p53null cancer cell-derived exosomes, but this ECM was significantly less adhesive [53]. These weaker cancer cell–matrix interactions allowed cancer cells to migrate and invade more readily over the ECM produced by p53mut-educated fibroblasts compared with their p53null counterparts. In addition, it was shown that exosomal PODXL released by p53mut cancer cells also significantly altered the ECM architecture of lung parenchyma in mice to be more amenable to metastatic colonization [53].

In line with this study, the existence of a p53-driven hierarchy in PDAC was recently reported, where GoF p53mut cancer cells educate CAFs partially via tumour necrosis factor-alpha (TNF-α) and nuclear factor-κB (NFκB) signalling to create a permissive environment with pro-invasive cues via deposition of perlecan, an ECM proteoglycan [47]. Strikingly, these p53mut-educated CAFs caused invasion of normally poorly invasive p53null tumour cells (to the same extent as the highly invasive p53mut cancer cells) through both direct and long-range paracrine signalling [47]. Furthermore, p53null-educated CAFs, which create an environment less permissive to invasion and metastasis than p53mut-educated CAFs, can be subsequently re-educated by either p53mut cancer cells or their matched CAFs to, in turn, behave like p53mut-educated CAFs [47]. These data show that this chain reaction of transferring aggressive phenotypes to less aggressive fibroblasts and cancer cells can lead to enhanced invasion and metastatic spread as well as chemotherapy resistance *in vivo* [47]. Overall, the specific genotype of tumour cells can therefore directly influence CAF functions, with CAFs exhibiting extensive plasticity, whereby they can be educated by, and respond to, cancer cells via local- and/or long-range bidirectional interactions to enhance tumourigenesis (Figure 2). Thus, a more nuanced, context-dependent fine-tuned treatment approach, where only certain subsets of CAFs, or their actions, are specifically targeted, should be investigated further as a therapeutic intervention to potentially improve clinical outcomes, as discussed in more detail later.

CAF Heterogeneity at the Epigenetic Level

While genetic aberrations, such as mutations or chromosomal rearrangements, are rare in CAFs [54,55], they can be epigenetically altered upon interactions with neighbouring cells via DNA and
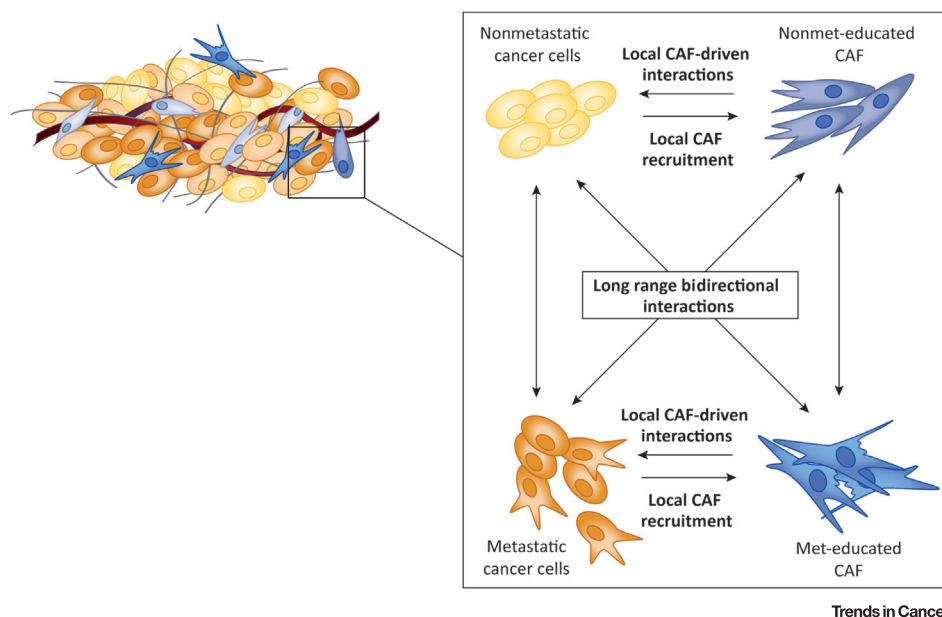

Trends in Cancer

Figure 2. Cancer-Associated Fibroblast (CAF) and Tumour Cell Crosstalk Can Occur through Both Local and Long-Range Paracrine Signalling.
Molecularly and phenotypically aggressive cancer cells and CAFs are able to confer protumourigenic characteristics in spatially distinct, less aggressive counterparts through short- and long-range secreted and exosomal factors. Abbreviation: met, metastasis/metastatic.

histone methylation (Figure 1C) [56,57]. Methylation is a reversible process that can be targeted with demethylating drugs, such as 5-aza-2'-deoxycytidine (DAC). In 2015, Albrengues et al. found that the CAF phenotype in head and neck, lung, and breast tumours was sustained through epigenetic modifications via a prominent proinflammatory cytokine called leukemia inhibitory factor (LIF), with this effect reversed by DNA methylation inhibitors. Interestingly, a recent paper by Biffi et al. implicated LIF in PDAC CAF heterogeneity, where it functions as an autocrine mediator of JAK/STAT signalling in PSCs, which then causes the PSCs to become more inflammatory (and pro-metastatic) in phenotype [26]. Xiao et al. reported that PDAC tumour cells promoted methylation of the SOCS1 gene in CAFs, leading to reduced expression of SOCS1, a known STAT inhibitor [56]. This resulted in phosphorylation of STAT3, which promoted the secretion of several protumourigenic growth factors, such as insulin-like growth factor 1 (IGF-1) [56]. Interestingly, it was shown that, in the absence of epigenetic reprogramming, the resident pancreatic fibroblasts were hostile to tumour development, indicating that this process may be a critical mechanism in PDAC tumourigenesis [56]. This reprogramming appears to occur via contact between cancer cells and CAFs [56], highlighting the importance of spatial location in contributing to specific CAF phenotypes. The preclinical efficacy for targeting DNA methylation in PDAC has already been shown, whereby early intervention treatment slowed tumourigenesis and increased survival in a stroma-rich GEMM of PDAC model, where CAFs would have been targeted [58]. Most recently, Eckert et al. identified nicotinamide N-methyltransferase (NNMT), a methyltransferase involved in metabolic regulation, as the master regulator of CAF phenotype and function in high-grade serous ovarian cancer (HGSC) [59]. Strikingly, NNMT maintained the CAF phenotype by metabolically reprogramming the epigenome of the stroma via DNA and histone hypomethylation [59]. Functionally, treatment with an NNMT inhibitor in an orthotopic model of HGSC reduced tumour cell burden and proliferation [59]. This result was mirrored in a cohort of patients with TCGA, where high NNMT expression was correlated with platinum-treatment resistance as well as poorer survival [59]. As such, the prognostic value and function of NNMT in PDAC should be investigated. Although there are several active clinical trials therapeutically targeting the epigenome of tumours, thus far most have focussed on blood-related malignancies and have yet to be assessed for cell-specific stromal reprogramming in solid tumours [60].

