Cancer-associated fibroblasts in gastrointestinal cancer

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Abstract | The tumour microenvironment, also termed the tumour stroma or tumour mesenchyme, includes fibroblasts, immune cells, blood vessels and the extracellular matrix and substantially influences the initiation, growth and dissemination of gastrointestinal cancer. Cancer-associated fibroblasts (CAFs) are one of the critical components of the tumour mesenchyme and not only provide physical support for epithelial cells but also are key functional regulators in cancer, promoting and retarding tumorigenesis in a context-dependent manner. In this Review, we outline the emerging understanding of gastrointestinal CAFs with a particular emphasis on their origin and heterogeneity, as well as their function in cancer cell proliferation, tumour immunity, angiogenesis, extracellular matrix remodelling and drug resistance. Moreover, we discuss the clinical implications of CAFs as biomarkers and potential targets for prevention and treatment of patients with gastrointestinal cancer.

[H1] Introduction

The classic concept of carcinomas as ‘wounds that never heal’ or ‘organs that never develop’ has its limitations, but as an analogy, it provides a helpful framework for understanding the inflammatory and developmental signalling between cancer cells and the activated tumour microenvironment (TME)[1]. Also termed the tumour stroma or tumour mesenchyme, the TME is composed of fibroblasts, inflammatory cells, blood vessels, extracellular matrix (ECM) and basement membrane (FIG. 1). Although most previous research has focused on the biology of cancer cells themselves, it is clear that the TME is a major contributor to cancer development[2–5]. For example, pancreatic ductal adenocarcinoma (PDAC) is characterized by a prominent desmoplastic reaction, a fibrotic stromal reaction accompanied by activated cancer-associated fibroblasts (CAFs) and extensive deposition of ECM, accounting for up to 90% of the tumour[6,7]. Among the heterogeneous components of the cancer mesenchyme, CAFs are probably one of the most relevant cell types but unfortunately also one of the least understood in terms of their origins, subtypes, biology and even definition. However, we are now developing the necessary understanding to help apply CAF biology to the treatment of patients with gastrointestinal cancer.

Studies investigating the function of CAFs are largely based on preclinical gastrointestinal cancer models. For instance, the role of CAFs in cancer restraint was first established in sophisticated studies using transgenic mouse models such as *Kras*$^{LSL-G12D/+}$;*Trp53*$^{LSL-R172H/+}$;*Pdx1*–Cre (KPC) mice, which recapitulate the desmoplastic features of human PDAC[8,9], and then later in studies using a colitis-associated colon cancer model induced by azoxymethane and dextran sulfate sodium[10,11]. Contrary to previous studies that showed that CAFs promote tumour growth[12,13], depletion of α-smooth muscle actin (αSMA)$^+$ CAFs in the mouse model of PDAC or blockade of Hedgehog signalling, a key signalling pathway necessary for activation of CAFs, in mouse models of PDAC and colon cancer accelerated cancer progression[8–11]. The concept that mesenchymal stem cells (MSCs) recruited from the bone marrow develop into CAFs that promote cancer progression was proposed on the basis of experiments using a mouse model of gastric carcinogenesis induced by *Helicobacter felis* infection[14]. Previous studies using subcutaneous injections of cancer cells lacked the effects of the TME in which gastrointestinal CAFs co-evolve with tumour cells. However, the development of physiologically accurate autochthonous cancer models and orthotopic injection of genetically edited organoids is providing the necessary understanding to translate basic gastrointestinal CAF research into the clinic[15,16].

Structurally and functionally, CAFs make a substantial contribution to the development of cancer through a variety of mechanisms. For instance, CAFs release various tumour-promoting factors such as cytokines and chemokines, which support cancer cell growth and angiogenesis[12,17]. Previous studies using genetically engineered mouse models (GEMMs) of pancreatic cancer have shown that CAFs, and ECM produced by CAFs, confer resistance to chemotherapy by impairing efficient drug delivery[18–20]. Furthermore, it has been demonstrated that CAFs could contribute to poor responses to immunotherapy in PDAC and colorectal cancer (CRC) mouse models[15,21]. Notably, CAFs are recruited to metastatic lesions at the nano-metastases stage[22] and also appear to create a favourable microenvironment for cancer growth at the secondary site[4,23,24], suggesting that CAFs could be a potential target for the development of new therapeutics against human malignancies.

The molecular subtyping of gastrointestinal cancers has highlighted the clinical importance of stroma-related genes as prognostic and predictive markers. In many types of gastrointestinal cancer including CRC, PDAC and hepatocellular carcinoma (HCC), stromal activation gene signatures are associated with poor prognosis[25–31]. Strikingly, the stromal gene signature, rather than epithelial gene signature, was found to more closely inform outcome in patients with CRC[28].

Notwithstanding the accumulating evidence showing the critical roles of CAFs in tumour progression, it has been challenging to therapeutically target CAFs, or at least the right CAFs or the right CAF-related factors. One example of this situation was the failure of a much-anticipated clinical trial of a Hedgehog inhibitor[32], which in combination with gemcitabine was initially shown to improve survival in preclinical mouse models of PDAC by improving drug delivery[18]. Hedgehog ligands, especially sonic hedgehog (SHH), secreted by cancer cells were shown to play a central role in activation of CAFs, leading to increased desmoplasia and PDAC progression[33]. However, consistent with the failure of the Hedgehog inhibitor in the PDAC clinical trial, a subsequent study using a mouse model of PDAC revealed that inhibition of the Hedgehog pathway unexpectedly resulted in increased PDAC progression with predominantly undifferentiated cancer cell histology, suggesting that some populations of CAFs activated by Hedgehog inhibit cancer progression[8,34]. One of the critical challenges in targeting CAFs for cancer treatment is the functional heterogeneity of CAFs[4,6,8]. For many years, CAFs

were considered to be a uniform entity that exerted a tumour-promoting effect as an accomplice of cancer cells by secreting pro-tumorigenic factors[12,13]. Interestingly, however, a growing number of studies have demonstrated that certain populations of CAFs actually inhibit tumour growth[8,9,35–38]. Here, we offer a novel nomenclature for CAFs based on function: tumour-promoting CAFs (pCAFs), tumour-retarding CAFs (rCAFs) and neutral CAFs (nCAFs) that neither promote or retard tumour progression.

In this Review, we summarize the current advances in our understanding of CAF origin and heterogeneity with a particular emphasis on local and recruited mesenchymal progenitor cells as one probable origin. We describe how CAFs can affect tumour progression from the viewpoint of stromal to epithelial interactions, tumour immunity, angiogenesis and ECM remodelling, particularly focusing on gastrointestinal cancers such as gastric cancer, CRC, PDAC and HCC. In addition, the mechanism by which CAFs confer resistance to chemotherapy, immunotherapy and radiotherapy is discussed. Lastly, we provide an overview of the clinical importance of gastrointestinal CAFs as biomarkers and therapeutic targets.

