




Heterocellular crosstalk and architecture of the pancreatic tumour microenvironment

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Abstract

A fibroinflammatory microenvironment coevolves with many tumour types and profoundly influences disease progression and response to therapy. Pancreatic cancer is the archetype of a fibroinflammatory tumour, with non-malignant stromal elements comprising the volumetric majority of the tumour tissue. A convergence of three factors – technological advances enabling deep understanding of heterocellular crosstalk in these complex tumours; therapeutic advances revealing meaningful vulnerabilities in this notoriously chemoresistant, immunosuppressive disease; and conceptual advances towards distilling the conserved features and key functions of stromal elements amid this complexity – has positioned the field in a promising era for discovery, wherein our ever-improving understanding of the pancreatic tumour microenvironment is poised for translational impact. Emerging pan-cancer analyses highlight features of tumour microenvironments conserved not only among pancreatic cancer specimens but also across anatomic sites, such that lessons learnt about the organization of tumour tissue architecture and the role of oncogenic KRAS signalling in this process in other tumours have shaped our understanding of heterocellular dependencies in pancreatic cancer and vice versa. Here, we review recent developments sculpting our current understanding of the diverse features of the pancreatic tumour microenvironment and emerging means to leverage these developments for the benefit of patients with pancreatic cancer.

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Key points

- Single-cell genomics methodologies have highlighted conserved transcriptional states among immune and non-immune stromal cells in mouse and human pancreatic ductal adenocarcinoma, motivating efforts to understand the underlying functions of these stromal states.
- Although complex and heterogeneous, the pancreatic ductal adenocarcinoma tumour microenvironment (TME) comprises recurrent, defined spatial units with distinct cellular and matrix compositions.
- Computational inference from single-cell genomics and spatial analytics, together with functional data, points to extensive heterocellular crosstalk among malignant cells and the diverse stromal cell types, motivating efforts to understand hierarchical relationships that may facilitate TME dismantling.
- Bidirectional relationships between KRAS–MAPK–MYC signalling and the TME suggest that non-malignant microenvironmental cell types may be key players in adaptation and resistance to the current suite of KRAS inhibitors and may therefore be crucial targets for effective combination therapy, which would overcome drug resistance.

Introduction

Pancreatic ductal adenocarcinoma (PDAC), which derives from the exocrine pancreas, is the most common form of pancreatic cancer and is associated with a poor prognosis, with an estimated 5-year survival rate of only 3% for patients diagnosed with metastatic disease^{1–3}. A hallmark of PDAC is the high frequency of KRAS mutations, found in about 95% of cases, and typically already present in early precursor lesions known as pancreatic intraepithelial neoplasias (PanINs)^{4,5}. In advanced stages of tumour progression, alterations in tumour suppressor genes such as *TP53*, *SMAD4* and *CDKN2A* are also present^{6,7}.

PDAC is distinguished by a dense and multifaceted tumour microenvironment (TME), comprising immune cells, cancer-associated fibroblasts (CAFs), extracellular matrix (ECM) components, vascular and lymphatic networks and a rich neural infiltrate⁸. The cellular and acellular features of the TME can account for up to 80% of PDAC volume in clinical specimens⁹. This highly structured and dynamic microenvironment has a key role in supporting tumour proliferation and progression and also contributes to therapeutic resistance through intricate interactions with cancer cells^{10,11}. The complexity of the PDAC TME results not only from the different cellular and acellular components but also from the spatial heterogeneity in how these components are arranged around tumour cells and the signalling networks among them^{12,13}. Given the profound influence of the TME on PDAC progression, in-depth investigation of the TME, including its individual components, the crosstalk mechanisms with tumour cells as well as its spatial distribution and heterogeneity, represents a promising strategy to identify novel therapeutic opportunities.

The past decade has ushered in technological advancements that have enabled major conceptual progress in our understanding of PDAC biology. This includes powerful single-cell genomic, proteomic and spatial approaches that have not only informed on PDAC heterogeneity but also uncovered recurrent niches and spatially conserved transcriptional programmes that may represent essentialities for

PDAC progression and thus harbour therapeutic targets. Major recent advances also include a suite of potent pharmacological inhibitors of mutant KRAS now available for preclinical investigation and in clinical trials¹⁴. This represents a substantial and pivotal moment for researchers of multiple solid tumour types, and particularly for the PDAC research community, given the frequency of *KRAS* driver mutations in this setting. To date, KRAS inhibitors (KRASi) – together with genetic mouse models – have illustrated the causal link between KRAS signalling and the fibroinflammatory TME^{15–21}, two cardinal features of these tumours (Fig. 1). *KRAS*-mutant, stroma-rich lesions in the pancreas are common in humans, compelling understanding of tumour-permissive mechanisms of stromal evolution. With these emerging tools and technologies, together with ever-improving mouse models to perturb and study complex TMEs, the field has made great strides towards identifying beneficial versus detrimental components of the perturbed wound-healing response that coevolves with pancreatic cancer at every step²² and in understanding cellular and acellular elements of these TMEs that were long mysterious and may now point the way to new therapeutic interventions. Although other subtypes of pancreatic cancer such as pancreatic neuroendocrine tumours feature rich TMEs as well, here we focus on PDAC for the sake of clarity and in light of the wealth of recent literature meaningfully informing on key features and functions of the PDAC TME. Therefore, in this Review, we discuss emerging principles of heterocellular crosstalk and tissue architecture in the PDAC microenvironment, which impact disease progression and may serve as meaningful targets for therapeutic intervention.

Adaptive immune compartment

Although the pancreatic TME is broadly characterized as immunosuppressive, the contextual basis for immune suppression varies considerably, reflecting both spatial and intertumoural heterogeneity in immune composition. Limitations on antitumour T cell activity against PDAC take at least three forms: (1) suppression of T cell infiltration into the tumour, (2) restriction of T cell spatial localization within the TME, such that interactions with and ability to kill tumour cells are limited and (3) activation of paracrine and T cell-intrinsic signalling nodes which hinder antitumour T cell function. To the first point, quantification of leukocyte infiltrate in human PDAC has indicated substantial heterogeneity in the total abundance of leukocytes in these tumours, with some tumours T cell-excluded whereas others harbour relatively abundant T cells^{23–25}. Taken together, quantification of the leukocyte infiltrate in human PDAC yielded immune phenotypes termed hypo-inflamed, myeloid-enriched and lymphoid-enriched. Patients whose resected tumours were lymphoid-enriched had a more favourable prognosis than other immune phenotypes²³, consistent with prior work demonstrating a correlation between quality and quantity of T cell infiltrate in PDAC resection specimens and patient survival²⁶. Preclinical models similarly demonstrate heterogeneity in T cell infiltrate into PDAC tissues²⁷, providing opportunities for mechanistic dissection of spatial regulation of lymphoid cells. The dense ECM produced by pancreatic CAFs restricts T cell extravasation and perivascular invasion into the TME, such that depletion of fibroblast activation protein (FAP)-expressing CAFs increases intratumoural CD8⁺ T cell abundance and antitumour activity²⁸. Overexpression of FAP in naive fibroblasts results in the production of ECM permissive to PDAC cell invasion in vitro²⁹, highlighting ECM production by FAP-expressing CAFs as a potential co-regulator of CD8⁺ T cell exclusion and cancer cell-invasive properties. In addition to physical barriers, T cell accumulation into the PDAC microenvironment is suppressed by the local production of