CAF Heterogeneity Based on Spatial Location

The location of CAF populations can have far-reaching consequences for PDAC development and progression. In 2017, Öhlund and colleagues described two spatially and functionally distinct CAF populations in PDAC: inflammatory CAFs (iCAFs), which have low expression of α-SMA and high expression of inflammatory mediators, such as interleukin-6 (IL-6), IL-11 and LIF, and myofibroblasts (myCAFs), which express high levels of α-SMA and low levels of inflammatory mediators [25]. iCAFs and myCAFs were found in distinct spatial locations, with myCAFs residing in close proximity to tumour foci, whereas iCAFs were located more distantly to tumour cells [25] (Figure 1D). Interestingly, the authors reported a small population of CAFs that expressed a combination of iCAF and myCAF markers, indicating that these populations may not be fixed in differentiated states but instead have the potential for plasticity and conversion from one state to another [25]. In a later study by the same group [26], it was found that this heterogeneity is reliant on tumour cell-derived IL-1 and transforming growth factor-beta (TGF-β) signalling cascades. Here, IL-1 drives the iCAF phenotype via JAK-STAT, while TGF-β antagonises this mechanism by downregulating stromal IL-1 receptor 1 expression (IL-1R1) to promote a more myofibroblastic CAF phenotype [26]. In this study, an intermediate population of CAFs was identified in vivo [26], supporting previous in vitro work that CAF subpopulations are plastic cell states [25] rather than terminally differentiated subtypes. This was further reinforced by the successful conversion of PDAC iCAFs to myCAFs with JAK inhibition in vivo [26]. This plasticity presents an exciting opportunity to target deleterious CAF subpopulations, such as iCAFs, by pushing them into a tumour-restraining state (myCAFs). Nevertheless, it remains to be seen whether targeting a particular CAF subpopulation will have lasting effects, considering the acute ability of fibroblasts to adapt to direct, local, and long-range factors [47]. In PDAC and other cancers, it is still not fully understood whether CAF subpopulations are intrinsically heterogenous (due to their cellular precursor or otherwise) or whether the presence of different CAF populations are due to paracrine and/or mechanical signalling alone. This also calls into question whether CAFs of different origin

are differentially amenable to therapeutic targeting (see Outstanding Questions). As such, further preclinical research is essential to fully assess the longevity of targeting CAF subpopulations.

Interestingly, Bernard et al. demonstrated that microenvironmental heterogeneity occurs in premalignancy and throughout cancer progression [61]. This study examined iCAF and myCAF populations in PDAC and in precursor low-grade and high-grade intraductal papillary mucinous neoplasms (LG- and HG-IPMN) [61]. iCAFs were only apparent in PDAC, whereas myCAFs were lowly abundant in LG-IPMN and highly abundant in HG-IPMN, indicating that myCAF activation can also occur in the non-invasive setting. Interestingly, the increase in iCAFs coincided with a clearly defined switch from immunosurveillance (LG-IPMN) to immunosuppression (PDAC) [61]. Considering that iCAFs produce high levels of chemokines and cytokines that are involved in tumourigenesis and disease progression, it is likely that these cells are also responsible for aggressive tumour spread, although this correlation has yet to be explicitly elucidated. Furthermore, most recently a third PDAC CAF subpopulation was identified using single-cell RNA sequencing (scRNA-seq), which was described as 'antigen presenting CAFs' (apCAFs) [135]. ApCAFs express major histocompatibility complex (MHC) class II family genes as well as CD74 and were reported to have an immunodulatory role, where they can interact with CD4$^+$ T cells [135]. In addition, another recent scRNA-seq study by Hosein et al. identified three subpopulations of fibroblasts in normal mouse pancreata, which then give rise to two distinct populations of fibroblasts (i.e., CAFs) in the pancreatic tumours from the $Kras^{LSL-G12D/+}$; $Ink4a^{fl/fl}$; $Ptf1a^{Cre/+}$ (KIC) GEMM [62]. These two populations were described as inflammatory and myofibroblastic in phenotype, with the latter also expressing some MHC-II-associated genes, perhaps indicating a hybrid population comprising myCAFs and apCAFs. This study again supports the notion of intratumoural CAF heterogeneity in PDAC, albeit with slightly different clustering. Although we know that there are myofibroblastic, inflammatory, and/or antigen-presenting CAFs in PDAC, it is possible that, with further advances in single-cell transcriptomic sequencing and other technologies, either more subpopulations will emerge or, more likely, that a spectrum of CAFs with intersecting and diverse roles in the pancreatic TME will be revealed that could be targeted in the future.

CAF Heterogeneity in Promoting Stemness

CAFs have been implicated in promoting stemness in adjacent tumour cells via the secretion of inflammatory cytokines in several tumour types [23,63–69], including PDAC [70,71]. More recently, it was reported that certain subpopulations of CAFs specifically regulate stemness in lung and breast tumour cells, thus conferring chemoresistance [72] (Figure 1E). Su et al. reported that a population of CAFs positive for the cell surface markers CD10 and GPR77 were responsible for promoting cancer formation and chemoresistance in cohorts of patients with either lung or breast cancer [72]. This secretory CAF subpopulation enhanced stemness in cancer cells through high secretion of IL-6 and IL-8 due to sustained NFκB signalling [72]. This is in line with other work where increased NFκB signalling in p53mut PDAC cancer cells caused an increase in pro-invasive perlecan production in adjacent CAFs via TNF-α secretion from the cancer cells [47,73]. Furthermore, CD10 is implicated in tumour–stroma interactions in PDAC, where CD10$^+$ PSCs can significantly increase the invasiveness of PDAC cancer cells compared with their CD10$^-$ counterparts [74]. Considering these studies, investigating the CD10$^+$GPR77$^+$ CAF subpopulation and NFκB crosstalk in PDAC therefore warrants further study. Conversely, Patel et al. reported a myofibroblastic CAF subpopulation that inhibited cancer cell stemness via secretion of bone morphogenetic protein 4 (BMP-4) in oral carcinoma [75], highlighting the tissue-dependent multifaceted role of CAF subpopulations in regulating cancer cell stemness and subsequent chemoresistance.