[H1] Definition of fibroblasts and CAFs

Fibroblasts are spindle-shaped, non-epithelial and non-immune cells embedded in the ECM that are easily propagated in adherent cell culture[4,39]. They are a major constituent of the stroma in gastrointestinal organs, and as in other tissues, they are highly organized. Throughout the gastrointestinal tract, a reticular network of stromal cells lies coincident with the epithelial basement membrane[40]. The subepithelial plexus, composed of reticular stromal cells, entirely surrounds the glandular axis from the stomach to the rectum[41]. This compartment is dynamic, with a radial axis of proliferation and differentiation, analogous to the epithelium, developing from gremlin 1-expressing intestinal reticular stem cells[42]. These cells give rise to intestinal reticular cells[42], probably overlapping with FOXL1$^+$ subepithelial telocytes and glioma-associated oncogene homologue 1 (GLI1)$^+$ mesenchymal cells, which constitute an essential mesenchymal niche to support the intestinal stem cells[41,43]. Beneath this highly compartmentalized population exist loose arrangements of fibroblasts within the lamina propria that interact with each other and deeper stromal elements including smooth muscle, vessels, nerves and inflammatory cells[40,44,45]. Functionally, fibroblasts are fundamental regulators of ECM synthesis and of paracrine and juxtacrine signalling to nearby epithelium to regulate growth and differentiation, and they are also ready to respond to tissue injury, either in wounding or tumorigenesis[4,46].

CAFs are generally accepted to be all of the fibroblasts found within and surrounding a cancer[47]. This group includes native, normal fibroblasts and activated, proliferating (Ki67$^+$) or recruited fibroblasts in response to stimuli from cancer. These new CAFs could, in turn, have originated via a number of possible mechanisms that we discuss below. Despite the rapid evolution of immunophenotyping and subtyping of immune cells, there is no single, precise positive discriminator of CAFs[4,47–49]. This lack of understanding has led to different studies reporting on overlapping, incomplete or discrete populations of CAFs and the use of markers that label both CAFs and other cell populations. These difficulties have complicated interpretation of several studies, which is discussed below.

[H1] Heterogeneity of CAFs

[H2] Marker heterogeneity

Representative CAF markers include but are not limited to, αSMA, the serine protease fibroblast activation protein (FAP; also known as prolyl endopeptidase FAP), fibroblast-specific protein 1 (FSP1; also known as S100A4), platelet-derived growth factor receptor-α (PDGFRα) and PDGFRβ. Some functions of well-established CAF markers and several cell types in which they are expressed are briefly summarized in TABLE 1. One of the most well-established CAF markers, αSMA, fails to distinguish all CAFs in the TME[48,50], and none of these CAF markers are specific to CAFs, as they are also expressed in other cell types and healthy tissues. For instance, αSMA expression is observed in smooth muscle cells in the muscular layer of the gastrointestinal tract and in vascular smooth muscle cells[5,45]. FAP$^+$CD45$^+$ cells also correspond to a subset of tumour-associated macrophages[51,52], and FSP1 has been demonstrated to mark epithelial cells undergoing epithelial-to-mesenchymal transition (EMT)[53–55] and inflammatory macrophages, but not αSMA$^+$ myofibroblasts, in liver fibrosis models[56]. Experimentally, using fluorescence-activated cell sorting, CAFs are isolated by their lack of expression of an epithelial marker (epithelial cell adhesion molecule (EPCAM)), a haematopoietic cell marker (CD45) or an endothelial marker (CD31) and/or their expression of CAF markers such as FAP and PDGFRα[21,28,50,57–60]. Moreover, CAFs are a heterogeneous population on the basis of both markers and functions, with a broad spectrum of different CAFs existing simultaneously in the cancer mesenchyme[48,50,61], adding further complexity to CAF definitions. Notably, contradictory results regarding whether CAFs promote or retard cancer progression can be obtained depending on the specific CAF markers used. Thus, future work is required to identify the right CAF marker(s) for the right therapy to make a major breakthrough in this area of study. Fundamentally, CAFs will be best understood and subtyped by biology and by function, with subgroups previously suggested including tumour-restraining CAFs, tumour-promoting CAFs, secretory CAFs, inflammatory CAFs and myofibroblastic CAFs[4,50].

It is possible that CAFs identified by a single marker are composed of a range of distinct CAF subtypes that have functionally opposing roles in cancer progression. Accordingly, it will be necessary to subdivide CAFs by the combination of several marker proteins to help better and prospectively characterize their biology and thus their therapeutic relevance. Interestingly, single-cell RNA sequencing analyses from human CRC samples revealed the presence of two major subtypes of CAFs[26]. On the basis of TGFβ pathway gene expression, CAFs in CRC could be divided into CAF-As, characterized by high expression of matrix metalloproteinase 2 (MMP2), decorin, collagen type I α2 (COL1A2) and FAP, and CAF-Bs, which were characterized by high expression of myofibroblastic markers such as αSMA, transgelin and PDGFα[26].

[H2] Functional heterogeneity

Studies suggest that CAFs are composed of various functionally heterogeneous subsets that either promote or restrain cancer growth[4]. Most previous studies have focused only on the pro-tumorigenic functions of CAFs on the basis that co-culture or in vivo co-implantation of cancer cells with CAFs facilitated tumour growth[12,13,58,62]. For instance, CAFs co-injected into mice with human breast cancer cells promote tumour growth and angiogenesis more than normal fibroblasts through secretion of CXC-chemokine ligand 12 (CXCL12; also known as SDF1)[12]. However, much of the previous work failed to address the functions of CAFs from the viewpoint of complicated TME interactions. In the past 5 years, the development of sophisticated GEMMs that spontaneously develop cancer has enabled CAFs to be fully incorporated into the complex TME interactions (FIG. 1) and has shed light on novel tumour-inhibiting roles of CAFs. For example, specific depletion of αSMA$^+$ cells, including CAFs, led to the progression of PDAC in mice by inducing immunosuppression, implying that αSMA$^+$ cells include a subset of rCAFs, at least in this experimental model[9]. Although Hedgehog signalling was shown to promote PDAC progression in an initial short-term assessment[18], subsequent analysis revealed that long-term genetic

and pharmacological inhibition of the Hedgehog pathway and stromal desmoplasia unexpectedly accelerated PDAC growth[8]. The antitumorigenic role of the Hedgehog pathway was corroborated by the failure of clinical studies of Hedgehog inhibitors[32,63] and a further preclinical study that used three distinct mouse models of PDAC[64]. In agreement with these results, more recent work has shown that blockade of Hedgehog signalling accelerated cancer progression in colitis-associated colon cancer models[10,11], further supporting the notion that a subset of CAFs marked by GLI1, a transcriptional factor involved in the Hedgehog signalling pathway, represent a population of rCAFs[65].