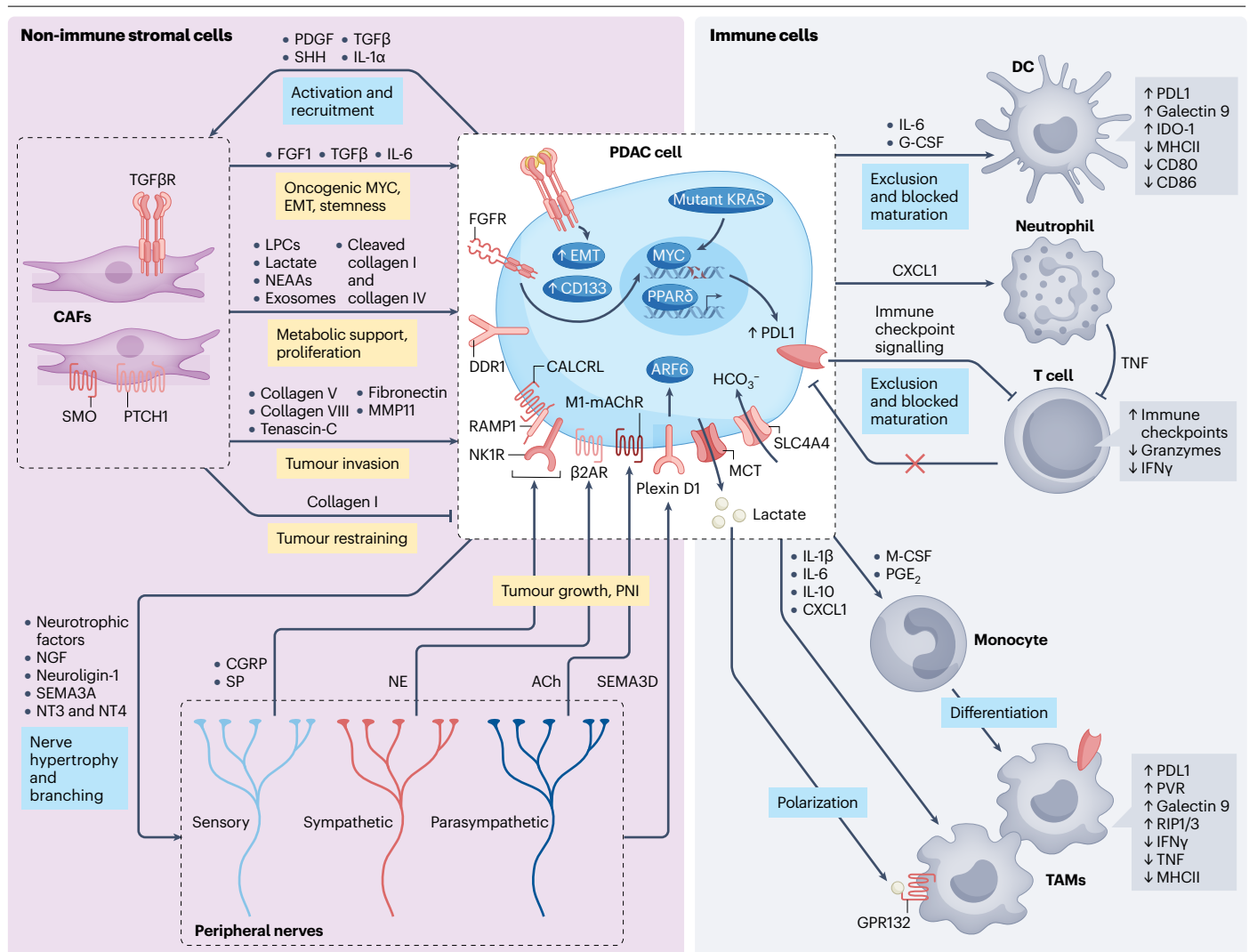


Fig. 1 | The bidirectional crosstalk between tumour cells and stromal cells in pancreatic ductal adenocarcinoma promotes tumour progression, fibrosis and immune evasion. In pancreatic cancer, heterocellular interactions between tumour cells and stromal cells, both immune and non-immune, facilitate fibrosis, tumour proliferation, invasion and immune evasion. Inflammatory mediators and growth factors secreted by tumour cells hamper the infiltration and maturation of dendritic cells (DCs), resulting in immature or tolerogenic DC phenotypes with reduced expression of costimulatory and antigen presenting factors and enrichment of immune-checkpoint ligands. Similarly, macrophages are reprogrammed towards an immunosuppressive, anti-inflammatory phenotype under the influence of tumour cell-derived cytokines such as macrophage colony-stimulating factor (M-CSF) and metabolites such as prostaglandin E2 (PGE₂), to promote immune evasion and tumour growth⁷³. Tumour cells reduce T cell infiltration and function via CXC-chemokine ligand 1 (CXCL1)-mediated secretion of tumour necrosis factor (TNF) by neutrophils, oncogenic KRAS and fatty acid signalling and inhibit their cytotoxic potential through the upregulation of immune checkpoint ligands. Neurotrophic factors and axon guidance molecules secreted by tumour cells promote peripheral nerve hypertrophy and axonal branching. In turn, sensory and autonomic nerves secrete neurotransmitters promoting tumour cell proliferation and perineural invasion (PNI). Nerves can indirectly promote anti-inflammatory macrophage polarization through the secretion of semaphorin 3D (SEMA3D), resulting in plexin D1-ARF6-mediated upregulation of lactate by tumour cells, acting on G protein-coupled receptor 132 (GPR132) on macrophages⁶⁸.

Cancer-associated fibroblasts (CAFs) are recruited and activated by tumour cell-derived growth factors and cytokines, such as platelet-derived growth factor (PDGF), transforming growth factor β (TGF β), sonic Hedgehog (SHH) and IL-1 α . CAFs promote epithelial-to-mesenchymal transition (EMT) and stemness in tumour cells via growth factors, provide nutrient support through secretion of lipids, lactate and amino acids and promote tumour invasiveness through secretion of components of the extracellular matrix (ECM) including collagens and fibronectin. CAF-derived IL-6 elevates glycolytic flux in PDAC tumour cells, leading to an enrichment of CD133⁺ tumour cells¹⁵². Moreover, CAF-derived fibroblast growth factor 1 (FGF1) increases oncogenic MYC expression, facilitating PDAC progression³¹³. ACh, acetylcholine; ARF6, ADP-ribosylation factor-6; CGRP, calcitonin gene-related protein; DDR1, discoidin domain receptor 1; FGFR, FGF receptor; G-CSF, granulocyte colony-stimulating factor; IDO-1, indoleamine 2,3-dioxygenase; IFN γ , interferon- γ ; LPC, lysophosphatidylcholine; M1-mAChR, muscarinic acetylcholine receptor M1; MCT, monocarboxylate transporter; MHCII, major histocompatibility complex class-II; MMP11, matrix metalloproteinase-11; NE, norepinephrine; NEAA, non-essential amino acid; NGF, nerve growth factor; NK1R, NK1 receptor (also known as substance P receptor); NT, neurotrophin; PDAC, pancreatic ductal adenocarcinoma; PPAR δ , peroxisome proliferator-activated receptor δ ; PTCH1, patched-1; PVR, poliovirus receptor; RAMP1, receptor activity-modifying protein 1; RIP, receptor-interacting protein; SLC4A4, solute carrier family 4 member A4; SMO, smoothened; SP, substance P; TAM, tumour-associated macrophage; TGF β R, TGF β receptor; β 2AR, β 2-adrenergic receptor.

cytokines and chemokines, including IL-33 produced by CAFs²⁰ and CXC-chemokine ligand 1 (CXCL1) produced by cancer cells^{27,30}. Beyond local immunomodulatory cues, systemic inflammation induced by primary PDAC burden induces signal transducer and activator of transcription 3 (STAT3) activation in the liver, resulting in serum amyloid A production and restriction of tumour-specific T cell infiltration into the primary tumour via Toll-like receptor 2 (TLR2) signalling³¹. In the light of the recent identification of tumour antigen-specific T cells^{32,33}, T cell neoantigens³⁴ and shared cryptic antigens³⁵ among patients with PDAC, efforts to increase T cell infiltration into these tumours may serve as important elements of combination immunotherapy.

Despite these notable barriers to T cell accumulation, some tumours or tumour regions demonstrate considerable infiltration by T cells including tumour-specific T cells, which face limits on cytotoxic potential upon arrival. Multiple mechanisms suppress their antitumour potential in this setting, including paracrine interactions with macrophages, fibroblasts and tumour cells (Fig. 2). The abundant macrophage population in the PDAC microenvironment, further discussed subsequently, has the potential to be reprogrammed by immunotherapies to support effector T cells and is essential for the antitumour effect of some immunotherapies such as agonistic anti-CD40 (ref. 36). Nevertheless, macrophages are generally immunosuppressive at steady state³⁷. Macrophages present in both human and mouse PDAC are frequently positive for the immunosuppressive marker arginase 1, which depletes local concentrations of the amino acid arginine and as a consequence inhibits T cell activation in this setting³⁸. Efferocytosis, or engulfment of apoptotic cells by macrophages, similarly restricts CD8⁺ T cell function in both primary PDAC and liver metastases^{39,40} by promoting an arginase-1⁺ immunosuppressive macrophage state, which in turn suppresses CD8⁺ T cell production of effector molecules such as interferon- γ (IFN γ) and granzyme-B. Similar to macrophages, CAFs enact a perturbed wound-healing response in PDAC, which limits T cell functions. CAFs secrete chemokines including CXCL12, which spatially restrict T cells in tumours and limit their contact with cancer cells⁴¹. Defined CAF subsets in PDAC further signal to CD8⁺ T cells either directly or indirectly to suppress effector function. Leucine-rich repeat-containing protein 15 (LRRCL15)-expressing CAFs positively regulate the expression of multiple T cell exhaustion markers including T cell immunoglobulin and mucin domain 3 (TIM3) and lymphocyte activation gene 3 (LAG3) and suppress T cell production of tumour necrosis factor (TNF) and IFN γ ⁴². This LRRCL15⁺ CAF population is transforming growth factor β (TGF β)-dependent, highlighting one of several ways by which TGF β signalling in the PDAC TME promotes immune suppression. A recently identified population of senescent CAFs in PDAC expresses multiple secreted factors, which, together, foster a dysfunctional T cell state⁴³. However, a population of PDAC CAFs negative for the cell surface marker CD105 (also known as endoglin) can promote antitumour immunity⁴⁴, motivating efforts to remodel the stroma to favour an inflamed microenvironment consistent with antitumour T cell function.

In addition to input from immune and non-immune stromal cells, T cells are suppressed by signals from PDAC cells themselves. Recent studies highlight a functional link between the core oncogenic circuitry driving pancreatic tumorigenesis – oncogenic KRAS signalling – and suppression of antitumour T cell responses^{17,21,45}. Consistent with these findings, MYC activity downstream of KRAS signalling promotes exclusion of T cells in PDAC, at least in part via the induction of epithelial PDL1 expression⁴⁶. These studies highlight the possibility that the suite of powerful KRASi currently under investigation in preclinical and clinical

settings may foster effective antitumour immunity. This notion is supported by recent preclinical evidence that tumour regressions in response to KRAS inhibition are T cell-dependent and that KRASi promotes CD8⁺ T cell-dependent PDAC cell killing in mouse models^{17,21,45}, although this may be a consequence of tumour cell apoptosis not directly related to KRAS inhibition. Additional PDAC cell-intrinsic mechanisms suppress antitumour T cell function, including fatty acid signalling to the nuclear receptor peroxisome proliferator-activated receptor δ in PDAC cells⁴⁷ and evident immunoeediting of high-quality neoantigens over the course of tumour evolution⁴⁸.

Adaptive immunity against pancreatic cancer is further limited by the relative paucity of immunostimulatory cell types and structures in these tumours. Dendritic cells (DCs), and conventional type 1 DCs (cDC1) in particular, are required for the therapeutic activity of adoptive T cell therapy or PDL1 blockade in mouse PDAC^{37,49}; however, DC abundance is low and limiting in these tumours^{50,51}. Furthermore, although plasma cell responses are generally associated with antitumour immunity and productive responses to immunotherapy via antibody production⁵², B cells in PDAC exhibit a dysregulated transcriptional programme driven by an IL-35–BCL6 axis, resulting in B cell effector dysfunction⁵³. These IL-35⁺ regulatory B cells are positively regulated by stimulator of interferon genes (STING) signalling and in turn reduce the proliferation and antitumour potential of natural killer (NK) cells⁵⁴. Finally, in some cases and spatially restricted regions, PDAC harbours tertiary lymphoid structures (TLSs), which are de novo lymphoid aggregates that regulate immunity in settings of chronic inflammation and cancer. TLSs can elevate endogenous and therapeutic antitumour immunity⁵⁵, and their presence correlates with longer survival among patients with PDAC⁵⁶. Recent evidence supports a role for group 2 innate lymphoid cells, activated by IL-33 signalling, in the engagement of putative lymphotoxin- β receptor-expressing myeloid organizer cells, which initiate TLS formation in PDAC⁵⁷. Further mechanistic studies may identify means to disentangle the immune-suppressive, when CAF-derived²⁰, from the lymphoneogenic functions of IL-33 to promote antitumour immunity.

Innate immune cells

Myeloid cells are major contributors to the pancreatic TME, orchestrating immune suppression, fibrosis, modulation of tumour and stromal cell activity and metastasis. The PDAC TME is characterized by a high abundance of terminally differentiated CD14⁺ monocytes and tumour-associated macrophages (TAMs), whereas DCs are relatively rare or spatially restricted to the tumour periphery⁵⁸. Elevated levels of both circulating and tumour-infiltrating myeloid cells correlate with poor patient outcomes^{58–60}. Myeloid cell infiltration and expression of various immune checkpoint ligands on macrophages and DCs inversely correlate with activated CD8⁺ T cell infiltration⁶¹. Given the essential role of myeloid cells in the initiation of the inflammatory response and activation of adaptive immunity (Fig. 2), as well as their highly plastic nature, myeloid cells are subject to constant reprogramming by tumour and stromal cells to varying phenotypes that promote immune evasion, desmoplasia and tumour growth throughout the stages of PDAC development. Although evidence in the field is mounting for the functional importance of additional innate immune cell types including neutrophils^{30,62} and eosinophils⁶³, here we focus on recent efforts to understand macrophage and DC functions in PDAC.