CAF Heterogeneity in Metabolic Reprogramming

Solid tumours, such as PDAC, are metabolically heterogeneous. In 1927, Warburg et al. described a metabolic phenomenon where tumour cells favour oxygen-independent glycolysis over mitochondrial oxidative phosphorylation, even in the presence of sufficient oxygen [76], termed the 'Warburg' effect. More recently, a new model of cancer metabolism has been described called the 'reverse Warburg effect', where tumour cells and CAFs become metabolically coupled [77] (Figure 1F). In this model, tumour cells induce oxidative stress in adjacent CAFs via reactive oxygen species (ROS) [78] or miRNAs [79]. CAFs, in turn, undergo a metabolic switch to glycolysis, producing energy-rich

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metabolites that the tumour cells then use to perform oxidative phosphorylation or glycolysis, producing abundant ATP and resisting cell death [77].

In 2010, Martinez-Outschoorn and colleagues found that loss of caveolin-1 (CAV1) in breast fibroblasts resulted in their metabolic reprogramming and acquisition of the CAF phenotype [78]. In 2012, this result was confirmed by others to be due to upregulated TGF-β signalling [80]. CAV1 loss is also observed in PDAC stroma and is associated with poor clinical outcomes in patients with pancreatic cancer [81]. Besides downregulation of CAV1, upregulation of monocarboxylate transporter 4 (MCT4) is observed in CAFs and tumour cells, where it effectively shuttles lactate into tumour cells to increase production of ATP. In 2016, Knudsen et al. found that PDAC CAFs express high levels of hypoxia inducible factor 1a (HIF1α), which then promotes high expression of MCT4, thereby aiding glycolysis [82]. In addition, Zhang et al. found that downregulation of the α subunit of the isocitrate dehydrogenase 3 complex (IDH3α) was responsible for a metabolic switch in CAFs from oxidative phosphorylation to glycolysis and also helped maintain an activated CAF phenotype in breast cancer [83]. In this study, it was established that CAFs undergo metabolic reprogramming to help provide a supportive niche for the adjacent tumour cells, rather than for their own proliferation [83]. In PDAC, Sousa et al. reported that 'activated' PSCs supported tumourigenesis via secretion of alanine, which aids tumour cell proliferation even in low-nutrient conditions. So far, little is known about heterogeneity in CAF metabolism and whether this is correlated with known CAF subtypes; however, it is likely that CAFs will have a spectrum of metabolic profiles that they and tumour cells take advantage of depending on the availability of metabolites. Improving our knowledge of heterogenous cancer cell–CAF metabolic coupling could prove to be an exciting new avenue for the treatment of PDAC. It would also be interesting to investigate whether cancer cells have variable reliance on metabolic coupling to CAFs.

Targeting the Protumourigenic Functions of CAFs: New Therapeutic Avenues in Pancreatic Cancer

Given the many tumour-promoting functions of CAFs, this stromal population and their actions represent a promising therapeutic target for cancer treatment. Over the past decade, many studies have attempted to target CAFs, either using direct targeting of CAFs, via reprogramming of CAFs towards a normal fibroblast phenotype, or by inhibiting crosstalk between CAFs and neighbouring cells. Although some promising results have emerged, CAF targeting still faces many obstacles and challenges due to the heterogeneity of CAF identity and functions, as well as a lack of CAF-specific markers, as highlighted earlier. Nevertheless, our increased knowledge of CAF function and biology has led to several preclinical studies, some of which have begun to be translated into ongoing clinical trials.