CAFs are more than inert cells; they actively modulate their environment. Several CAF-derived proteins have been suggested to have tumour-inhibiting functions, but conflicting results have also been reported[35,36,60,66,67]. For instance, IκB kinase-β (IKKβ)-mediated nuclear factor-κB (NF-κB) activation in CAFs is responsible for inducing tumour-promoting inflammation in a mouse model of skin carcinogenesis[66]. Consistent with this finding, the genetic deletion of IKKβ in collagen type VI (COLVI)$^+$ fibroblasts resulted in reduced tumour growth and immune cell infiltration in a mouse model of colitis-associated cancer (CAC) via decreased IL-6 production by IKKβ-deficient CAFs[60]. However, genetic deletion of IKKβ in a larger population of COL1A2$^+$ fibroblasts in a similar CAC model unexpectedly accelerated tumour growth through enhanced hepatocyte growth factor (HGF) secretion[35].

Asporin, a CAF marker, has been suggested to promote the coordinated invasion of gastric cancer cells and CAFs through activation of RAC1 (REF.[67]). Asporin in breast cancer CAFs, however, exerts a tumour-restraining effect by inhibiting the TGFβ pathway and EMT of cancer cells, and high expression of asporin in human breast cancer stroma is associated with better clinical outcome in patients with breast cancer[36]. Taken together, these conflicting results underline a broad spectrum of CAF functions, with one molecule exerting pleiotropic effects in distinct CAF subpopulations. Thus, caution is warranted in generalizing CAF therapies, as context in terms of both native organ and tumour stage is probably critical.

Conversely, consistent evidence exists for the tumour-promoting function of FAP. Specific depletion of FAP$^+$ cells using transgenic ablation or targeting FAP$^+$ cells via chimeric antigen receptor (CAR) T cells inhibited tumour growth in a mouse model of PDAC by enhancing antitumour immunity and reducing desmoplasia and vascular density[21,68,69]. Indeed, FAP knockout impaired development of PDAC in KPC mice and subcutaneously injected colon cancer in mice[70,71], suggesting that FAP has a tumour-promoting function. In humans, elevated expression of FAP in the stroma of CRC and PDAC has been shown to correlate with poor patient prognosis[28,70]. In this regard, FAP could be a candidate marker for pCAFs.

[H2] Intratumoural heterogeneity

Analogous to phenotypic heterogeneity among cancer cells[72], CAF phenotypes are different not only between tumours (intertumoural heterogeneity) but also within tumours (intratumoural heterogeneity)[73]. Notably, Ohlund and colleagues identified two spatially and functionally distinct subtypes of CAFs in human and mouse PDAC: αSMA^{hi}IL-6^{low} myofibroblastic CAFs, which are marked by expression of myofibroblast genes and TGFβ-responsive genes such as *ACTA2*, *CTGF* (also known as *CCN2*) and *COL1A1* and are located adjacent to cancer cells; and αSMA^{low}IL-6^{hi} inflammatory CAFs, which secrete inflammatory cytokines and chemokines such as IL-6, IL-11, CXCL1 and LIF and are located distantly from cancer cells[50]. A subsequent study has revealed that TGFβ signalling and IL-1–JAK–STAT signalling are responsible for inducing differentiation of pancreatic stellate cells (PSCs), which serve as precursors for CAFs as they transition into myofibroblastic CAFs and inflammatory CAFs, respectively[74].

Currently, the heterogeneity of CAFs and their multifaceted roles remain to be fully elucidated. Further studies using single-cell RNA sequencing, translatable in vivo cancer models, discrete transgenic targeting and new stromal reagents, such as specific CAR T cell approaches, will provide novel insights into these different types of CAF heterogeneity.

[H1] Origin of CAFs

Although studies have begun to illustrate the heterogeneous nature of CAFs, little is known about the origins of CAFs. Different pathways probably exist for development of different CAF subpopulations. Fundamentally, cancer develops within an initially normal organ[2]. Depending on the stage of tumorigenesis, there will, at least in very early stages, exist some remnant native fibroblasts[4,5,61]. Thereafter, these cells are increasingly replaced by new CAFs that are different from native fibroblasts within normal tissue[4,5,61]. These new CAFs arise through one of several of the following processes (FIG. 2): transdifferentiation, in which CAFs can develop from a non-fibroblastic lineage such as epithelial cells[54,55], blood vessels[75] or serosa[76–78], with gene expression and biology changed to adopt a fibroblastic phenotype (FIG. 2 (step 1)); activation, in which altered gene expression and phenotype are induced within the existing resident fibroblasts in response to the TME (BOX 1; FIG. 2 (step 2)); recruitment, in which cells can arise from remote circulating populations, most often suggested to be bone marrow mesenchymal stem cells (MSCs)[14] (FIG. 2 (step 3)); and finally differentiation, in which CAFs might arise in a typical stem cell–progenitor cell hierarchy, as has been shown to occur within the periepithelial mesenchymal sheath of the mouse intestine[42] (FIG. 2 (step 4)). Elucidating the contribution of these four routes is vital for understanding the therapeutic challenges and opportunities to influence mesenchymal remodelling in cancer. The reality is that these pathways are not mutually exclusive and all might be operating in the development of CAFs. The key consideration is whether CAF ontogeny informs CAF biology, a question that requires further study.

Numerous publications show that CAFs originate from local fibroblasts, bone marrow MSCs or, depending on how they were experimentally defined, pericytes[4,45,79]. Interestingly, Arina et al. have shown using bone marrow transplantation, parabiosis and skin graft models that COL1A1$^+$ and αSMA$^+$ CAFs predominantly derive from local precursors and not bone marrow precursors[80]. Using traditional, transgenic lineage tracing experiments, others have shown that epithelial cells and endothelial cells differentiate to CAFs through EMT and endothelial-to-mesenchymal transition, respectively[54,55,75].

Studies using bone marrow transplantation have shown that MSCs (BOX 2) have a remarkable feature called tumour-specific tropism, in which they actively migrate to tumour sites[14,81,82]. In fact, bone marrow transplantation of αSMA-reporter MSCs revealed that at least 20% of CAFs arise from bone marrow-derived MSCs in a mouse model of inflammation-driven gastric carcinoma[14]. Notably, the presence of bone marrow-derived cells in tumour mesenchyme was confirmed in human gastric adenocarcinomas and rectal adenomas in patients who developed tumours following bone marrow transplantation[82]. The recruitment of MSCs to the TME is dependent on CXCL12, CXCL16, TGFβ, CC-chemokine ligand 2 (CCL2) and CCL5 secreted by cancer cells, inflammatory cells and CAFs[14,83,84]. In response to the soluble factors secreted from the TME, these recruited MSCs are converted to pCAFs expressing high levels of IL-6, WNT5A, bone morphogenetic protein 4 (BMP4) and CCL5 (REFS[14,83,85]). It is also conceivable that specific immature CAF subpopulations arising from MSCs could potentially exert a tumour-inhibitory effect. Indeed, it was indicated that high stromal expression of CD271 (also known as NGFR), a human bone marrow MSC marker, predicts a favourable prognosis in human PDAC[86]. In the fore-

seeable future, lineage tracing of MSC markers in the development of cancer will help elucidate the cellular origin and evolution of CAFs and could identify MSC or CAF markers of therapeutic value.