Macrophages

TAMs have been extensively described in PDAC, regulating antitumour immunity, modulating tumour and stromal cell activity and shaping

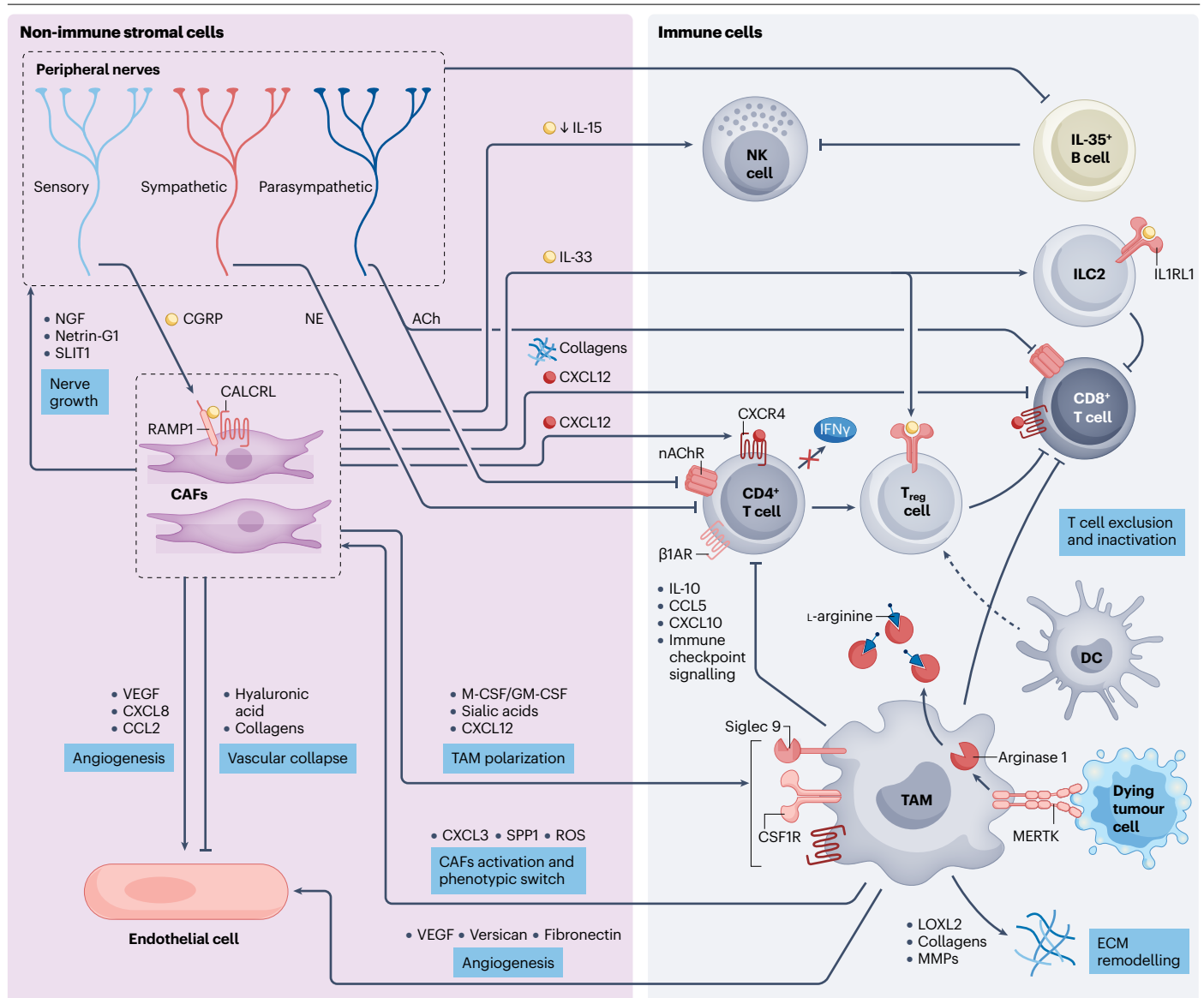


Fig. 2 | Functional interactions between immune and non-immune stroma in pancreatic ductal adenocarcinoma, which drives an immunosuppressive microenvironment. Cancer-associated fibroblasts (CAFs) maintain an immunosuppressive microenvironment in pancreatic cancer via the release of cytokines, chemokines and extracellular matrix (ECM) components, which activate tumour-associated macrophages (TAMs) and limit T cell infiltration. The increased tissue stiffness facilitated by CAF-derived hyaluronic acid and collagens causes vascular collapse favouring a hypoxic and nutrient-depleted environment, which CAFs can partially counteract through the secretion of pro-angiogenic factors. CAFs further communicate with sensory nerves to suppress a natural killer (NK) cell-mediated immune response via reduced IL-15 secretion. Additionally, sympathetic and parasympathetic nerves further suppress the adaptive immune response (B cells and T cells) via the secretion of neurotransmitters. Similarly, TAMs and dendritic cells (DCs) contribute to the immunosuppressive microenvironment in pancreatic

cancer by suppressing local T cell responses. Moreover, efferocytic macrophages in PDAC liver metastases have been shown to engulf dead cells via the MERTK receptor, leading to arginase 1 expression and subsequent suppression of CD8⁺ T cells³⁹. ACh, acetylcholine; CCL, CC-chemokine ligand; CGRP, calcitonin gene-related peptide; CSF1R, colony-stimulating factor 1 receptor; CXCL, CXC-chemokine ligand; CXCR, CXC-chemokine receptor; GM-CSF, granulocyte-macrophage colony-stimulating factor; IFN γ , interferon- γ ; ILC2, group 2 innate lymphoid cell; LOXL2, lysyl oxidase-like 2; M-CSF, macrophage colony-stimulating factor; MERTK, MER proto-oncogene, tyrosine kinase; MMP, matrix metalloproteinase; nAChR, nicotinic acetylcholine receptor; NE, norepinephrine; NGF, nerve growth factor; RAMP1, receptor activity-modifying protein 1; ROS, reactive oxygen species; SPPI, secreted phosphoprotein 1; T_{reg}, regulatory T; VEGF, vascular endothelial growth factor; β 1AR, β 1 adrenergic receptor.

the ECM. A highly heterogeneous cell type, TAMs are classified by their developmental origins and transcriptional states with distinct functions. TAMs derive both from tissue-resident macrophages, which are

long-lived cells of the pancreas, and monocyte-derived macrophages (MDMs) differentiated from bone-marrow-derived monocytes⁶⁴. Tissue-resident macrophages-TAMs drive fibrosis through high

expression of ECM remodelling genes^{64,65}, whereas MDM-TAMs express inflammatory gene signatures, associated with the modulation of antigen presentation and regulation of T cell activation^{64,66}. Transcriptionally, TAMs exist along a continuum of states under the influence of tumour^{67–69} or stromal^{70–72} cell-derived signals. High-resolution profiling of human and mouse identified several TAM subtypes by marker and functional gene expression including metabolically active secreted phosphoprotein 1 (SPP1)⁺ or arginase 1⁺ TAMs, ECM remodelling folate receptor 2 (FOLR2)⁺ or CX3C-chemokine receptor 1 (CX3CR1)⁺ TAMs and multiple immune modulating TAM subtypes such as macrophage receptor with collagenous structure (MARCO)⁺, complement C1q subcomponent subunit C (C1QC)⁺, triggering receptor expressed on myeloid cells 2 (TREM2)⁺ or IL-1 β ⁺ TAMs^{61,73–75}.

Functionally, TAMs primarily mediate the formation of a highly immunosuppressive TME and contribute to the desmoplastic reaction. Immunosuppressive features of TAMs include reduced antigen presentation, production of secretory factors hindering cytotoxic T cell infiltration and expression of various immune checkpoints^{38,61,76,77}. MDM-TAMs are sustained via the macrophage colony-stimulating factor 1 (M-CSF)–M-CSF receptor axis, the inhibition of which reprogrammed TAMs to enhance antigen presentation and improved responses to immune checkpoint blockade therapies⁷⁸. Immunosuppressive, anti-inflammatory TAMs are also characterized by reduced expression of IFN γ and TNF and upregulation of specific factors which reduce T cell infiltration and activation, promoting tumour cell survival and growth⁷⁸. Numerous factors expressed by or regulating TAMs promote reduced T cell activity, such as the sodium bicarbonate cotransporter solute carrier family 4 member A4 (SLC4A4), which maintains pH homeostasis, apolipoprotein E (APOE), which mediates cholesterol metabolism, and the aryl hydrocarbon receptor activated by tryptophan-derived microbial metabolites^{79–81}. Arginase-1 expressed by TAMs reduces arginine stores in T cells required for their proliferation, enabling the progression of PanINs to advanced, invasive stages³⁸. TAMs directly mediate fibrosis as they express collagens and ECM remodelling genes, such as matrix metalloproteinases and lysyl oxidase-like 2 (LOXL2), mediating collagen stabilization^{64,65,82}. High putative interactions between TAMs and CAFs have been denoted by the expression of ligand–receptor pairs, for example, CXCL12–CXC-receptor 4 (CXCR4), CXCL3–CXCR2 and sialic acid-binding immunoglobulin-like lectins (siglecs)–sialic acids, promoting CAF-mediated macrophage recruitment as well as CAF phenotypic switches^{83,84}. SPP1⁺APOE⁺ TAMs colocalize with collagen triple helix repeat-containing protein 1 (CTHRC1)⁺GREM1⁺ myofibroblastic CAFs (myCAF), promoting deposition of ECM components, epithelial-to-mesenchymal transition in surrounding tumour cells and immune suppression via osteopontin–integrin signalling⁸⁵. Macrophage mannose receptor 1 on TAMs leads to collagen internalization and subsequent high production of reactive nitrogen species, promoting CAF activation and enhanced intratumoural collagen deposition⁸⁶. In liver metastases, CC-chemokine ligand 2 (CCL2)-triggered granulins secretion^{87,88} and IL-33-mediated CXCL3 secretion⁸⁴ by TAMs promote myofibroblast activation, enhanced fibrosis and metastatic tumour growth.

Although the immunosuppressive roles of PDAC TAMs are well documented, so too is their potential for phagocytosis of live cancer cells, a desirable property of immune cells in the TME. For example, stimulation of macrophages with a CpG oligodeoxynucleotide to signal through TLR9 alters their metabolism and fosters their antitumour potential to engulf CD47⁺ cancer cells⁸⁹. Macrophages also respond to the induction of non-canonical nuclear factor- κ B signalling, achieved

through antagonists of cellular inhibitor of apoptosis 1 (cIAP1) and cIAP2, resulting in tumoricidal phagocytosis of live cancer cells, via a mechanism dependent on T cells but independent of direct T cell recognition of tumour cell-expressed major histocompatibility complex class I (MHCI)⁹⁰. Overall, TAMs have a pivotal role in modulating antitumour immunity and primary and metastatic tumour progression. Therapies reprogramming their pro-fibrotic, immunosuppressive activity may hold potential in sensitizing PDAC to immunotherapies.