Depleting CAFs

Direct depletion of CAFs has been attempted in GEMM of PDAC models using CAF-related cell surface markers, such as α-SMA or FAP (Figure 3). For instance, depletion of cells expressing α-SMA can be achieved using the α-SMA-thymidine kinase mouse, where all αSMA-expressing cells are ablated upon ganciclovir administration. This model was crossed with the $Ptf1a^{cre/+}$; $LSL-Kras^{G12D/+}$; $Tgfbr2^{flox/flox}$ (PKT) or $Pdx1^{cre/+}$; $LSL-Kras^{G12D/+}$; $p53^{R172H/+}$ (KPC) mouse models of spontaneous PDAC. Surprisingly however, in these mice, selective depletion of α-SMA$^+$ cells resulted in poorly differentiated primary tumours, increased metastatic spread, enhanced intratumoural infiltration of immunosuppressive regulatory T cells (T$_{reg}$), and, thus, reduced survival (Figure 3A) [18]. Similarly, when Rhim et al. generated a Hh-knockout PDAC GEMM, it was reported that the resulting tumours were more aggressive, less differentiated, and highly proliferative despite having lower stromal content [19]. Conversely, the depletion of FAP-expressing cells reduced tumour growth and re-established tissue homeostasis [84]. In this study, conditional ablation of FAP$^+$ cells using diphtheria toxin resulted in an enhancement of antitumourigenic cytotoxic CD8$^+$ T cells and slowed pancreatic tumour growth [84] (Figure 3B). Moreover, in 2010, Duda and colleagues reported that the depletion of CAFs through systemic administration of diphtheria toxin decreased both primary tumour growth and overt metastatic colonization [85]. The authors showed that this was due to a reduction in CAFs co-migrating to the metastatic niche, a phenomenon that has also
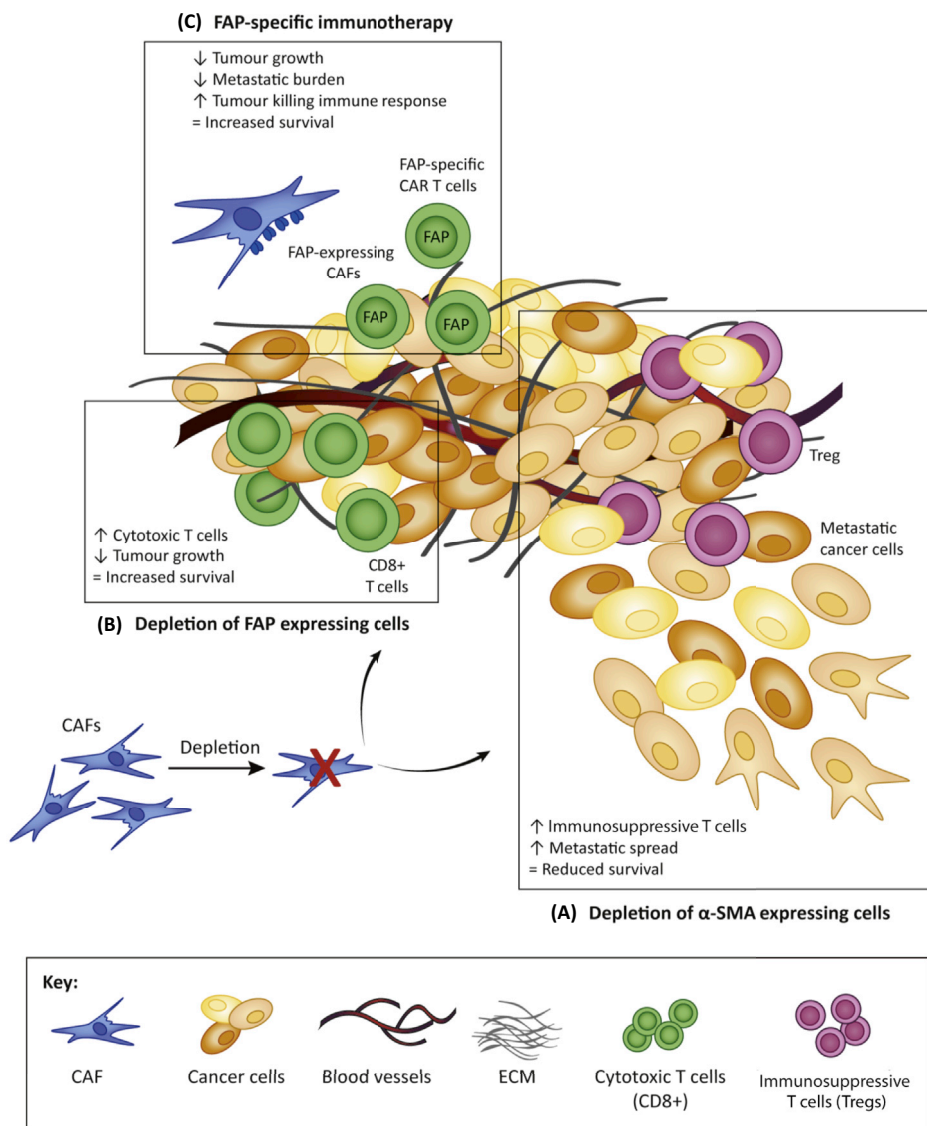
Figure 3. Therapeutic Potential of Targeting Cancer-Associated Fibroblasts (CAFs) via Widespread Depletion Highlights the Heterogeneity of CAF Populations.
(A) Widespread ablation of alpha smooth muscle actin (α-SMA)-expressing CAFs results in poorly differentiated, immunosuppressive, and metastatic tumours. (B) Depletion of fibroblast activation protein (FAP)-expressing CAFs through genetic deletion, pharmacological targeting or (C) chimeric antigen receptor (CAR) T cell therapy can decrease tumour growth and metastatic spread. Targeting FAP can also increase cytotoxic immune infiltration and, therefore, survival. Abbreviation: Treg, regulatory T cell.

been observed in breast and lung cancer models [85–87] (see Outstanding Questions). Furthermore, genetic deletion or pharmacological targeting of FAP-expressing cells reduced tumour growth in mouse models of pancreatic cancer [88] as well as colorectal, lung, and breast cancer [89–92]. Considering these promising results, targeting FAP in patients with cancer was assessed using sibrotuzumab, a FAP-specific antibody. While sibrotuzumab was found to be well tolerated and to halt tumour progression in Phase I trials [89,90], it failed to improve survival in a Phase II trial for patients with metastatic colorectal cancer [93].

This suggests that further efforts are needed to develop therapeutically efficient agents against FAP before use in patients with PDAC.

Interestingly, the development of novel immunotherapies also offers new routes to deplete CAFs. For instance, strategies for vaccination against the FAP antigen were shown to reduce tumour growth, decrease metastatic burden, and overcome immune tolerance in mouse models of colon, lung, and breast cancer [94,95]. In addition, FAP-specific chimeric antigen receptor T (CAR T) cells were shown to induce an immune response against FAP-expressing cells, while also reducing tumour growth in pancreatic and lung cancer [96–98] (Figure 3C). Considering this, targeting FAP-expressing cells in human pancreatic tumours with immunotherapy could be beneficial; however, there are inherent issues with this approach because PDAC tumours are often classed as immunologically 'cold', with relatively low basal cytotoxic T cell infiltration. Therefore, future work should include investigating how to enhance the infiltration of T cells into PDAC tumours to maximise the efficacy of this approach. One way this can be done is by improving the vascular patency of tumours, which allows better infiltration of cytotoxic T cells. For example, Johansson-Percival et al. reported that targeted treatment with LIGHT (a TNF family cytokine) normalised and activated the vasculature of tumours [99]. This led to a significant influx of macrophages, CD4$^+$ T cells, and CD8$^+$ T cells, which sensitised the tumours to immunotherapy [99]. Alternatively, the innate immune system could be targeted to enhance cancer cell killing, avoiding any adverse effects caused by overstimulating the adaptive immune system. Furthermore, targeting of the CXCR4/CXCL12 axis has been shown to improve T cell infiltration in PDAC tumours [84]. Overall, targeting FAP in the clinic, whether via antibodies or immunotherapy, remains challenging. In the context of stromal targeting, FAP is not solely expressed by CAFs and, as such, global and/or whole-body inhibition strategies are likely to be accompanied by toxicity. Consequently, the identification of novel, CAF subpopulation-specific markers is required to develop more effective treatments. This could be achieved by targeting cells expressing both CD10 and GPR77, which were recently shown to identify a population of CAFs with protumourigenic and chemoresistance functions in human tumours [72], as mentioned earlier. However, it is yet to be seen whether targeting this subpopulation alone would be sufficient to increase survival in PDAC models and patients, or whether it exists in PDAC tumours. In addition, because of the heterogeneity and plasticity of CAFs, profiling the CAF signature of individual patients may identify patient- or cancer subtype-specific changes in CAF subpopulations that could help with the development and optimisation of novel targeted stromal therapies, in line with the current efforts to develop a personalised medicine approach to cancer treatment [4,5] (see Outstanding Questions).