[H1] Function of CAFs

In this section, we discuss how CAFs functionally modulate cancer progression through interaction with other compartments in the TME.

[H2] Stromal and epithelial interactions

CAFs directly confer growth advantages to cancer cells via paracrine signalling, exosome transfer and physical interaction[4,45,87] (FIG. 3).

[H3] Paracrine signalling. Chemokines, cytokines and growth factors secreted by CAFs, such as CXCL12, HGF, epidermal growth factor (EGF), insulin-like growth factor (IGF), IL-6, IL-8 and IL-11, have an essential role in stimulating epithelial cell growth and maintaining cancer stem cells (CSCs)[12,88–100] (FIG. 3 (step 1)). For instance, CXCL12 produced by activated CAFs exacerbates breast cancer growth by binding to its cognate receptor, CXCR4, which is expressed in cancer cells[12]. Activation of the HGF receptor, MET, induces cancer stemness and chemoresistance in models of HCC and colon cancer[88–90] and upregulates keratin 19 expression in HCC, which is a predictor of poor patient survival[91]. It has been demonstrated that hepatic myofibroblasts and colon cancer CAFs secrete EGF family proteins and promote cancer progression through activation of ERBB receptors, including EGFR[92,93]. IGF2 secreted by CAFs maintains the stemness of cancer cells[94,95]. Additionally, IL-6 and IL-11 increase cancer cell proliferation and liver metastases, respectively, in models of colon cancer by augmenting STAT3 signalling[45,60,99]. Notably, $CD10^+GPR77$ (also known as $C5AR2)^+$ CAFs were identified as a novel subset of stemness-sustaining CAFs that provides IL-6 and IL-8 to maintain CSCs and promote chemoresistance[100].

The WNT pathway and BMP signalling have crucial roles in controlling intestinal stem cell fate in health and cancer[46]. HGF expressed by CAFs plays a vital role in maintaining colon CSCs by augmenting WNT signalling[89]. The expression of a ligand-sequestering BMP antagonist, gremlin 1, distinguishes intestinal reticular stem cells that give rise to the periepithelial mesenchymal sheath[42] and is upregulated in CAFs of human gastrointestinal cancers including oesophageal cancer, PDAC and CRC[101]. Interestingly, disruption of BMP morphogen gradients by aberrant epithelial gremlin 1 expression induces ectopic crypt formation and progressive intestinal polyps in a transgenic mouse model[102]. Furthermore, gremlin 1 knockout ameliorated tumorigenesis in the mutant APC mouse model of intestinal cancer[102].

[H3] Exosome transfer. Several studies offer evidence that bidirectional communication between CAFs and cancer cells is mediated in part by exosomes[4,103]. For instance, $TGFβ^+$ exosomes released by gastric cancer cells can convert MSCs to $αSMA^+$ activated CAFs[104]. Intriguingly, a similar secretion of exosomal miRNA-1247-3p by metastatic HCC cells results in activation of fibroblasts, which in turn secrete inflammatory cytokines such as IL-6 and IL-8 that promote lung metastasis of HCC[105]. Conversely, exosome transfer from CAFs to cancer cells confers a survival advantage to the cancer cells[106–108]. In this regard, in vitro experiments have shown that gemcitabine treatment increases the release of exosomes containing miRNA-146a and SNAIL from human PDAC CAFs, leading to increased cancer cell proliferation and chemoresistance[108].

Given that the classic concept that soluble factors secreted by CAFs promote cancer cell proliferation has already been well established, further research should concentrate on identification of candidate molecules that can be taken advantage of for cancer treatment. In view of the heterogeneity of CAFs, it is also vital to identify the CAF subpopulations that produce each soluble factor.

[H3] Physical interaction. In addition to the aforementioned biochemical crosstalk, direct physical interactions between CAFs and cancer cells play a critical part in cancer cell migration. Co-culture of CAFs and skin cancer cells has shown that leading CAFs can generate a track by ECM remodelling to facilitate the collective migration of cancer cells behind the CAFs[62,109]. CAFs can also directly lead the collective invasion of cancer cells by generating pulling forces on cancer cells through a mechanism mediated by N-cadherin and E-cadherin in the CAFs and cancer cells, respectively[87] (FIG. 3 (step 1)).

[H2] Tumour immunology

CAFs are major contributors to an immunosuppressive TME that might act as a restitution programme to help support epithelium in acute injury but promotes cancer growth in a tumour setting[4,110]. Mechanistically, cytokines and chemokines such as IL-6 and CCL2 produced by CAFs directly recruit immune cells and modulate both innate and adaptive immune systems[4]. Furthermore, CAFs impede trafficking of T cells indirectly by remodelling ECM, thereby suppressing antitumour immunity[111]. Several studies suggest that CAFs are one of the mediators of response to immune checkpoint inhibitors[6,15,21,57].

CAFs and tumour-associated MSCs produce chemokines such as CXCL1, CCL2, CCL5 and CXCL12 and induce recruitment of polymorphonuclear myeloid-derived suppressor cells (MDSCs), monocytic MDSCs and regulatory T cells (T_{reg} cells), all of which restrain tumour immunity and promote tumour progression[57,81,112–114] (FIG. 3 (step 2)). The expression of the granulocytic chemokine CXCL1 by CAFs is negatively regulated by crosstalk between cancer cells and CAFs through colony-stimulating factor 1 (CSF1)–CSF1 receptor signalling[113]. CSF1 receptor inhibitors have been used as antitumour-associated macrophage agents in clinical trials for solid tumours including gastrointestinal cancers; however, they have shown limited response[113,115]. This outcome was attributed to the ability of CAFs to neutralize the therapeutic effect of the agents by recruiting polymorphonuclear MDSCs to tumour sites and shaping an immunosuppressive TME[113].

Some CAFs appear to preferentially recruit $CD4^+CD25^+$ T cells by secreting CCL5 and CXCL12 and to increase their differentiation to tumour-promoting $CD25^{hi}FOXP3^{hi}$ T_{reg} cells by CXCL12 (REFS[57,114]), a finding consistent with the histological observation that $αSMA^+$ CAFs are in close proximity to $FOXP3^+$ T_{reg} cells[114]. Another example of the immunosuppressive roles of CAFs in immune cell recruitment is that activated PSCs, which are equivalent to activated CAFs in PDAC, sequester antitumour $CD8^+$ cytotoxic T cells and prevent their migration to pancreatic cancer cells[116]. However, this finding was challenged by a computational analysis of multiplex immunohistochemistry using human PDAC samples, which demonstrated that high or low cytotoxic T cell infiltration around the tumour cells was not associated with αSMA and COL1 expression levels[117]. These seemingly contradictory observations might be explained in part by the heterogeneity of CAFs.