Dendritic cells

DCs are typically either excluded or functionally dysregulated in PDAC, via mechanisms including low levels of the DC growth factor Fms-related tyrosine kinase 3 ligand (FLT3L)^{51,91}. Higher levels of circulating or tumour-infiltrating DCs were associated with the infiltration of CD4⁺ and CD8⁺ T cells and improved overall survival in patients with resected pancreatic cancer^{49,92,93}. Levels of mature DCs are substantially reduced during early-onset disease and continue to decline throughout disease progression, owing to tumour cell-derived factors, such as IL-6 (ref. 91) and granulocyte CSF (G-CSF)⁹⁴, lack of the NK cell-derived cytokines CCL5, CXCL1 and CXCL5 (ref. 95), as well as high levels of nitric oxide produced by myeloid-derived suppressor cells (MDSCs)⁹⁶. Various subsets of DCs have been identified in PDAC, including cDC1 and cDC2 subtypes, plasmacytoid DCs and Langerhans-like DCs^{61,97}. cDCs in tumour tissues are immunosuppressive owing to impaired antigen presentation⁵⁰ and the capacity to recruit and prime migratory and tumour antigen-specific T cells, preventing efficient T helper 1 and cytotoxic T cell responses⁵¹. cDCs have reduced expression of costimulatory factors including CD80 and CD86, and increased expression of various immune checkpoint ligands such as galectin 9 and PDL1 (refs. 51,61), as well as increased expression of indoleamine-2,3-dioxygenase-1 (refs. 50,98), which inhibits T cell activity. CD11b⁺CD103[−] DCs induce the formation of regulatory T (T_{reg}) cells, which in turn reduce the expression of costimulatory markers in DCs required for effector T cell activation^{99,100}. Reintroduction of DCs into the TME restores antitumour immunity as the administration of CD40 agonists combined with FLT3L results in increased DC maturation and proliferation coupled with enrichment of NK and tumour antigen-specific T cells, ultimately reducing tumour burden and fibrosis in mouse models of PDAC⁵¹. Equally, cDC1s stimulated with tumour antigens *ex vivo* can sensitize tumours to various immune checkpoint blockade therapies resulting in a high diversity of T cell receptors, efficient priming and migration of T cells to tumours, and tumour regression in orthotopic and autochthonous mouse models of PDAC^{49,101}. Clinical studies currently underway will reveal the applicability of cDC-based vaccinations, which hold promise in reactivating immune surveillance in PDAC.

Fibroblasts and the extracellular matrix

CAFs are abundant within the PDAC TME, transcriptionally distinct from normal pancreatic fibroblasts⁵, and exhibit tumour-promoting and tumour-restraining functions in enacting perturbed wound-healing responses. Enabled in part by single-cell genomics, substantial heterogeneity among CAFs has been uncovered which can be classified by (1) transcriptional state, (2) cell surface or protein marker expression and (3) cell of origin. Although the heterogeneous CAF population as a whole shares certain core functions, such as the capacity to produce ECM, these defined subtypes may conduct specific functions that represent targets for stroma-directed therapy. We note that most of these CAF subtype designations are not mutually exclusive nor unique to the TME, often representing functions and states observed

in physiological and pathological tissue repair or other contexts of acute or chronic stress. Furthermore, these CAF subtypes or states exist along a dynamic continuum, likely reflecting functional fibroblast transitions across diverse contexts of acute versus chronic wound healing. While dynamic transitions of intrinsically tissue-protective fibroblastic cells to potentially tumour-promoting CAFs accompanies tumorigenesis, these transitions may be reversible and represent avenues for therapeutic intervention.

Transcriptional CAF subtypes

The most common transcriptional CAF subtypes include myCAFs, inflammatory CAFs (iCAFs) and antigen-presenting CAFs (apCAFs)⁹⁸, with independent studies identifying similar states albeit with some distinctions in gene programmes and nomenclature^{98,102–107}. apCAFs express MHCII while lacking costimulatory molecules (CD40, CD80 or CD86)^{98,105}, enabling T_{reg} cell formation and contributing to immunosuppression in PDAC¹⁰⁵. Further study of apCAFs may reveal that they have both pro-tumour and antitumour potential, given the established roles of fibroblastic reticular cells (FRCs) in the lymph node: FRCs present antigen to T cells under steady-state and inflamed conditions, and although early IFN-driven functions are immunostimulatory, FRCs assume a suppressive state during chronic inflammation^{108,109}. TGF β -regulated myCAFs¹¹⁰, marked by high expression of α -smooth muscle actin (α SMA), are typically located near tumour cells and impair tumour progression potentially through cell-to-cell contact¹¹¹. Spatial transcriptomics confirmed TGFBI-expressing¹¹² and CXCL10⁺ (ref. 113) myCAFs adjacent to aggressive basal-like tumour cells. Epidermal growth factor receptor (EGFR)-activated myCAFs enhance epithelial-to-mesenchymal transition in PDAC cells promoting metastasis¹¹⁴, whereas LRRC15⁺ myCAFs suppress CD8⁺ T cell function, enabling tumour growth⁴². Conversely, α SMA⁺ CAFs – including but perhaps not limited to all myCAFs – restrain tumour progression by enforcing collagen I-mediated physical constraints^{115,116}. IL-1 α -induced iCAFs¹¹⁰, spatially located at a distance from tumour cells¹¹¹, show low expression of α SMA, but secrete cytokines, chemokines, complement components and growth factors^{103,111,117} supporting tumour-promoting and immunomodulatory functions through paracrine signalling. CAF transcriptional states can shift in response to changes in microenvironmental cues including paracrine factors and metabolic states (for example, hypoxia)^{103,110,118}, which are common during PDAC progression. CAF state switching exhibits temporal dynamics during PDAC progression: Meflin⁺ iCAFs surround preneoplastic lesions at early stages of PDAC development but transition into α SMA⁺ myCAFs at advanced stages¹¹⁹. Similarly, complement-secreting iCAFs¹⁰⁴ are found exclusively at early stages of PDAC¹¹⁷. Analysis of surgical resection specimens identified a tumour-restraining ‘restCAF’ signature and a tumour-permissive ‘permCAF’ signature in human PDAC; the restCAF signature overlaps with iCAF subpopulations and associates with better outcomes than the myCAF-like permCAF subtype¹²⁰. Although these CAF transcriptional states have been recognized, their precise functions are still under investigation.

Protein and/or cell surface markers

CAF heterogeneity expands with PDAC progression⁵, with clear heterogeneity in defined CAF markers evident at the protein level in invasive tumours. Surface markers such as podoplanin⁹⁸, platelet-derived growth factor receptor α (PDGFR α) or PDGFR β ^{5,121} or FAP^{28,106,122} are used to identify and discriminate CAFs, with podoplanin and/or PDGFRs expressed

by most if not all CAFs; however, none is entirely cell-type-specific. FAP⁺ CAFs are predominantly associated with tumour-promoting features¹¹⁵ and correlate with poor clinical outcome¹²². A recent comparison of CAF profiles in surgical resection specimens from patients with PDAC with no recurrence at least 24 months post-surgery versus patients with local or metastatic recurrence revealed enrichment for α SMA⁺ CAFs in patients with no recurrence and for FAP⁺ CAFs in patients who recurred with peritoneal metastases and had particularly poor outcomes¹²³, potentially indicating distinct roles for these CAF types in patients. Nevertheless, rare INF-responsive FAP⁺ CAFs suppress mesenchymal features of tumour cells and reprogramme tumour-associated neutrophils towards an antitumour phenotype¹⁰⁶. Mass cytometry identified the surface marker CD105 denoting two functionally distinct fibroblast populations in PDAC⁴⁴. Although CD105-positive CAFs promote tumour growth, CD105-negative CAFs restrain tumour progression through improved adaptive antitumour immunity⁴⁴. The surface protein netrin-G1 also distinguishes a functionally important CAF subpopulation, implicated in both immune suppression and metabolic support for PDAC cells¹²⁴. Finally, CAFs can be distinguished based on the expression of proliferation versus senescence markers^{43,103,125}. CAFs expressing senescence-associated protein p16 exert tumour-promoting immunosuppressive functions by facilitating alternative activation or anti-inflammatory responses from TAMs⁴³ and cytotoxic T cell exclusion¹²⁵, with a secretory profile partially overlapping that of iCAFs.