Reprogramming CAFs into Quiescent Fibroblasts

Rather than complete CAF depletion, which can have adverse effects, researchers have begun to investigate reprogramming CAFs into quiescent fibroblasts to hinder pancreatic cancer progression and to render cancer cells more responsive to treatments (Figure 4). For instance, reprogramming CAFs can be achieved using all-trans retinoic acid (ATRA), which switches both PSCs and CAFs into more quiescent fibroblasts [100,101] (Figure 4). For example, Han et al. treated 'activated' PSCs with PEG-grafted polyethylenimine (PEI)-coated gold nanoparticles loaded with ATRA and heat shock protein 47 (HSP47), which is a collagen-specific molecular chaperone [102] (Figure 4). Using this nanoparticle treatment approach, quiescence was induced in the activated PSCs and ECM production was reduced to normal levels. This resulted in a reversal of the desmoplastic reaction, which was shown to be antitumourigenic in vitro and in vivo [102]. Clinically, ATRA is being tested in combination with standard-of-care gemcitabine and Abraxane in a Phase I clinical trial for pancreatic cancer (STARPAC^{iv}). Interestingly, ATRA treatment can also enhance T cell infiltration, which prolonged survival in KPC mice [101].

CAF reprogramming can also be achieved using vitamin D or calcipotriol (a synthetic form of vitamin D), where CAF activation is reversed via stimulation of the vitamin D receptor (of which ATRA is also a ligand) [103] (Figure 4). Such a strategy reduced inflammation and fibrosis in PDAC tumours. In line with this, treating CAFs with Minnelide, a novel prodrug of a plant-derived diterpenoid epoxide called triptolide, reduced TGF-β signalling and pushed CAFs into a more quiescent state [104]

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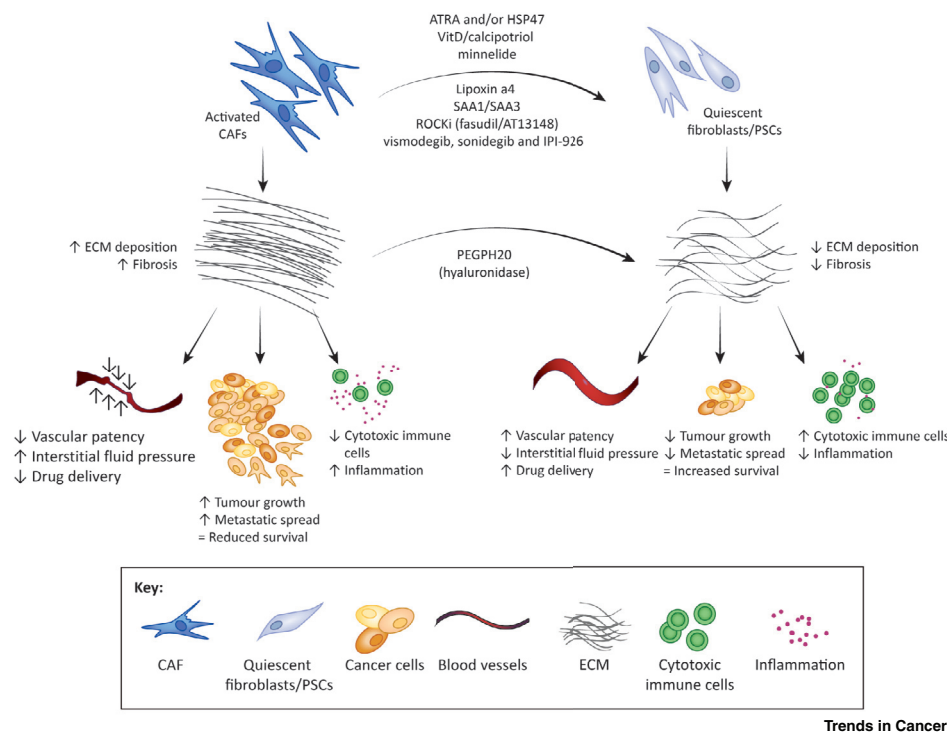
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Figure 4. Reprogramming Cancer-Associated Fibroblasts (CAFs) into Quiescent Fibroblasts.
Activated CAFs produce increased amounts of extracellular matrix (ECM), leading to desmoplasia and poorer outcomes for patients. Reverting the phenotype of CAFs to a more quiescent state ablates this increase in fibrosis. It is also possible to target fibrotic ECM directly with enzymes, such as pegvorhyaluronidase alfa (PEGPH20). These treatments improve drug delivery, vascular patency, and/or increase cytotoxic immune cell infiltration. Overall, this results in decreased metastatic burden and improves survival. Abbreviations: ATRA, all-*trans* retinoic acid; HSP47, heat shock protein 47; PSC, pancreatic stellate cell; ROCKi, Rho-associated protein kinase inhibitor; SAA1/3, serum amyloid A1/3; VitD, vitamin D.

(Figure 4). This treatment also resulted in reduced collagen and HA deposition in the stroma of mouse and patient-derived pancreatic tumours, while improving vasculature patency and drug delivery due to reduced interstitial fluid pressure (IFP) in the tumour [105]. Similarly, lipoxin a4 (LXA4), a bioactive lipid associated with antifibrotic properties in the kidney [106] and lung [107], was demonstrated to revert the myofibroblastic state of pancreatic CAFs without affecting their viability [108] (Figure 4). Here, treatment with LXA4 restricted crosstalk between cancer cells and CAFs, resulting in reduced cancer cell growth *in vitro* and *in vivo* [108]. Serum amyloid A3 (SAA3), an inflammatory apolipoprotein, was recently found to be specifically expressed in protumourigenic murine CAFs and to be a key mediator of interactions between CAFs and cancer cells [109] (Figure 4). In line with this, high stromal *SAA1* expression (the ortholog of murine *SAA3*) in human PDAC tumours was correlated with worse survival [109], suggesting that specifically targeting SAA1^{+} CAFs is a promising approach to revert the TME to a tumour-restraining status [109].