In addition to recruiting immune cells, CAFs also modulate the immunosuppressive properties of these cells. For example, both CAFs and colonic myofibroblasts express programmed cell death 1 ligand 1 (PDL1), an immune checkpoint molecule that plays an essential role in inhibiting activation and proliferation of T cells through binding to programmed cell death 1 (PD1) on T cells[110,118,119]. Moreover, in vitro experiments indicate that CAFs induce an immunosuppressive TME by secreting CXCL12, IL-6 and IL-8 and promoting M2 polarization of macrophages[120,121] (FIG. 3 (step 2)). In the liver, granulin secretion by metastasis-associated macrophages converts quiescent hepatic stellate cells to periostin$^+$ pCAFs, resulting in increased metastatic tumour burden in a PDAC model[122]. Notably, CAFs are also one of the major producers of the immunosuppressive cytokine TGFβ in the cancer mesenchyme[15,110], and multiple studies have underscored the importance of TGFβ in shaping the immunosuppressive TME[15,123,124] (FIG. 3 (step 2)). The development of a novel mouse model that recapitulates the scarce T cell infiltration of human microsatellite-stable CRC has revealed that TGFβ inhibits the T helper 1 (T_H1) cell effector phenotype, thereby limiting benefit from anti-PD1–PDL1 therapy[15]. Notably, combination therapy with a TGFβ receptor inhibitor (galunisertib) and an anti-PDL1 antibody unleashed the cytotoxic immune response and eradicated most liver metastases in this model[15]. The combination therapy with galunisertib and an anti-PDL1 antibody is currently being assessed in a phase Ib clinical trial for metastatic pancreatic cancer[125].

Among a variety of CAF subtypes, the immunosuppressive role of FAP$^+$ CAFs has been investigated by multiple groups[21,57,69,112]. FAP$^+$ CAFs induce immunosuppression by secreting CXCL12 (REFS[21,57]). Specific depletion of FAP$^+$ cells in a mouse model of PDAC resulted in enhanced antitumour immunity, and the combination of the FAP$^+$ CAF depletion and immune checkpoint inhibitors (anti-cytotoxic T lymphocyte antigen 4 (CTLA4) or anti-PDL1) exerted a synergistic effect in reducing tumour volume[21]. Consistent with this finding, Costa et al.[57] have identified four different subsets of human breast cancer CAFs by fluorescent-activated cell sorting and found that the CAF-S1 subset, characterized by high FAP expression, is responsible for generating an immunosuppressive TME by recruiting CD4$^+$CD25$^+$ T cells and promoting their differentiation to T_{reg} cells.

Although there has been growing interest in cancer immunology, especially in regard to immune checkpoint inhibitors, we are only beginning to understand how CAFs participate in tumour immunosurveillance. Further CAF research is required to identify promising target molecules or CAF subpopulations and develop novel therapeutics that can improve clinical responses to current immunotherapies.

[H2] Angiogenesis

Neovascularization in cancer is regulated not only by tumour cells but also by stromal cells[126]. Indeed, CAFs promote tumour angiogenesis directly by secreting pro-angiogenic factors (FIG. 3 (step 3)) and indirectly by producing ECM[4,126]. Besides cancer cells, CAFs are a major source of vascular endothelial growth factor A (VEGFA), the most potent pro-angiogenic factor that promotes angiogenesis by acting on its cognate receptor, VEGF receptor 2 (VEGFR2), expressed on endothelial cells[126,127]. Moreover, CAFs induce angiogenesis by secreting several pro-angiogenic factors such as CXCL12, fibroblast growth factor 2 (FGF2) and PDGFC[12,128–130]. In turn, the leaky vasculature in tumours results in platelet extravasation and the subsequent degranulation of pro-angiogenic factors such as PDGF and TGFβ, which in turn activate fibroblasts[126,131]. In addition to paracrine signalling, CAFs also contribute to angiogenesis indirectly via remodelling ECM proteins such as periostin, tenascins, fibronectin, osteopontin and collagens[4,126,132].

Importantly, the crosstalk between CAFs and endothelial cells confers resistance to anti-VEGF therapy and chemotherapy[130,133,134]. PDGFC and FGF2 secreted by CAFs and bone marrow-derived COL1A1$^+$CXCR4$^+$ fibrocyte-like cells, respectively, are crucial mediators in the acquisition of resistance to anti-VEGF therapy in several mouse models of solid tumours[130,133]. Microfibrillar-associated protein 5 (MFAP5) has also been described as a novel CAF-derived pro-angiogenic factor that upregulates lipoma-preferred partner (LPP) in endothelial cells and confers resistance to chemotherapy by increasing microvessel leakiness[134]. Collectively, these studies provide a rationale for targeting CAFs as a potential therapeutic strategy to alter tumour vasculature and improve drug delivery to tumour cells.

[H2] Extracellular matrix remodelling

Activated CAFs are the main producers of ECM constituents such as collagen, fibronectin, proteoglycans, periostin and tenascin C, and the ECM is degraded primarily by CAF-derived MMPs[4,135] (FIG. 3 (step 4)). CAFs can exacerbate tumour progression indirectly by generating a mechanically stiff ECM[136]. A considerable amount of the literature suggests that ECM stiffness plays a central part in cancer progression[7,135,137,138]. Collagen crosslinking and increased ECM stiffness promote cancer cell proliferation, EMT, metastasis and resistance to chemotherapy[139–141]. Additionally, increased ECM stiffness leads to the generation of a dysregulated, leaky vasculature[142], and a dense ECM impedes migration of T cells to cancer cells[111]. One study has revealed that SPOCK1, a member of the SPARC family, which is predominantly expressed in the PDAC stroma, facilitates invasive pancreatic cancer cell growth by modifying collagen fibre patterns[143]. The ECM remodelling mediated by YAP activation in CAFs and collagen crosslinking by the lysyl oxidase (LOX) family substantially contribute to tissue stiffness[136,139,144,145]. In turn, matrix stiffness elevates YAP activity in CAFs, resulting in activation of CAFs and further matrix stiffening[136,144]. Notably, ECM stiffness is also crucial for inducing differentiation of MSCs to CAFs, which then support cancer cell proliferation[146]. Importantly, targeting the ECM has shown to improve the effectiveness of standard chemotherapy in KPC mice[19,20,147]. For instance, enzymatically depleting hyaluronic acid, an ECM component, in combination with gemcitabine significantly prolongs the median overall survival of KPC mice compared with gemcitabine monotherapy (91.5 days versus 55.5 days)[19] through improved drug delivery[19,20]. Combining a LOX-neutralizing antibody with gemcitabine was shown to reduce fibrillar collagen and increase tumour-free survival in KPC mice with early-stage tumours[147]. Further development of methods to manipulate the ECM and tissue stiffness will probably improve the therapeutic response to conventional chemotherapy.