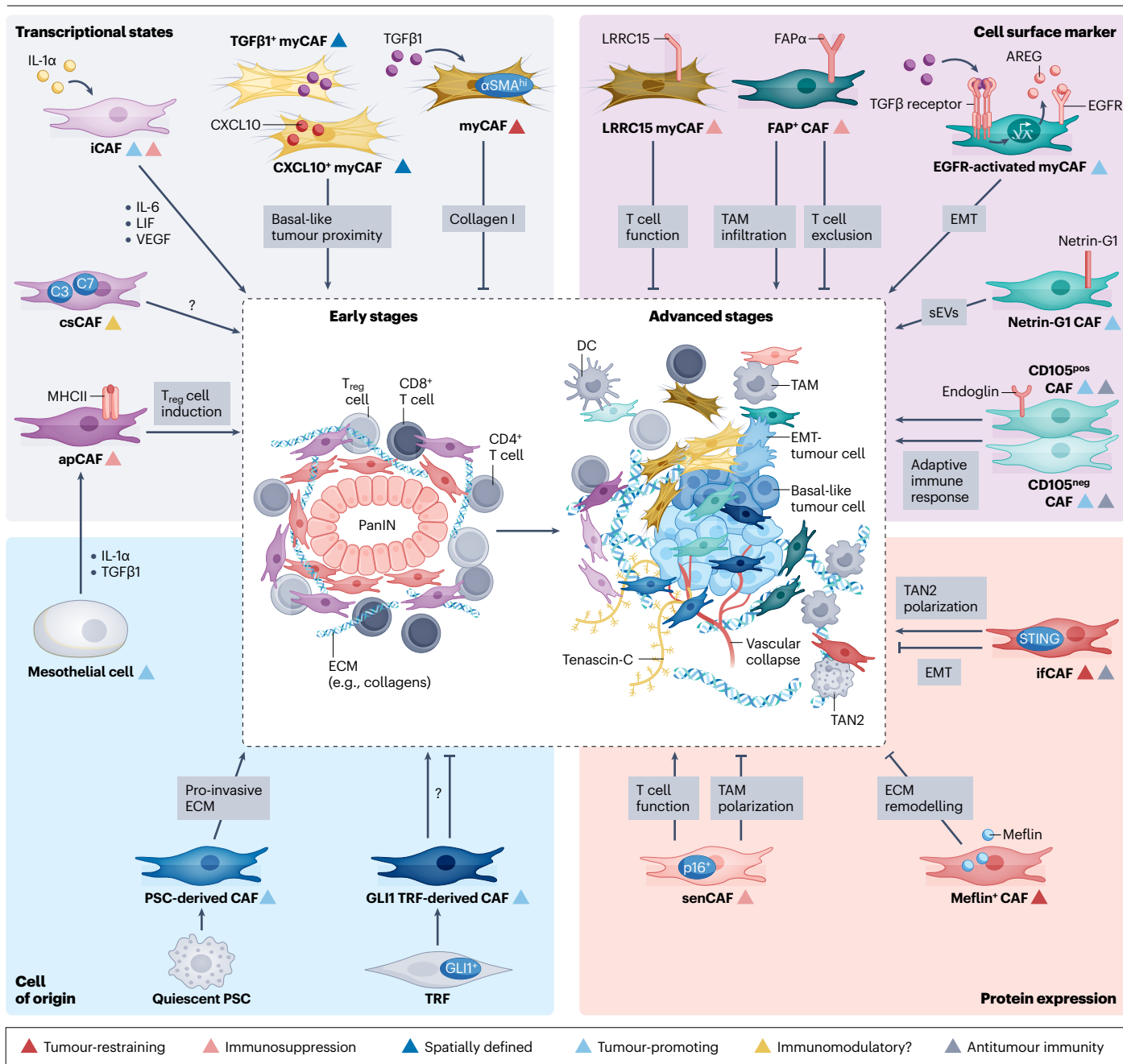
CAF origin

Recent efforts using different lineage tracing models confirmed the contribution of distinct cellular origins to PDAC CAFs as an orthogonal basis for heterogeneity^{105,121,126,127}. The healthy pancreas contains pancreatic stellate cells (PSCs) and tissue-resident fibroblasts (TRFs), both likely originating from the insulin gene enhancer protein 1 (ISL1)-positive splanchnic mesenchyme¹²⁸. PSCs, a vitamin A-storing and lipid-storing quiescent cell population, were originally thought to be the main source of CAFs in PDAC, but surprisingly they only give rise to a minor subpopulation of ~10–20% of CAFs in primary PDAC, albeit with non-redundant and tumour-promoting functions with respect to other CAFs¹²⁶. By contrast, hepatic stellate cells are the predominant source of CAFs in PDAC liver metastases, exhibiting tumour-promoting and tumour-restraining roles¹²⁷. Distinct TRF populations are different from PSCs and are defined by *Gli1* or *Hoxb6* expression within the healthy pancreas; however, only *Gli1*⁺, but not *Hoxb6*⁺, TRFs expand during PDAC progression and contribute to the CAF population¹²¹. Furthermore, mesothelial cells differentiate into apCAFs, which is orchestrated by both IL-1 α and TGF β 1 (ref. 105). Other sources of CAFs may include mesenchymal stem cells^{72,129–132}, bone-marrow-derived macrophages¹³³, endothelial cells¹³⁴ and pericytes¹³⁵, all of which warrant further investigation.

Bidirectional crosstalk and matrix production

CAFs and tumour cells engage in bidirectional, functionally relevant crosstalk^{136,137} (Fig. 3). Tumour cell-secreted factors such as PDGF¹³⁸, Hedgehog ligands^{137,139}, TGF β 1 (refs. 110,140) and IL-1 α ¹¹⁰ facilitate CAF recruitment and activation. Recent evidence suggests that the mutational status of tumour cells influences CAF reprogramming, indicating genotype-specific tumour cell secretomes. Although oncogenic *Kras*^{20,141} and germline BRCA-mutated tumours¹⁴² promote an iCAF phenotype, p53 status alters PSC-derived CAF frequency in mouse allograft models¹²⁶. Activated CAFs support PDAC growth^{143,144} under hypoxia

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and nutrient scarcity through paracrine secretion of growth factors (for example, fibroblast growth factor 1 (ref. 46)), cytokines (for example, TGF β 1 (ref. 144)), lipids^{145,146}, non-essential amino acids^{147,148}, lactate¹⁴⁹ and CAF-derived extracellular vesicles^{150,151}. Stromal-derived IL-6 further promotes cancer ‘stemness’¹⁵², tumour cell invasion¹⁵³ and premetastatic niche formation within the liver¹⁵⁴. CAF-secreted cytokines (M-CSF¹⁵⁵ and IL-33 (ref. 20)), chemokines (CXCL12 (refs. 41,156)) and sialic acids⁸³ support an immunosuppressive TAM state^{83,155} and limit cytotoxic T cell infiltration into PDAC tissue^{20,41,152,156}. Matrix-producing CAFs restrict vascular perfusion¹⁵⁷, which limits nutrient supply, and thus, restricts tumour growth. However, CAFs can increase angiogenesis by secreting factors such as

CXCL8, CCL2 (ref. 158) and vascular endothelial growth factor (VEGF)¹¹⁸, with potential implications for leukocyte infiltration and metastasis.

CAFs are the major source of ECM components in PDAC¹⁵⁹, which increase tissue stiffness and potentially tumour invasion¹⁶⁰. Individual ECM components including collagens and tenascin-C enhance tumour cell contractility and movements via integrin–focal adhesion kinase (FAK) signalling^{161–164}. Moreover, CAFs secrete and align fibronectin around PDAC cells to dictate their migration¹⁶⁵. Matrisome analyses linked tumour cell-derived, but not stromal-derived ECM proteins, with poor outcome of patients with PDAC¹⁵⁹. Similarly, stromal-derived fibrillar collagen I, which is the most abundant collagen in PDAC, restricts tumour progression at the primary site¹⁶⁶

Fig. 3 | Functional and phenotypic heterogeneity of cancer-associated fibroblasts in pancreatic ductal adenocarcinoma. Cancer-associated fibroblasts (CAFs) are widely classified by their (1) transcriptional states, (2) cell of origin and (3) cell surface and protein marker expression. The most recognized transcriptional states are myofibroblastic CAFs (myCAFs), inflammatory CAFs (iCAFs) and antigen-presenting CAFs (apCAFs) exhibiting tumour-restraining, tumour-promoting and immunosuppressive functions. Different cell sources have been identified as origins of CAFs including quiescent pancreatic stellate cells (PSCs), tissue-resident fibroblasts (TRFs) and mesothelial cells, with the latter one specifically giving rise to apCAFs. CAFs marked by the expression of specific cell surface receptors include tumour-promoting epidermal growth factor receptor (EGFR)-activated myCAFs, netrin-G1⁺ CAFs, CD105^{pos} CAFs and immunosuppressive leucine-rich repeat-containing protein 15 (LRRC15)⁺ myCAFs and fibroblast activation protein (FAP)⁺ CAFs. Senescent CAFs (senCAF), positive for p16 expression, support tumour growth by suppressing the function of local T cells. Most CAF subtypes support tumour progression via specific mechanisms; however, α -smooth muscle actin (α SMA)⁺ CAFs and meflin⁺ CAFs both restrain tumour growth potentially by enforcing extracellular matrix

(ECM)-mediated physical constraints. In addition, CD105^{neg} CAFs and interferon-responsive CAFs (ifCAF) can enhance an antitumour immune response. The central panel in this figure depicts the spatial location of the described functional CAF subsets during pancreatic ductal adenocarcinoma (PDAC) progression. Although most CAFs are presumably present across all stages, meflin⁺ CAFs and complement-secreting CAFs (csCAFs) are found only during early stages of PDAC development. Lineage-tracing studies revealed that meflin⁺ CAFs transition from a GLII⁺ platelet-derived growth factor receptor α (PDGFR α)⁺, meflin-expressing subtype to an α SMA⁺ myofibroblast with low-to-absent expression of meflin in advanced stages¹¹⁹, underscoring that CAF functional heterogeneity is a dynamic and context-dependent feature over the course of PDAC progression. AREG, amphiregulin; CXCL, CXC-chemokine ligand; DC, dendritic cell; EMT, epithelial-to-mesenchymal transition; LIF, leukaemia inhibitory factor; MHCII, major histocompatibility complex class II; PanIN, pancreatic intraepithelial neoplasia; sEV, small extracellular vesicle; STING, stimulator of interferon genes; TAM, tumour-associated macrophage; TAN2, tumour-associated neutrophil type 2; TGF β 1, transforming growth factor β 1; TGFBR, TGF β receptor; T_{reg}, regulatory T; VEGF, vascular endothelial growth factor.

and within liver metastases¹²⁷, whereas tumour-derived collagen I promotes tumour growth¹⁶⁷. Matrix metalloproteinases (MMPs) remodel the ECM to facilitate invasion. Membrane-bound MT1-MMP (also known as MMP14) on tumour cells¹⁶⁸ cleaves collagens allowing PDAC tumour cells to invade the underlying tissue^{169,170}. Additionally, cleaved collagen fragments activate the collagen sensor discoidin domain receptor 1 (ref. 171) or support cancer cell metabolism¹⁷² to promote tumour growth. Moreover, secreted MMPs can impact PDAC progression, as tumour cell-secreted MMP3 (ref. 173) and CAF-derived MMP11 (ref. 174) promote tumour invasion, whereas systemic MMP9 restricts PDAC invasion¹⁷⁵. Beyond influencing tumour cell behaviour, the dense ECM impedes immune cell infiltration, specifically limiting T cell infiltration^{28,176} and chemokine-guided T cell migration towards tumour cells¹⁷⁷, thereby restricting antitumour immunity. ECM barriers also hinder the infiltration of tumour-promoting MDSCs¹⁶⁶. Finally, collagens and hyaluronic acid (HA) produced by CAFs increase interstitial pressure in the PDAC TME resulting in vascular dysfunction¹⁷⁸ and reduced drug delivery^{178–180}. CAFs further reduce therapy efficiency by reducing vascular perfusion through secreted factors¹⁸¹ and promoting drug inactivation^{115,163,182,183}. Taken together, the functional and phenotypic complexity of CAFs has a central role in PDAC progression, immune evasion and therapy resistance, underscoring the importance of dissecting CAF subpopulations for potential therapeutic targeting.

Endothelial compartment

Although tumour-associated vasculature generally sustains neoplastic growth by supplying nutrients and oxygen while enabling exchange of metabolic waste and carbon dioxide, pancreatic cancer is generally characterized as hypovascular and hypoxic. Intraoperative oxygenation measurements revealed hypoxia in human pancreatic cancer compared with adjacent normal pancreas tissue¹⁸⁴. Consistent with this finding, PDAC is among the most hypovascular of human cancers per a recent analysis of tumour vascular landscape¹⁸⁵, although PDAC endothelial cell abundance exhibited heterogeneity across the patient population. Mouse models of PDAC similarly exhibit hypoxia and hypovascularity, which associate with an aggressive tumour phenotype^{181,186–188}. Hypoxia and the associated stabilization of hypoxia-inducible factor 1 α are evident from the early, pre-invasive stages of pancreatic tumorigenesis¹⁸⁹. Labelling of functionally perfused vessels by intravenous injection of

fluorescence-conjugated lectins in PDAC-bearing mice followed by analysis of cancer cell proliferation revealed an inverse relationship between cancer cell proliferative capacity and distance from the nearest patent vessel¹⁹⁰. Although these results implicate endothelial cells in support of tumour growth, cancer cells relatively far from patent vessels adapt in part via upregulation of anti-apoptotic BCL-2 family member BCL-xL¹⁹⁰. Poor vascularization fosters formation of a slow-cycling and chemoresistant tumour cell state enabled by BCL-xL expression. PDAC cells engage additional metabolic adaptations to survive hypoxia and nutrient deprivation in a hypovascular context, which in turn fuel tumour growth, including uptake of proteins from the extracellular space by macropinocytosis and scavenging of fatty acids to negate the need for oxygen-dependent steps of lipid synthesis^{191,192}. These results highlight the complex relationship between PDAC vasculature and tumour progression.