Rho-associated protein kinase (ROCK) is a small GTPase that regulates cell contraction and is commonly upregulated in PDAC [110]. Fasudil, a potent ROCK inhibitor, also reduced PSC activation in a murine model of PDAC, and led to decreased collagen deposition in the TME, increased gemcitabine delivery, and improved survival [111] (Figure 4). Moreover, inhibition of ROCK-based contractility via the small-molecule inhibitor AT13148 resulted in antitumourigenic effects in both *in vitro* and *in vivo* models of PDAC (Figure 4) [112]. The same group also established that activation of ROCK in KPC cancer cells promoted protumourigenic ECM remodelling via matrix metalloproteinase (MMP) secretion [113]. In the KPC mouse model, this aberrant ECM remodelling was abolished upon

treatment with Fasudil, improving overall survival [113]. This aligns with a previous study that demonstrated that transient short-term ROCK inhibition in murine and patient-derived xenograft (PDX) models of PDAC improved the efficacy of gemcitabine and Abraxane and impaired metastatic spread [114] (Figure 4). Moreover, in this study, the grade of fibrosis in the PDXs was associated with the level that this dual-targeting approach was effective. Considering these studies, it would be advisable to further investigate the use of ROCK inhibitors in stratified patients with PDAC, particularly in those where high levels of tumour fibrosis are evident.

Targeting Interactions between CAFs and Their Surrounding Microenvironment

Over the past decade, numerous studies have demonstrated that the intricate crosstalk between CAFs and their surrounding environment can influence tumour progression. This new knowledge may facilitate the development of therapeutic strategies whereby targeting signalling molecules supporting the interactions between CAFs and other tumour compartments may impair cancer progression (Figure 5). For example, targeting CAF education by cancer cells could have therapeutic benefits. As such, IL-6 and JAK/STAT have been shown to promote CAF activation by cancer cells, with several drugs targeting IL-6, its receptor IL-6R, or JAK being tested in cancer clinical trials [115–117]ᵛ. Similarly, IL-1 and TGF-β were recently demonstrated to shape CAF heterogeneity in murine models of PDAC, as mentioned earlier [26]. This study reported that IL-1 and JAK/STAT signalling induces LIF expression to generate inflammatory CAFs, while TGF-β inhibits the IL-1 receptor and pushes CAFs towards a myofibroblastic phenotype [26] (Figure 5A). Interestingly, Shi et al. also recently found that LIF is a key mediator of interactions between pancreatic cancer cells and CAFs [118]. Here, pharmacological targeting and genetic depletion of LIF impaired pancreatic tumour growth and enhanced chemotherapy efficacy [118]. In mouse and patient-derived models of pancreatic cancer, high levels of circulating LIF correlated with poor response to therapy and worse survival, suggesting that targeting LIF could improve the outcome of patients with pancreatic cancer [118]. Furthermore, in the KPC mouse model, Pinho and colleagues found that fibroblast activation and protumourigenic T cell infiltration was enhanced via the loss of ROBO2 in adjacent tumour cells, thereby making it a 'stroma suppressor' gene [119]. This supports earlier work by Biankin et al., who reported that genes involved in SLIT/ROBO signalling are frequently mutated in PDAC tumours [120]. In the Pinho et al. study, it was found that TGF-β signalling was upregulated in ROBO2-knockout cultures, leading to aberrant stromal reprogramming. These effects were effectively blocked using galunisertib, a small-molecule TGF-β receptor inhibitor [119]. The authors also reported that loss of ROBO2 with concomitant increased ROBO1 expression signalling was correlated with poor disease-free survival in patients with PDAC [119]. In addition, Ligorio and colleagues recently reported that mitogen-activated protein kinase (MAPK) and STAT3 signalling is upregulated in a subpopulation of highly proliferative and invasive PDAC cancer cells, and that this phenotype is due to CAF-derived paracrine TGF-β signalling [121]. Collectively, these studies suggest that IL-1 and/or TGF-β could be targeted to reduce the proportion of the prometastatic CAF subpopulations in vivo, therefore impairing tumourigenesis (Figure 5A).

Blocking CAF activation can also be achieved using imatinib, a broad-spectrum tyrosine kinase inhibitor that targets BCR-ABL, c-KIT (CD117), PDGFRα/β, and the discoidin domain receptors (DDRs). Pietras et al. reported that PDGFR inhibition with imatinib reduced CAF-derived fibroblast growth factor 2 and 7 (FGF2 and 7) secretion, which in turn reduced angiogenesis and decreased cancer cell proliferation in cervical cancer [122]. Recently, it was reported that GoF p53mut cancer cells activated the NFκB pathway in CAFs via paracrine TNF-α signalling, as discussed earlier, and this pathway could also be targeted to impair tumour-CAF crosstalk, thus normalising the PDAC TME [47] (Figure 5B). Importantly, the diversity in secreted factors activating CAFs alludes to the potential for redundancy in tumour-promoting CAF pathways, highlighting the necessity for homing in on downstream targets, as well as identifying novel tumourigenic mechanisms (see Outstanding Questions).