[H1] CAFs and therapeutic resistance

Despite advances in chemotherapy, molecularly targeted drugs and immunotherapy, these treatments offer survival benefits only to a small group of patients[110,148]. Emerging evidence has demonstrated that CAFs confer substantial resistance to cancer therapeutics via impaired drug delivery and biochemical signalling[4,83]. The ECM produced by CAFs acts as a physical barrier to prevent the penetration of drugs by increasing interstitial fluid pressures and inducing vascular collapse[19]. One study using mass spectrometry revealed that pancreatic cancer CAFs entrap an active gemcitabine metabolite[149], demonstrating another mechanism to impair drug delivery to cancer cells. Important CAF-derived soluble factors that mediate resistance to chemotherapy include IL-6, IL-17A, IGF1, IGF2, nitric oxide and platinum-induced polyunsaturated fatty acids[150–154]. In regards to molecularly targeted drugs, HGF and IGF2 released by CAFs contribute to the resistance to tyrosine kinase inhibitors[90,95]. Indeed, dual inhibition of EGFR and MET or insulin and IGF1 re-

ceptors (IR or IGF1R) that mediate the IGF2–IR–IGF1R signalling axis enhanced the therapeutic response to an EGFR inhibitor in xenograft models of colon cancer and cholangiocarcinoma, respectively[90,95]. Interestingly, Wang and colleagues found that CAF-released cysteine and glutathione lead to reduced intracellular cisplatin content in ovarian cancer cells, conferring resistance to platinum-based chemotherapy[155]. Furthermore, CAFs influence the responsiveness to immune checkpoint inhibitors by shaping the immunosuppressive TME, as discussed above. Remarkably, a high compound stromal score defined by three stromal components (CAFs, leukocytes and endothelial cells) can predict resistance to radiotherapy in patients with rectal cancer[31]. In oesophageal squamous cell carcinoma, the expression of the long non-coding RNA DNM3OS in cancer cells is increased by CAFs through PDGFβ–PDGFRβ–FOXO1 signalling, leading to radioresistance by regulating DNA damage response[156]. The radioresistance is attributed, in part, to an altered interaction between cancer cells and CAFs following radiation[157]. Indeed, radiation induces CRC CAFs to secrete IGF1, thereby supporting cancer cell growth[158].

[H1] Clinical implementation

[H2] Biomarkers

In addition to the functional contribution of CAFs to cancer progression described above, CAFs and their gene expression patterns have diagnostic and prognostic value in clinical oncology[159]. Surprisingly, the presence of circulating FAP^+ CAFs was confirmed in peripheral blood of patients with cancer, including those with metastatic CRC[160]. Furthermore, increased levels of circulating stroma-related molecules such as MMP7 and connective tissue growth factor (CTGF) can help discriminate patients with PDAC from healthy individuals or patients with chronic pancreatitis when combined with CA19.9, a commonly used PDAC biomarker[161]. Gene expression analyses and proteome profiling of cancer tissues have revealed that stromal gene signatures predict poor patient outcome in multiple types of gastrointestinal cancer[25–31,162,163]. In particular, it has been shown that elevated stromal expression of TGFβ-related genes is associated with poor prognosis in CRC[28,99]. In support of this finding, histological observations revealed that high expression of αSMA or high stromal proportion are predictive of poor clinical outcome in patients with CRC, PDAC and HCC[164–167]. Because CAFs accumulate at the tumour site at an early stage of tumorigenesis[4], future investigations will probably identify valuable CAF markers that might facilitate early detection of cancer.

[H2] CAF-targeting therapy

In the past 5 years, there has been considerable interest in therapeutic strategies to target CAFs, and numerous clinical trials for gastrointestinal cancers are ongoing to assess their benefits[7,45]. Elimination of FAP^+ pCAFs by CAR T cells or vaccination has been shown to inhibit tumour progression in several different mouse models of cancers including PDAC and CRC[68,168] (FIG. 4). CAF reprogramming by vitamin D and vitamin A, which revert pCAFs to rCAFs, has attracted much attention in the fields of PDAC and colon cancer[169–172]. Administration of a vitamin D analogue inhibits tumour-promoting signalling in activated PSCs, resulting in substantially improved therapeutic efficacy of gemcitabine in KPC mice[169]. A phase II clinical trial of concomitant treatment with a PD1 inhibitor and a vitamin D analogue in PDAC is now underway[173]. FAP has been used in a xenograft model of PDAC and in clinical trials for solid tumours to target drug delivery specifically to tumour sites using antibody–drug conjugates or immunocytokines, in which, for example, a cytotoxic drug (maytansinoid DM1, a tubulin inhibitor) or an IL-2 variant is conjugated with FAP antibodies[174–176]. A highly anticipated approach, which utilizes the tumour-specific tropism of MSCs, is the administration of MSCs that are engineered either to express enzymes that metabolize pro-drugs to active drugs or to secret tumour-inhibitory molecules such as TRAIL and IFNα[83,177–180]. Acceptable safety and tolerability have been reported in the first phase I clinical trial for gastrointestinal cancers using autologous MSCs genetically engineered to express herpes simplex virus–thymidine kinase, which converts the prodrug ganciclovir into its active cytotoxic metabolite[181]. Investigators are also using TGFβ inhibitors or Hedgehog inhibitors in combination with standard chemotherapies or immunotherapies in an attempt to block pro-tumorigenic signalling relevant to CAFs in gastrointestinal cancers[7,15,45].

[H1] Conclusions

CAFs are important in the development of gastrointestinal cancers, both in their promotion and, as we increasingly appreciate, in their antagonism. CAFs are not one entity but rather contain heterogeneous functional subpopulations including pCAFs, rCAFs and probably also a neutral subset that neither promotes nor retards (nCAFs). CAF biology is mediated through the direct and paracrine interactions of CAFs with both cellular (tumour cells, immune cells and vascular cells) and acellular (ECM) compartments. Despite the importance of CAFs, we are still in the infancy of CAF-directed approaches to cancer care. To help accelerate the integration of CAF science into CAF clinical care, we encourage future work in this field to precisely define the CAF population being studied through careful characterization including, where appropriate, immunophenotyping, multiplex immunofluorescence, discrete transgenic markers and single-cell transcriptional analysis and to combine these characteristics with CAF biology. We offer the biological nomenclature of pCAF, rCAF and nCAF but expect these terms, as in the case of lymphocyte and myeloid cellular classifications, to be replaced by a precise, biologically rooted and clinically translatable immunophenotypic classification. Furthermore, future studies should carefully consider the tumour context of their experimental models. We speculate that CAFs have considerable plasticity in terms of their function and marker expression, analogous to other important cell populations including CSCs[72]. The interconversion of CSCs and the inherent difficulties in reproducing specific CSC markers have hindered progress in this area of cancer research[72]. We are encountering similar obstacles in CAF research. Future genetic fate-mapping of CAFs, more widely accepted CAF subclassifications and a better understanding of the context-specific behaviour of CAFs will offer novel insights into the heterogeneity, hierarchy and plasticity of CAFs. These scientific discoveries will help drive the more rapid translation of CAF basic science into CAF clinical practice and the development of new diagnostics, prognostics, preventives and therapeutics for patients with gastrointestinal cancer.

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Author contributions

All authors contributed substantially to discussion of content for the article and reviewed and/or edited the manuscript before submission. H.K., A.E. and D.L.W. researched data and wrote the manuscript.

Competing interests

The authors declare no competing interests.