As vessel dysfunction and consequent hypoxia have detrimental consequences, multiple efforts in the field have investigated and identified mechanistic bases for hypovascularity and poor perfusion in PDAC. Recent spatial analysis of the human PDAC vascular niche identified a population of CD44-positive macrophages enriched for proangiogenic gene expression in association with vessels, raising the possibility that this defined macrophage subpopulation contributes to vascular heterogeneity¹⁹³. Excessive deposition of ECM components by PDAC CAFs results in substantial physical pressure in the TME, in part driven by a specific matrix component HA^{179,180}. Enzymatic degradation of HA in PDAC-bearing mice reduced intratumoural pressure, increased vascular perfusion and increased delivery of intravenously administered chemotherapy. Vascular perfusion and drug delivery were also increased by inhibition of the sonic Hedgehog (SHH)–Smoothed (SMO) signalling pathway via pharmacological inhibition of SMO or by genetic inhibition of *Shh* in PDAC cells, which drives activation of a substantial proportion of matrix-producing CAFs^{181,194}. SHH signalling also limits the PDAC endothelial compartment by inhibiting non-canonical WNT signalling in both CAFs and epithelial cells, ultimately limiting VEGF receptor 2-dependent endothelial sprouting¹⁹⁴. In healthy tissues and tumours, endothelial cells receive signalling inputs and structural support from pericytes. Although pericytes have been subject to relatively little study in the pancreatic cancer setting, recent work revealed induction of an oxidative stress response programme in PDAC-associated pericytes¹⁹⁵.

Induction of the contractility marker α SMA among pericytes was associated with aberrant vasculature across PDAC tissues¹⁹⁶, and these intratumoural pericytes may also foster infiltration of immune-suppressive myeloid cells from circulation¹⁹⁷. The complexity of the vascular niche and vessel function in PDAC impels further effort to understand cell–cell and cell–matrix interactions in this setting.

The aforementioned studies motivated efforts to normalize tumour vasculature for combination therapy. In multiple solid tumour types including PDAC, angiotensin signalling in tumour cells positively regulates solid stress, promoting CAF activation and matrix deposition as well as vessel compression¹⁹⁸. In mouse models of PDAC, treatment with the angiotensin receptor blocker losartan reduced solid stress and local fibrosis, decompressed tumour vessels, increased drug and oxygen delivery and improved chemotherapy responses¹⁹⁹. As a result, vascular normalization by losartan has been incorporated into clinical trials, as discussed subsequently. Promoting PDAC vascularization via less direct means has been achieved through the administration of senescence-inducing therapy including inhibitors of MEK and cyclin-dependent kinase 4 (CDK4) and CDK6, both of which promoted intravenous drug delivery to enhance the efficacy of cytotoxic chemotherapy, and activated endothelial cells to stimulate CD8⁺ T cell accumulation and sensitization of tumours to anti-PD1 immune checkpoint blockade²⁰⁰. Furthermore, a recent study aimed to normalize the PDAC vasculature using a derivative of the C-type natriuretic peptide (dCNP)²⁰¹. Endogenous CNP acts on endothelial cells and adjacent stromal cells to regulate angiogenesis and vascular remodelling²⁰², which motivated testing of the dCNP compound. dCNP administration stimulated the formation of functional tumour vessels, reduced hypoxia and reinvigorated the antitumour immune response in PDAC-bearing mice, underscoring the link between the tumour endothelium and tumour immunity. Although inhibiting vascular suppression mechanisms may foster improved leukocyte recruitment and immune surveillance, together with a less-aggressive tumour cell state, such efforts must also pay mind to the role of the vasculature in metastatic spread.

Despite its hypovascular nature, PDAC is characterized by frequent histopathological evidence of venous and lymphatic invasion, which correlates with metastases^{203,204}. Lymphatic vessels have been subject to relatively little study in PDAC, but lymphangiogenesis and cancer cell invasion into lymphatic vessels as a potential route to lymph node metastasis are observed in patients with PDAC and genetically engineered mouse models²⁰⁵. Preclinical modelling implicates paracrine signalling downstream of oncogenic KRAS in the epithelial compartment to the vasculature in positive regulation of lymphangiogenesis²⁰⁶, although further mechanistic studies would help parse out impacts of KRAS-driven lymphangiogenesis on metastatic spread and tumour immunity. Finally, recent efforts to model tumour–blood vessel interactions and PDAC vascular invasion on a biomimetic chip identified the activin–activin receptor-like kinase 7 (ALK7) pathway as a mechanism by which PDAC cells ablate endothelial cells once outside the vessel lumen after invasion²⁰⁷, potentially reconciling the observation of frequent PDAC venous invasion with its hypovascularity. Taken together, these studies illustrate the complex consequences of vascular dysfunction in PDAC and suggest that we require further granularity in mechanistic understanding as the field develops therapeutic approaches targeting the PDAC vasculature.

PDAC innervation

Besides the formation of a desmoplastic stroma, a hallmark feature of the pancreatic TME is its dense infiltration by reprogrammed and

hypertrophic peripheral nerves along with perineural invasion (PNI), which is invasion of tumour cells into the peripheral nerve sheath²⁰⁸. More than 70% of patients with PDAC present with PNI at diagnosis, and both PNI and nerve hypertrophy are associated with post-operative recurrence and reduced overall survival^{208,209}. Additionally, severe pain is a common symptom of pancreatic cancer²¹⁰. Despite these clinical associations, little is known of the underlying mechanisms facilitating nerve-mediated pancreatic tumour growth, invasion, metastasis and immunosuppression. To date, several foundational studies have highlighted evidence of the complex crosstalk between nerves and tumour, stromal and immune cells, underscoring the importance of nerves in PDAC and the potential for nerve-targeted therapies.

PDAC-innervating neurons comprise coeliac ganglion-derived sympathetic, noradrenergic nerves as well as dorsal-root ganglia-derived and jugular-nodose ganglia-derived sensory, glutamatergic neuronal subtypes²¹¹. To facilitate nerve outgrowth, branching and PNI, tumour, stromal and glial cells secrete various neurotrophic factors and axon guidance molecules^{212–217}. The infiltration of sensory nerves increases throughout PDAC progression and is associated with disease severity and pain²¹⁸. As early as the PanIN stage, neurotrophic factor expression increases and transformed cells invade along nerves and into the dorsal-root ganglia and spinal cord^{218,219}. Sensory neurons promote proliferation and PNI of PanIN and PDAC cells through activation of the substance-P–neurokinin-1 axis^{218,220–222}. Sympathetic nerves are extensively reprogrammed, fostering PNI and tumour growth through catecholamine secretion. Noradrenaline promotes tumour cell proliferation, migration and PNI via binding to the β 2-adrenergic receptor on tumour cells, activating various downstream pathways including cAMP-responsive element-binding protein–ERK–nerve growth factor²²³, protein kinase A–STAT3 (ref. 224), poly(rC)-binding protein 2–MYC²²⁵ and CDC42–p21-activated kinase 1–Lim kinase 1–Merlin²²⁶. Patients with PDAC experience high levels of chronic, psychological stress²²⁷ owing to sympathetic nerve activation, and several clinical studies have highlighted favourable outcomes for patients with PDAC treated with β -blockers, which are antagonists of β -adrenergic receptors^{228,229}. Chronic stress induces tumour proliferation, invasion and dissemination, as well as suppressed immune responses by diminishing CD4⁺ T cells, B cells and enriching for T_{reg} cells and MDSCs^{230–232}. Combination therapy using propranolol, a non-selective β -blocker, with immune checkpoint inhibitor therapy demonstrated a synergistic positive effect on CD8⁺ T cell effector function, underlining the potential benefit of nerve-targeted therapies²³³. Although infrequently present in PDAC tumours per analysis of mouse models, infiltration of parasympathetic nerves and elevated acetylcholine-mediated signalling were found in tumours of patients with high PNI and shown to enhance tumour growth by impairing CD4⁺ and CD8⁺ T cell infiltration in mouse models of PDAC^{234,235}. Conversely, parasympathetic nerves may hamper tumour progression through activation of the acetylcholine receptor, muscarinic acetylcholine receptor M1 (M1-AChR), on tumour cells reducing cancer stem-like cell growth, immunosuppressive myeloid cell recruitment and liver metastases²¹⁷, revealing a context-dependent effect of parasympathetic nerve signalling. In addition to their direct influence on tumour cells, nerves can interact with CAFs to modulate antitumour immunity. CAF-derived nerve growth factor facilitates the release of the neuropeptide calcitonin gene-related peptide from sensory nerves, causing the suppression of receptor activity-modifying protein 1-mediated IL-15 secretion by CAFs and subsequent suppression of NK cell infiltration and cytotoxic function²³⁶. Additionally, CAFs facilitate tumour invasion in sensory

nerves, via the production of lactate leading to L1 cell adhesion molecule and SLIT1 release by tumour cells²³⁷. Sympathetic nerves also directly impact antitumour immunity by activating the β 1-adrenergic receptor on terminally exhausted, CD8⁺PDI⁺ T cells, suppressing T cell receptor signalling²³³.

The nerve subtypes discussed earlier, along with their associated glial cells, known as Schwann cells, secrete factors locally into the TME involved in promoting PNI. Key factors, TGF β 1, GDNF family receptor α 1 (GFR α 1), chemokines including CCL2 and CXCL12 and metabolites such as glutamate have all been reported to support PNI^{238–242}. PDAC cells were shown to utilize amoeboid migration patterns, assisted by Schwann cells that form tracks to facilitate their invasion along axons^{243,244}. Despite mounting evidence of the crucial involvement of nerves in tumour progression, the causal link between PNI and metastasis to distant organs, most commonly the liver and the lung, has not yet been investigated. Given the hypovascular nature of the pancreatic TME, tumour cells may use nerves as an alternative route of dissemination, supported by cells within the nerve sheath, such as Schwann cells, endoneurial macrophages and perineural fibroblasts. Neuronal electrical activity may additionally modulate tumour and stromal cells to promote tumour growth and PNI, as has been demonstrated in subtypes of gliomas^{245–247}, but has yet to be investigated in pancreatic cancer. Overall, it is evident that peripheral nerves have a pivotal role in PDAC progression and further investigation is warranted to explore the potential of nerves as therapeutic targets in PDAC.