Inhibiting the signals produced by CAFs to shape the TME and to promote cancer development is another viable therapeutic strategy. For instance, CAFs were recently shown to secrete high levels of IL-33, which can induce tumour-associated macrophages (TAMs) to switch from a tumour-suppressing M1 phenotype
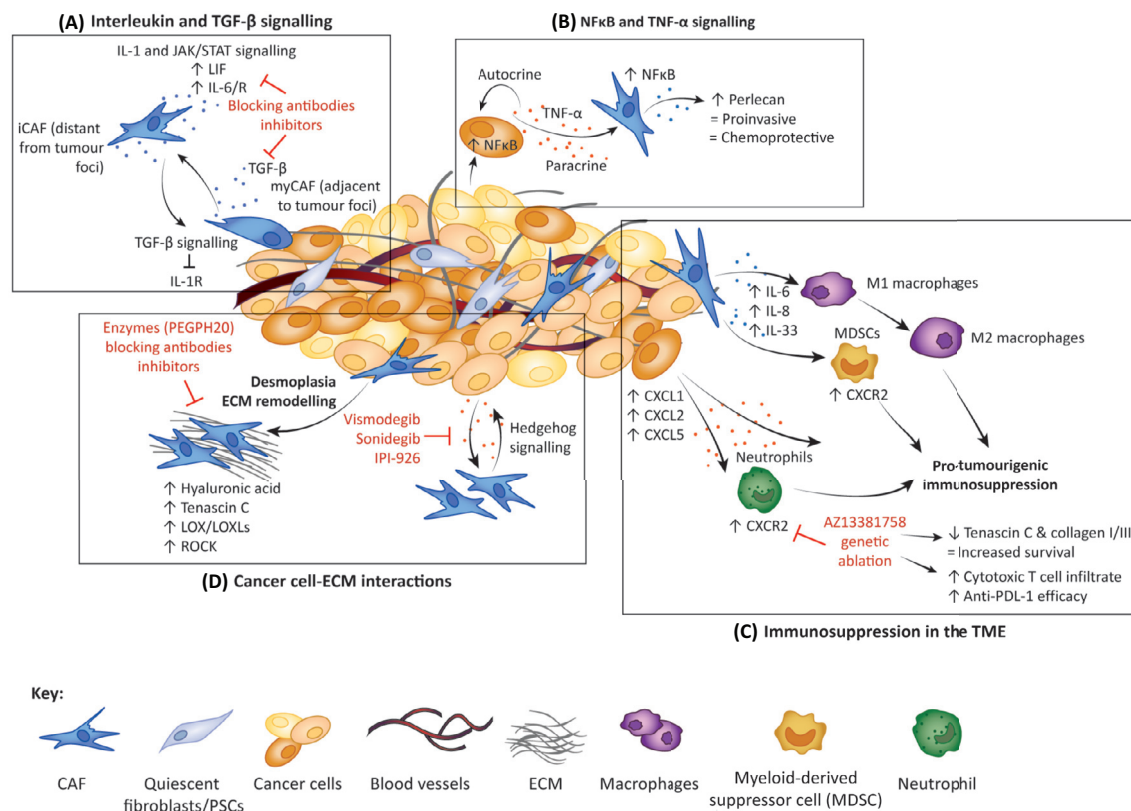
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Figure 5. Targeting Interactions between Cancer-Associated Fibroblasts (CAFs) and Their Surrounding Microenvironment.
(A) Two distinct populations of CAFs in the PDAC tumour microenvironment (TME) have been identified; inflammatory CAFs (iCAFs; found distally to tumour foci) and myofibroblastic CAFs (myCAFs; adjacent to tumour cells), which may have distinct functions *in vivo*. Targeting this interleukin (IL)-1 and Janus kinase-signal transducer and activator of transcription (JAK/STAT)-driven mechanism pushes the iCAFs towards a more myCAF-like phenotype, impeding tumourigenesis and improving survival. (B) In PDAC, mutant p53 cancer cells have upregulated nuclear factor κB (NFκB) signalling, which results in increased secretion of tumour necrosis factor (TNF)-α. TNF-α acts in an autocrine and paracrine manner, stimulating increased NFκB signalling in cancer cells and CAFs alike. Increased NFκB signalling in CAFs results in increased secretion of perlecan, which subsequently promotes invasion and metastasis. (C) CAF-driven aberrant cytokine signalling promotes an immunosuppressive microenvironment, resulting in increased numbers of M2 macrophages, myeloid-derived suppressor cells (MDSCs) and neutrophils. Increased expression of checkpoint blockage ligands (e.g., programmed death-ligand 1; PDL-1) is also observed. (D) PDAC typically exhibits excessive CAF-dependent extracellular matrix (ECM) deposition and remodelling, which can be therapeutically targeted. Abbreviations: CXCL1/2/5, chemokine (C-X-C motif) ligand 1/2/5; CXCR2, C-X-C chemokine receptor type 2; IL-1R/6R, interleukin 1 receptor/6 receptor; LIF, leukemia inhibitory factor; LOX/LOXLs, lysyl oxidase/lysyl oxidase-like; PEGPH20, pegvorhyaluronidase alfa; PSC, pancreatic stellate cell; ROCK, Rho-associated protein kinase; TGF-β, transforming growth factor beta.

to a tumour-promoting M2 phenotype [123] (Figure 5C). This suggests that IL-33 would be an interesting target to block the protumourigenic functions of CAFs. Similarly, Steele *et al.* reported that CXCR2 is commonly upregulated in the neutrophils and myeloid-derived suppressor cells (MDSCs) of human PDAC tumours and that this is associated with poor prognosis [124] (Figure 5C). When CXCR2 was targeted using a small-molecule inhibitor of CXCR2 (AZ13381758) in the KPC mouse model, it was observed that there was a reduction in tenascin C and collagen I/III, which are largely produced by CAFs. This treatment also lowered metastatic burden and increased overall survival [124]. Similarly, Chao *et al.* reported that genetically ablating CXCR2-mediated neutrophil recruitment in PDAC resulted in T cell-dependent suppression of tumour growth. Furthermore, inhibiting CXCR2 also significantly enhanced the effects of anti-PD1 immunotherapy, promoting cytotoxic T cell entry into the tumours [124]. Considering the effects on the stroma with this treatment, investigating CAF–neutrophil/MDSC crosstalk could be another

promising avenue to explore for the development of stromal treatments in PDAC. Feig *et al.* previously reported that CAFs secrete increased levels of CXCL12, also known as stromal cell-derived factor 1 (SDF1). Interestingly, CXCL12 can bind to the cell surface of adjacent cancer cells, rendering them resistant to T cell-mediated killing [84]. Here, treatment with AMD3100, an inhibitor of CXCR4 (the receptor for CXCL12) increased T cell infiltration into the tumour tissue and reduced tumour growth when combined with immune checkpoint inhibition [84]. CAFs can secrete increased levels of IL-6 and IL-8, which can induce the differentiation of tumour-infiltrating myeloid cells into MDSCs or M2 TAMs, both of which are involved in immunosuppression in the TME (Figure 5C). Hence, blocking IL-6 and IL-8 signalling could be used to increase the antitumourigenic activity of the immune system *in vivo*. In line with this, pasireotide, a somatostatin analogue, was previously shown to activate the somatostatin type 1 receptor (SSTR1) in CAFs, causing inhibition of the mTOR/4E-BP1 pathway and, thus, reduced IL-6 secretion [125]. The authors also reported that pasireotide reduced tumour growth and decreased chemoresistance in mouse and patient-derived models of pancreatic cancer [125].