Key points

- Cancer-associated fibroblasts (CAFs) include all fibroblasts in the tumour and are involved in functionally controlling cancer progression.
- CAFs are composed of heterogeneous subpopulations arising from distinct cellular origins such as local fibroblasts and mesenchymal stem cells.
- Distinct CAFs influence cancer cell proliferation, tumour immunity, angiogenesis, extracellular matrix remodelling and metastasis.
- Functionally, CAFs can be classified into subpopulations such as tumour-promoting CAFs and tumour-retarding CAFs.
- An improved understanding of CAF biology could lead to the development of novel stroma-based diagnostics, prognostics and therapeutics.

Box 1 | Fibroblast activation

Tissue-resident fibroblasts in health are activated in response to a plethora of stimuli from the tumour microenvironment (TME). Transforming growth factor-β (TGFβ), platelet-derived growth factor (PDGF), sonic hedgehog (SHH), bone morphogenetic protein (BMP), IL-1, IL-6, TNF and reactive oxygen species are important biochemical activators of fibroblasts[4,66,182,183]. Notably, the *KRAS*^{G12D} mutation, one of the most common driver mutations in pancreatic ductal adenocarcinoma (PDAC), induces SHH secretion by pancreatic cancer cells, thereby activating pancreatic stellate cells, which are fibroblast-like cells in the pancreas, and supporting cancer cell growth[183]. The co-evolution of cancer-associated fibroblasts (CAFs) with cancer cells seems to be analogous to the developmental biological crosstalk between mesenchymal cells and epithelial cells. Indeed, soluble factors necessary for CAF activation, such as Hedgehog and BMP, have a critical role in defining the epithelial and stromal niche in developmental gastrointestinal organs[44,65,184]. Mechanical stiffness generates a positive feedback loop for CAF activation[136]. Activated CAFs express α-smooth muscle actin (αSMA), a cytoskeleton molecule required for cell contraction, and are therefore regarded as myofibroblasts[4,47]. Activated CAFs acquire a highly contractile, extracellular matrix (ECM)-synthesizing, proliferative and secretory phenotype and are epigenetically and metabolically distinct from quiescent fibroblasts[4,185,186]. Functionally, fibroblasts initially facilitate wound healing, a biological response beneficial for tissue regeneration[4]. However, chronic activation of fibroblasts leads to organ fibrosis[4], which is deleterious for maintaining organ function, as exemplified by liver cirrhosis. In this regard, perpetually activated CAFs might play a key part in fostering cancer progression. In contrast to the mechanism by which activated CAFs promote tumour progression, how quiescent CAFs restrain the development of cancer is largely unknown. Importantly, CAFs with different degrees of activation (for example, quiescent CAFs and activated CAFs) exist in the TME, contributing to the heterogeneity of CAFs[45,48].

Box 2 | Mesenchymal stem cells and their markers

Mesenchymal stem cells (MSCs) have stellate morphology and represent a rare subset of stromal cells, which are localized mainly in the bone marrow, adipose tissue and umbilical cord[4,187]. Importantly, MSCs or MSC-like cells are also observed in the perivascular regions of many organs, implicating their potential overlap with pericytes classically labelled by neuron-glial antigen 2 (NG2) and platelet-derived growth factor receptor-β (PDGFRβ)[188,189]. MSCs play a vital part in the maintenance of haematopoietic stem cells in the bone marrow, and interest has been growing in how MSCs maintain epithelial stem cell niches in other tissues[190]. MSCs are developmentally derived mainly from embryonic mesoderm[187] and demonstrate a self-renewal capability and a capacity to differentiate into osteocytes, adipocytes and chondrocytes[191]. Numerous studies have shown that MSCs give rise to α-smooth muscle actin $(αSMA)^{+}$ myofibroblasts in fibrosis in various organs such as the liver, heart and lung[187,192,193]. Notably, it has been shown that administration of MSCs is a promising therapeutic strategy for acute graft-versus-host disease, intestinal ulcers and inflammatory bowel diseases owing to the immunomodulatory and pro-angiogenic properties of MSCs[194,195].

Fig. 1 | **Cellular components of the tumour microenvironment.** Fibroblasts are a vital component of the tumour microenvironment (TME). The TME comprises cancer-associated fibroblasts (CAFs), cancer cells, normal epithelial cells, endothelial cells, pericytes, mesenchymal stem cells (MSCs), extracellular

matrix (ECM), the basement membrane and inflammatory cells such as T cells, natural killer (NK) cells, macrophages and myeloid-derived suppressor cells (MDSCs). CAFs are a highly heterogeneous population and include quiescent CAFs and activated CAFs. RBC, red blood cell; T_{reg} cell, regulatory T cell.

Fig. 2 | **The origins of CAFs.** Cancer-associated fibroblasts (CAFs) are considered to arise through four non-mutually exclusive mechanisms. Non-fibroblast lineage cells such as epithelial cells and endothelial cells become a part of the CAF population through transdifferentiation (1). Local fibroblasts acquire CAF phenotypes that are distinct from a normal fibroblast phenotype via activation (BOX 1) (2). Mesenchymal precursor cells, typically bone marrow mesenchymal stem cells (MSCs), are recruited to the tumour by cytokines and chemokines secreted from the tumour microenvironment, such as transforming growth factor-β (TGFβ) and CXC-chemokine ligand 12 (CXCL12) (3)[4,45]. It is also conceivable that a CAF stem cell exists (4). In this scenario, a minor subpopulation of CAF progenitors in a hierarchical organization could have self-renewal capacity and could also give rise to progeny CAFs, such as cancer-promoting CAFs (pCAFs) and cancer-retarding CAFs (rCAFs). Some of the CAF stem cells probably overlap with subpopulations of MSCs, the lineage of which is committed to CAFs during cancer progression. We speculate that CAFs from different cellular origins have functionally distinct phenotypes; however, the relationship between the CAF subtypes and their cellular origins is not fully elucidated. EMT, epithelial-to-mesenchymal transition; EndMT, endothelial-to-mesenchymal transition.

Fig. 3 | **The functions of CAFs.** Cancer-associated fibroblasts (CAFs) orchestrate the development of cancer. CAFs promote cancer cell proliferation by secreting a plethora of pro-tumorigenic factors. Transfer of proteins and RNAs is mediated in part by exosomes. CAFs also physically pull cancer cells to guide collective cancer cell migration (1). CAFs secrete numerous chemokines and cytokines such as CXC-chemokine ligand 12 (CXCL12) and transforming growth factor-β (TGFβ), thereby inducing immunosuppression in the tumour microenvironment (TME). Of note, CAFs express programmed cell death 1 ligand 1 (PDL1), a target protein for immune checkpoint inhibitors (2). Vascular endothelial growth factor A (VEGFA), CXCL12, fibroblast growth factor 2 (FGF2) and platelet-derived growth factor C (PDGFC) produced by CAFs facilitate the formation of new blood vessels in the TME (3). CAFs synthesize extracellular matrix (ECM) components such as collagen and fibronectin, and the ECM is degraded by matrix metalloproteinases (MMPs) secreted by CAFs. Collagen cross-linking is mediated by members of the lysyl oxidase (LOX) family produced, in part, by CAFs[139,196]. CAFs contribute to increased ECM stiffness, which in turn promotes cancer progression, for example, by increasing cancer cell proliferation and invasion[135,136,139] (4). BMP, bone morphogenetic protein; CCL, CC-chemokine ligand; EGF, epidermal growth factor; HGF, hepatocyte growth factor; IGF, insulin-like growth factor; MDSC, myeloid-derived suppressor cell; RBC, red blood cell; T_{reg} cell, regulatory T cell.