Spatial architecture

The PDAC TME exhibits an extremely complex and heterogeneous spatial organization. Its architecture varies depending on the stage of tumour progression and the molecular subtype, which can be classified as classical and epithelial or basal-like, squamous and quasi-mesenchymal^{248–250}. The spatial profile of the TME in PDAC also influences treatment responses²⁵¹. Understanding how TME organization contributes to tumour progression is crucial for elucidating the biological mechanisms underlying PDAC development and aggressiveness. A landmark study combining laser capture microdissection with multiomic analysis of human PDAC identified recurrent, histologically defined ‘sub-TMEs’ across these spatially heterogeneous tumours. These ‘reactive’ versus ‘deserted’ sub-TMEs differed in fibroblast abundance and profile, immune contexture and tumour cell states, with the deserted sub-TME enriched upon chemotherapy and associated with worse prognosis¹². This spatial subtype heterogeneity has been attributed at least in part to PDAC cell-intrinsic activator protein 1 and to TNF⁺ macrophages²⁵², and ever-improving technologies leave us poised to further uncover mechanisms and consequences of emerging spatial patterns in tumours.

In recent years, the advent of new spatial profiling technologies has led to considerable advances in understanding the transcriptional heterogeneity of the cellular components within the PDAC TME as well as the presence of defined heterocellular niches. The gene expression patterns of different stromal cell types are heavily influenced by how close they are to the tumour, allowing for mapping of molecular profiles based on their spatial position. In the first application of spatial transcriptomics to human PDAC, together with single-nucleus RNA sequencing, Hwang et al.²⁵³ identified three multicellular communities based on fibroblast, immune and cancer cell profiles: classical, squamoid–basaloid and treatment-enriched. This enabled the identification of a neural-like progenitor PDAC cell state enriched after chemotherapy and radiotherapy and associated with poor prognosis²⁵⁴,

with these treatments later shown to pervasively remodel inferred ligand–receptor interactions between PDAC cells and CAFs. Alver et al.²⁵⁵ used spatial transcriptomics to analyse transcriptional differences between the central and peripheral (invasive) regions of PDAC and found that tumour cells located in the central region of the tumour exhibit a gene expression signature associated with cell proliferation, whereas the peripheral regions are enriched in transcriptional programmes related to hypoxia and oxidative phosphorylation. Moreover, myCAFs were found in close proximity to tumour cells, whereas iCAFs were more abundant in the peripheral areas of the tumour²⁵⁵, a finding supported by complementary approaches^{256–258}. Further illustrating conserved spatial relationships between PDAC and CAF states, two recent studies applied spatial transcriptomics to human PDAC and found heterocellular niches with inferred functional importance, including proximal myCAFs and basal-like cancer cells which correlated with plasma cell exclusion likely mediated by CXCR4–CXCL12 signalling^{112,259}.

The tumour immune microenvironment in PDAC is characterized by low immunogenicity and a complex structural organization. Mi et al.²⁴ identified two distinct immune subgroups in PDAC through spatial analysis, each displaying unique spatial patterns correlated with patient survival. Notably, CD4⁺ T cells were more prevalent in regions with a high density of myelomonocytic cells, whereas CD8⁺ T cells were found to colocalize spatially with B cells, specifically within intraepithelial regions^{24,260}. TAMs predominantly accumulate in the stroma surrounding tumour cells^{261–263}. However, accumulating studies on nerve biology in PDAC have shown that macrophages also infiltrate perineural areas, actively contributing to PNI and tumour spread along nerves^{264–266}. The vasculature is a critical conduit for intratumoural immune cell infiltration, such that the spatial distribution of immune cells (and other cellular components) within the TME is undoubtedly influenced by the poor vascularization²⁶⁷. The dense and fibrotic stroma, rich in collagen, present in the TME creates a physical barrier that prevents blood vessels from reaching the tumour core^{267,268}. Moreover, even in areas where blood vessels manage to penetrate, the high interstitial pressure generated by the stroma causes collapse of the endothelial walls, compromising perfusion. This condition leads to inefficient drug delivery and hinders the proper recruitment of immune cells to the tumour centre^{180,269,270}.

Studying spatial organization and architecture within the PDAC TME is crucial for understanding the transcriptional differences underlying its heterogeneity. It is now well established that spatial distribution substantially influences the crosstalk between microenvironmental components and tumour cells. Thus, as the spatial distribution of microenvironmental components influences tumour progression, it may be critically important for the development of new therapeutic strategies.

TME-targeting clinical trials

Over the past decade, PDAC clinical trials have increasingly incorporated agents targeting non-malignant features of the TME (Table 1), building on our improved understanding of heterocellular crosstalk mechanisms enabling PDAC progression. Several of these trials targeted components of the non-immune stroma including CAFs, endothelial cells and the ECM. As examples of the latter, several clinical trials tested the benefit of enzymatic degradation of the PDAC ECM using a PEGylated human hyaluronidase, PEGPH20, to degrade hyaluronan. However, combining PEGPH20 with different chemotherapy regimens or with anti-PDL1 immune checkpoint blockade did not show clinical activity in patients with PDAC^{271–275}, but may warrant further

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Table 1 | Summary of pancreatic ductal adenocarcinoma clinical trials from the past 10 years targeting cancer cell-extrinsic components of the tumour microenvironment

Tumour microenvironment target	Therapeutic approach	Clinical trial identifier	Phase	Key findings	Refs.
Hyaluronan	PEGPH20 + chemotherapy nab-paclitaxel and gemcitabine or PEGPH20 + chemotherapy regimen mFOLFIRINOX	NCT01959139 NCT02715804 NCT01839487	I, II, III	No improvement in OS or PFS, increased ORR in PEGPH20 + nab-paclitaxel and gemcitabine arm compared with chemotherapy alone	271–274
Hyaluronan	PEGPH20 + anti-PDL1 atezolizumab	NCT03193190 NCT03281369	Ib/II	Limited clinical activity	275
FAK	Defactinib + anti-PD1 pembrolizumab + chemotherapy gemcitabine	NCT02546531	I	Promising preliminary efficacy, biomarker activity with infiltrating T cells	279
α V integrins + neuropilin 1	CEND-1 + nab-paclitaxel and gemcitabine	NCT03517176	I	Encouraging ORR	280
HGF	Ficlatuzumab + nab-paclitaxel and gemcitabine	NCT03316599	Ib	Some toxicity, durable treatment responses	281
LOXL2	Simtuzumab + gemcitabine	NCT01472198	II	No improvement in clinical outcomes	292
VDR	Paricalcitol + pembrolizumab	NCT03331562	II	Short half-life noted for intravenous paricalcitol, no improvement in clinical outcomes	285
RAR	All-trans-retinoic acid + nab-paclitaxel and gemcitabine	NCT03307148	I	Stromal modulation evident by MRI	287
Angiotensin receptor	Losartan + FOLFIRINOX or chemoradiotherapy	NCT01821729	II	Downstaging of locally advanced disease, increased CD8 ⁺ T cell infiltration	288,289
TGF β	Galunisertib + anti-PDL1 durvalumab	NCT02734160	Ib	Limited clinical activity	293
PD1 + CTLA4	Nivolumab + ipilimumab \pm MEK inhibitor cobimetinib	NCT01928394	I/II	Few objective responses in triplet arm, no objective responses with ICIs only	290
PD1 + CTLA4	Durvalumab + tremelimumab	NCT02558894	II	Very low ORR	291
PD1	Nivolumab + nab-paclitaxel and gemcitabine	NCT02309177	I	Minimal impact on PFS and OS	292
PD1	Neoadjuvant pembrolizumab + chemotherapy capecitabine + external beam radiation	NCT02305186	Ib/II	Safe but no clear effect on CD8 ⁺ tumour-infiltrating lymphocytes	301
CD40 + PD1	Sotigalimab + nivolumab + nab-paclitaxel and gemcitabine	NCT03214250	Ib, II	No clear benefit for ORR or PFS, potential prognostic biomarkers identified in the individual ICI + chemotherapy arms	294,295
CCR2 and CCR5 + PD1	BMS-813160 + nivolumab + nab-paclitaxel and gemcitabine	NCT03496662	I/II	Prolonged PFS and OS in locally advanced disease	297
IL-1 β + PD1	Canakinumab + spartalizumab + nab-paclitaxel and gemcitabine	NCT04581343	Ib	Modest reactivation of peripheral CD8 ⁺ T cells, decreased levels of circulating monocytic MDSCs	296
BTK	Ibrutinib + nab-paclitaxel and gemcitabine	NCT02436668	III	No improvement in OS or PFS	303
CCR2	PF-04136309 + FOLFIRINOX	NCT01413022	Ib	Local tumour control and objective responses observed	298
CCR2	PF-04136309 + nab-paclitaxel and gemcitabine	NCT02732938	Ib	Concerning safety profile, no efficacy signal above chemotherapy alone	299

Table 1 (continued) | Summary of pancreatic ductal adenocarcinoma clinical trials from the past 10 years targeting cancer cell-extrinsic components of the tumour microenvironment

Tumour microenvironment target	Therapeutic approach	Clinical trial identifier	Phase	Key findings	Refs.
Antigen-presenting cells	GVAX ± chemotherapy cyclophosphamide	NCT00727441	II	Safe and feasible, longer OS associated with higher density of intratumoural tertiary lymphoid aggregates	304
Antigen-presenting cells	GVAX + cyclophosphamide + anti-mesothelin vaccine CRS-207 + nivolumab	NCT02243371	II	OS comparable with standard chemotherapy, increased CD8 ⁺ T cells and decreased CD68 ⁺ myeloid cells in long-term survivors	305
Antigen-presenting cells	GVAX + ipilimumab	NCT01896869	II	Clinical responses and biological effects on immune cells observed, no improvement in OS	306
Antigen-presenting cells	GVAX + cyclophosphamide + nivolumab + anti-4-1BB urelumab	NCT02451982	II	Modest improvements in DFS and OS, increased intratumoural activated T cells	307,308
Patient-specific neoantigens	Autogene + atezolizumab + mFOLFIRINOX	NCT04161755	I	Patients with vaccine-expanded T cells have longer recurrence-free survival	34
Antigen-presenting cells in lymph nodes	ELI-002 2P	NCT04853017	I	Encouraging relapse-free survival, considerable T cell responses	309

BTK, Bruton's tyrosine kinase; CCR, CC-chemokine receptor; CTLA4, cytotoxic T lymphocyte-associated antigen 4; DFS, disease-free survival; FAK, focal adhesion kinase; HGF, hepatocyte growth factor; ICI, immune checkpoint inhibitor; LOXL2, lysyl oxidase-like 2; MDSC, myeloid-derived suppressor cell; ORR, objective response rate; OS, overall survival; PFS, progression-free survival; RAR, retinoic acid receptor; TGFβ, transforming growth factor β; VDR, vitamin D receptor.

investigation in combination with improved agents targeting cancer cells. The dense stroma in PDAC activates mechanosignalling mediator FAK, which was shown to mediate immune suppression and disease progression in preclinical studies^{176,276–278}. A recent clinical trial combining FAK inhibition with anti-PD1 therapy showed promising preliminary efficacy as well as an increase in tumour-infiltrating T cells²⁷⁹. Taking a different approach to targeting cell–matrix adhesions, treatment with the cyclic peptide CEND-1, which targets both αV integrins and the transmembrane cytokine co-receptor neuropilin-1, yielded an encouraging objective response rate in patients with PDAC²⁸⁰.