Lastly, targeting CAF-derived ECM has been assessed to deprive cancer cells from their protective niche. Hh signalling and HA are two of the most clinically explored stromal targeting approaches in PDAC. In solid tumours, the Hh pathway drives ECM remodelling and promotes crosstalk between CAFs and cancer cells [12,23] (Figure 5D). So far, several Hh inhibitors, such as vismodegib, sonidegib, and IPI-926, have been tested in combination with chemotherapy for the treatment of PDAC [20] (NCT01383538[vi] and EDALINE trial[iii]) (Figure 5D), with a small number of patients responding to the treatment. In line with this, Cazet *et al.* reported a clinical benefit (including one complete response) in three out of 12 patients with triple-negative breast cancer using sonidegib in combination with docetaxel chemotherapy in a Phase I clinical trial [23]. Furthermore, ongoing trials with PEGPH20 (pegvorhyaluronidase alfa), an enzyme that depletes HA, showed improved progression-free survival (PFS) in some patients with high HA-expressing PDAC tumours in combination with standard-of-care chemotherapy (Figure 5D) (HALO 202[vii]) [126]. PEGPH20 has also been tested in combination with chemotherapy and anti-programmed death-ligand 1 (PD-L1) immunotherapy in pancreatic cancer[viii]; however, these treatment approaches have had limited clinical benefit thus far. Furthermore, tenascin C can be targeted using recombinant antibodies, which were shown to prolong survival when combined with chemotherapy in a Phase II trial with patients with glioma (NCT00002753[ix]) (Figure 5D). Interestingly, the matrix can also provide a metastatic niche in PDAC, where it supplies metabolites, such as proline, to tumour cells in low-nutrient conditions [127]. Inhibition of the lysyl oxidase (LOX) family, a group of enzymes that promote collagen synthesis and crosslinking [128–131], was also shown to be beneficial in PDAC GEMMs [132]. Here, targeting LOX with a blocking antibody during the early stages of cancer development reduced ECM deposition, decreased metastatic spread, and enhanced efficacy of gemcitabine chemotherapy [132] (Figure 5D). It was also recently reported that infiltrating mast cells potentiate the protumourigenic effects of CAFs in prostate cancer via secretion of tryptase, a serine protease [133]. In this study, mast cell-derived tryptase remodelled the stromal compartment of tissue-engineered tumours, promoting a malignant phenotype in normally benign epithelial cells [133]. This was abrogated with treatment of nafamostat mesylate, a potent tryptase inhibitor [133]. Furthermore, TAMs have been implicated in determining the subtype of PDAC tumours, where a high number of TAMs promotes a dense stroma and low T cell infiltrate, characteristic of the squamous subtype [134], which has worse prognosis [50]. Considering this, further studies of the tissue-remodelling role of innate immune cells, such as mast cells and macrophages, is warranted in PDAC. Lastly, 'priming' pancreatic tumours by pulsing the dosage of ROCK inhibitor (fasudil) reduced ECM crosslinking before treatment with chemotherapy (Figure 5D). This fine-tuned approach improved chemotherapy response at the primary tumour site, while also reducing metastasis [114]. This suggests that the timing and dosing of stromal treatments in combination with other interventions is important for therapeutic success, where we can maximise response, while minimising toxicity (see Outstanding Questions).

Concluding Remarks

Together, these studies show that we currently have several preclinical strategies to target subpopulations of protumourigenic CAFs. Further investigation using relevant mouse and patient-derived

models as well as clinical trials are needed to establish the benefits of these treatments (see Outstanding Questions). However, considering the level of CAF heterogeneity that has been recently reported in solid tumours, such as PDAC, we propose that precise profiling of CAF identity and function both temporally and spatially should be undertaken when designing and deploying treatments. We argue that expanding our knowledge of CAF heterogeneity will help to develop more personalised approaches to cancer treatment, where mutational status as well as stromal features can be used to stratify patients more effectively. However, further research is required to understand the origin/s of CAFs. It is still not clear which cells can (and do) readily transition into CAFs or whether the identity of these precursors can dictate phenotype, function, and/or drug targetability upon activation. Moreover, the rapid discovery of new CAF subpopulations using single cell sequencing approaches has progressed the field considerably. With further advances, it is possible that new markers for CAF subpopulations will be identified, which may relate to cellular origin. As these technologies develop further, it is conceivable that redundancy in CAF-dependent protumourigenic signalling pathways will be identified, thereby highlighting potential 'master regulators' of CAF phenotype and function. Lately, there has been renewed interest around whether CAFs co-migrate with tumour cells to the metastatic niche and, if so, what their function is at these sites. Further research is required to understand this in more depth as well as how and when these co-migratory events could be therapeutically targeted to hinder metastatic spread. In line with this, we argue that precise and/or transient delivery of stromal treatments will be critical to their efficacy. Given that CAFs exhibit acute dynamic plasticity, shifting the ratio of specific CAF subpopulations at certain timepoints rather than chronic ablation might be a more suitable approach to impeding tumourigenesis and metastasis.

Overall, considering the intricate interactions between tumour cells, CAFs, and other TME components, it is crucial to obtain a precise understanding of the effects of anti-CAF therapies at the molecular, cellular, and systemic levels to optimise outcomes and avoid adverse effects.

Acknowledgements

Resources

i https://clinicaltrials.gov/ct2/show/NCT01195415

ii https://clinicaltrials.gov/ct2/show/NCT01064622

iii https://clinicaltrials.gov/ct2/show/NCT02027376

iv https://clinicaltrials.gov/ct2/show/NCT03307148

v https://clinicaltrials.gov/ct2/show/NCT00385827

vi https://clinicaltrials.gov/ct2/show/NCT01383538

vii https://clinicaltrials.gov/ct2/show/NCT01839487

viii https://clinicaltrials.gov/ct2/show/NCT03481920

ix https://clinicaltrials.gov/ct2/show/NCT00002753

Outstanding Questions

How are CAF phenotype and function spatiotemporally regulated during solid tumour progression?

Do all CAFs originate from a common lineage? If not, which cell types can transition into CAFs?

Does the lineage of a CAF subpopulation determine its function or how well it can be targeted therapeutically?

Can we further identify novel markers delineating different CAF subsets?

Can CAFs and tumour cells co-migrating to metastatic sites be targeted therapeutically to delay and/or affect metastatic spread?

How does CAF heterogeneity vary both within and between cancer types, subtypes, and patients? How can we utilise knowledge of CAF heterogeneity to stratify patients for improved clinical outcomes across PDAC and other solid tumours?

Can we map the CAF landscape to identify redundancy in CAF-dependent protumourigenic pathways?

Are there stage and/or regionally dependent cancer-promoting CAF subsets with time-dependent windows of therapeutic opportunity?

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