Multiple recent trials have assessed the utility of targeting either the apparent tumour-promoting functions of CAFs or CAF transcriptional states. These included inhibition of hepatocyte growth factor, which yielded some durable treatment responses in combination with chemotherapy²⁸¹; inhibition of collagen crosslinking factor LOXL2, which did not improve clinical outcomes²⁸²; and activation of nuclear receptors including the vitamin D receptor and retinoic acid receptor as these factors promoted stromal remodelling in preclinical models^{283,284} and may do so in patients^{285–287}. As alluded to above, attempts to normalize the PDAC vasculature using losartan resulted in downstaging of locally advanced disease and increased infiltration of CD8⁺ T cells^{288,289}, suggesting that combination treatment with immunotherapies could perhaps be successful. Overall, our ever-improving grasp on functional heterogeneity within the PDAC stroma has the potential to instruct new and effective clinical combinations in the years ahead. Although single agents have shown somewhat modest success so far, efforts to understand TME dynamics and mechanisms of adaptation to treatment are poised to uncover rational targets for combination therapy.

The pronounced immune suppression in PDAC has stimulated many efforts to identify effective immunotherapies in this setting. Immune checkpoint inhibitors targeting PD1–PDL1 and cytotoxic T lymphocyte-associated antigen 4 have shown limited activity against PDAC^{290–292}, but may be effective as part of combination regimens. To date, these agents have been combined with drugs targeting fibrosis in the form of TGFβ inhibition²⁹³, which did not improve outcomes; or with drugs targeting myeloid cells such as agonistic anti-CD40 (refs. 294,295), anti-IL-1β²⁹⁶ or an inhibitor of CCR2 or CCR2 and CCR5 – of these agents, only the CCR2 and, CCR2 and CCR5 inhibitors showed clinical activity^{297,298}; although a subsequent trial of CCR2 inhibition failed²⁹⁹. Although anti-CD40 and anti-IL-1β did not improve patient outcomes, these agents may be beneficial in combination with immunotherapies beyond immune checkpoint blockade. As neoadjuvant chemoradiotherapy can increase tumour-infiltrating lymphocyte density³⁰⁰, a recent trial tested neoadjuvant treatment with the chemotherapeutic agent capecitabine, external beam radiation and PD1 blockade, which while safe did not impact numbers of CD8⁺ tumour-infiltrating lymphocytes³⁰¹. In the light of preclinical evidence that Bruton's tyrosine kinase (BTK) signalling fosters immunosuppressive crosstalk between B cells and macrophages to T cells³⁰², a clinical trial tested BTK inhibitor ibrutinib in PDAC, which unfortunately showed no improvement in outcomes³⁰³. In addition to these agents, several trials have tested the efficacy of cancer vaccines in patients with PDAC. These include multiple combinations built on the backbone of the granulocyte–macrophage CSF-producing vaccine GVAX, which have shown some promising impact on clinical outcomes as well as intratumoural immune contexture such as the formation of

Glossary

Cryptic antigens

Peptide antigens arising from non-coding or not previously annotated genomic loci.

Desmoplasia

Formation of connective, fibrous tissue, often in the context of invasive cancer.

Endoneurial macrophages

Resident or blood-derived immune cells found within the connective tissue of peripheral nerves.

Epithelial-to-mesenchymal transition

A cell state switch along a continuum with respect to markers and morphology wherein epithelial cells lose epithelial features but acquire mesenchymal traits, as is seen in several cancer types and is associated with invasion and metastasis.

Extracellular matrix

(ECM). The proteins external to and between cells which can form insoluble fibres and interconnected networks. Includes major components such as collagens, cell-binding glycoproteins and proteoglycans, all with distinct biochemical and physical properties.

Immunoediting

A dynamic process evident in cancer through which antitumour immune responses shape cancer evolution through phases of elimination, equilibrium and escape, ultimately yielding immune-resistant tumour cells.

Interstitial pressure

Physical pressure within the area surrounding cells where fluid and solutes are exchanged, with implications for drug delivery within tissues, nutrient and waste transport and fluid balance.

Matrisome

Collection of diverse proteins, including structural elements such as collagens as well as remodelling enzymes, which together comprise the extracellular matrix.

Neurotrophic factors

Stimulate nerve outgrowth, as may be observed in the tumour microenvironment by acting on nerve endings of adrenergic, cholinergic or sensory origin.

Oxidative stress response

Adaptation to an imbalance of accumulation and elimination of reactive oxygen species in cells.

Pancreatic neuroendocrine tumours

Relatively rare cancers in the pancreas arising from the endocrine or hormone-producing cellular compartment.

Paracrine signalling

A form of cell–cell communication wherein a cell produces and releases a signal that can act on and affect nearby cells.

Parasympathetic nerves

Nerves of the autonomic nervous system which generally use acetylcholine as the main neurotransmitter and serve to regulate the body's unconscious actions.

Patent vessel

An open or unobstructed blood vessel allowing normal fluid flow.

Perineural invasion

(PNI). A form of tumour progression wherein cancer cells invade into and track along nerves, typically a feature of aggressive disease.

Sensory, glutamatergic neuronal subtypes

Sensory neurons which transduce diverse stimuli from the environment into electrical signals, including nociceptors for pain sensing, mechanoreceptors for physical pressure, proprioceptors for spatial cues and thermoreceptors for temperature changes. All are generally responsive to glutamate as their primary excitatory neurotransmitter.

Solid stress

Internal mechanical stress generated within a solid material, such as tumour tissue, due to forces acting on it, potentially arising from growth or external forces.

Sympathetic, noradrenergic nerves

Nerves that release the neurotransmitter noradrenaline from their nerve endings, which can act through adrenergic receptors on target tissues to produce diverse physiological effects.

Tumour-associated macrophages

(TAMs). Macrophages found in the tumour microenvironment which may exist anywhere along a continuum of inflammatory to immunosuppressive phenotypic states and may derive from the host tissue or the bone marrow.

Vascular dysfunction

Disruption of normal blood and/or lymphatic vessel function owing to reduced numbers of vessels, reduced vessel perfusion or both, as common features of dense tumours.

tertiary lymphoid aggregates^{304–308}. The development of a personalized neoantigen vaccine approach for patients with resectable PDAC has demonstrated clear improvements in recurrence-free survival with evidence of a vaccine-expanded T cell repertoire³⁴. In a similar vein, treatment with a lymph node-targeted vaccine against mutant KRAS peptides showed encouraging increases in relapse-free survival and considerable T cell responses in patients with PDAC³⁰⁹. These innovative clinical trials coincide with the development of powerful KRASi, currently under intense preclinical and clinical investigation for the treatment of PDAC¹⁴. Although treatment resistance is likely to emerge and has been reported in the preclinical setting^{310,311}, these inhibitors will likely motivate efforts to further understand the molecular and cellular relationships between cancer cell-intrinsic KRAS signalling and the TME, which will hopefully enable the development of effective combination therapies that meaningfully improve outcomes for patients with PDAC.

Several promising results of preclinical studies may inform clinical trial design in the near or medium term. For example, our improved understanding of CAF heterogeneity has motivated efforts to target specific CAF subsets marked by FAP or LRRC15 as targeting either subtype fosters impressive responses to immune checkpoint blockade in mouse models of PDAC^{28,42}. Moving towards clinical translation, a recent study used patient-derived models to test anti-mesothelin chimeric antigen receptor T cells, which secrete T cell-engaging molecules targeting CAFs through FAP and T cells through CD3, an approach known as mesoFAP chimeric antigen receptor-TEAM cells³¹². This approach modified the PDAC stroma and increased elimination of tumours in these patient-derived models, motivating further study of combination therapy targeting immune-suppressive CAF subsets and immunotherapy. DC paucity is an additional limitation to productive antitumour immunity in PDAC, and the recent demonstration that an engineered cDC1 vaccine can sensitize PDAC

to immune checkpoint blockade in mice⁴⁹ has prompted efforts to test this combination in patients. Steady advances in the field towards understanding the fundamental mechanisms behind resistance to available cancer therapies are poised to drive innovative PDAC clinical trials in the years to come.

Conclusions and future perspectives

Over the past decade, technological advances in spatial profiling, single-cell analytics and lineage-tracing models have notably improved our understanding of the cellular and spatial heterogeneity of the PDAC TME. The functional diversity of CAFs and the spatial distribution of stromal components such as CAFs, TAMs and peripheral nerves orchestrate a fibroinflammatory TME in PDAC and critically contribute to disease progression, immune evasion and therapeutic resistance. Recent multicancer analyses highlight conservation of defined CAF subtypes among PDAC and other tumour types that respond relatively well to treatments such as chemotherapy (that is, colon cancer) and immunotherapy (that is, melanoma and lung cancer)²⁵⁹. This conservation extends to the cellular neighbours and spatial neighbourhoods of these CAF subtypes. These observations raise the possibility that a causal link between PDAC CAFs and therapy resistance would reflect an anatomically specific function of these CAFs – for example, a functional relationship with pancreatic lymph nodes or pancreatic cancer cells. Therapies targeting the tumour-promoting or immunosuppressive roles of individual stromal elements, such as CAFs or TAMs, present potentially promising strategies to boost local immune responses and to improve responses to immunotherapy and perhaps to targeted therapy. To achieve this goal, we need to better understand how TME interactions dynamically shift during disease progression and in response to therapy, particularly those dynamics which promote treatment resistance. This remains challenging owing to the difficulty in obtaining comprehensive longitudinal data from patients that capture the diverse, spatially restricted and evolving cell populations driving therapeutic resistance. Standard biopsy and histology approaches provide merely static snapshots, failing to capture constant shifts in the TME, which responds to intrinsic tumour cues, heterocellular interactions, immune pressures, metabolic changes and therapeutic interventions. Additionally, most TME research focuses on primary tumours, even though the majority of patients with PDAC present with metastatic disease at diagnosis¹. Thus, future studies need to dissect metastatic TMEs alongside the primary stroma to identify shared and site-specific dependencies.

The advent of mutant KRASⁱ has the potential to transform PDAC treatment. However, the efficacy of targeted therapies such as KRASⁱ is often limited by acquired resistance impacted by the profound PDAC microenvironment. Oncogenic KRAS signalling actively modulates features of the PDAC TME, including immune suppression and fibroblast activation¹⁹. A critical goal is to understand how KRASⁱ impacts the stroma and integrates TME modulation with KRAS-targeted therapy to overcome resistance. Overall, by integrating multiomics data and exploring spatial and temporal relationships within primary and metastatic niches, the field is poised to identify next-generation treatment strategies reshaping the spatial organization of the tumour–stroma ecosystem, prevent resistance and ultimately improve patient outcomes including those with distant metastases.

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