Fig. 4 | **Therapies that target CAFs.** Cancer-associated fibroblasts (CAFs) and mesenchymal stem cells (MSCs) present multiple therapeutic approaches for cancer treatment. Fibroblast activation protein $(FAP)^+$ cancer-promoting CAFs (pCAFs) can be eliminated by chimeric antigen receptor (CAR) T cells targeting FAP. A DNA vaccine against FAP can also induce $CD8^+$ T cell-mediated killing of pCAFs (1). A reprogramming therapy such as vitamin D and vitamin A can be used to dedifferentiate pCAFs to cancer-retarding CAFs (rCAFs) (2). Further studies are necessary to identify molecules that can effectively reprogramme CAFs. An antibody–drug conjugate or immunocytokine against FAP, a membrane marker expressed in CAFs, enables effective delivery of drugs to tumour sites (3). Administration of MSCs engineered to express antitumorigenic molecules such as TRAIL, IFNα and herpes simplex virus–thymidine kinase (HSV–TK) leads to the accumulation of MSCs in the tumour site, thereby inducing cancer cell death (4). Blocking the biochemical interaction between cancer cells and CAFs using transforming growth factor-β (TGFβ) inhibitors or Hedgehog inhibitors might prevent cancer progression (5). Manipulation of the extracellular matrix (ECM), which in the TME is produced predominantly by CAFs, leads to improved drug delivery (6). HA, hyaluronic acid; LOX, lysyl oxidase.

Table 1 | Some representative CAF markers in cancer and homeostasis

CAF marker	Fibroblast type	Description	Expression pattern (excluding fibroblasts)	Refs
Membrane proteins				
FAP	Activated fibroblasts	Serine protease	Macrophages	51,52
PDGFRβ (CD140β)	Activated fibroblasts	Growth factor receptor	Pericytes and cancer cells	4,79,197
Podoplanin (GP38 in mice)	Activated fibroblasts	Transmembrane glycoprotein	LECs and cancer cells	198,199
PDGFRα (CD140α)	Quiescent fibroblasts	Growth factor receptor	BM-MSCs and cancer cells	66,197,200–202
Intracellular proteins				
αSMA	Activated fibroblasts	Cytoskeletal protein crucial for cell contraction	SMCs and pericytes	4,45
Desmin	Activated fibroblasts	Intermediate filament	Skeletal muscle cells, SMCs and pericytes	4,45
Vimentin	Activated fibroblasts	Intermediate filament	SMCs, endothelial cells, neural cells and cancer cell	4,5,45,203
FSP1 (S100A4)	Quiescent fibroblasts	Calcium-binding protein	Cancer cells and macrophages	48,54–56
GLI1	Miscellaneous	Transcription factor in Hedgehog signalling	Perivascular fibroblasts, BM-MSCs and cancer cells	8,18,32,63,192,204
Secreted proteins				
CXCL12	Activated fibroblasts	Chemokine	BM-MSCs, BECs, osteoblasts and haematopoietic cells	12,205
Gremlin 1	Activated fibroblasts	BMP antagonist and VEGFR2 agonist	BM-MSCs and iRSCs	42,101,102,206

ECM proteins				
COL1A1	Activated fibroblasts	Component of collagen type I	Osteoblasts and tendon	190,207
Periostin	Activated fibroblasts	Matricellular protein	Periosteum, osteoblasts and tendon	208,209

αSMA, α-smooth muscle actin; BEC, blood endothelial cell; BM-MSC, bone marrow mesenchymal stem cell; BMP, bone morphogenetic protein; CAF, cancer-associated fibroblast; COL1A1, collagen type I α1; CXCL12, CXC-chemokine ligand 12; ECM, extracellular matrix; FAP, fibroblast activation protein; FSP1, fibroblast-specific protein 1; GLI1, glioma-associated oncogene homologue 1; iRSC, intestinal reticular stem cell; LEC, lymphatic endothelial cell; PDGFR, platelet-derived growth factor receptor; SMC, smooth muscle cell; VEGFR2, vascular endothelial growth factor receptor 2.

Glossary

Extracellular matrix
(ECM). An intricate network of fibrous proteins in the extracellular space, such as collagen, laminin and fibronectin.

Basement membrane
A highly specialized extracellular matrix that separates epithelial cells or endothelial cells from underlying connective tissue.

Desmoplastic reaction
An increase in a stromal component especially with prominent fibrous tissue in cancer.

Angiogenesis
The formation of new blood vessels to satisfy increased demand for nutrients and oxygen.

Gene signatures
Patterns of gene expression that are characteristic of a certain biological process.

Telocytes
Mesenchymal cells that have extending cytoplasmic processes termed telopodes.

Tumour-associated macrophages
A heterogeneous population of macrophages in the tumour that contribute to tumour progression.

Epithelial-to-mesenchymal transition
(EMT). A process by which epithelial cells gain a mesenchymal phenotype, leading to their migration and invasion.

Myofibroblasts
A specific type of fibroblast that is characterized by high expression of α-smooth muscle actin.

Single-cell RNA sequencing
Gene expression analysis of an individual cell instead of diverse cell populations.

Chimeric antigen receptor (CAR) T cells
T cells engineered to recognize a tumour-associated antigen and induce target-specific killing.

Stellate cells
Fibroblast-like cells characterized by their vitamin A storage. They are found in the pancreas and liver.

Pericytes
Fibroblast-like cells that wrap around the wall of capillaries.

Parabiosis
Two organisms joined together surgically to share blood circulation.

Lineage tracing
A method to genetically label cells of interest and all of their progenies, also known as genetic fate-mapping.

Endothelial-to-mesenchymal transition
A process by which endothelial cells lose their endothelial phenotype and acquire a mesenchymal phenotype.

Exosome
An extracellular vesicle (30–150 nm in size) that is released from many types of cells and contains proteins and RNAs.

Cancer stem cells
(CSCs). A minor subpopulation of cancer cells that have self-renewal capability and drive cancer progression, metastasis and resistance to treatment.

Immunosuppressive TME
A tumour microenvironment (TME) in which antitumour immunity is inhibited and cancer immunotherapy is ineffective.

Immune checkpoint inhibitors
Agents that unleash antitumour immunity through blocking an immune checkpoint, which is a ligand–receptor-mediated pathway to suppress an immune response.

Myeloid-derived suppressor cells
(MDSCs). A heterogeneous population of bone marrow-derived immune cells that suppress T cell activity.

Regulatory T cells
(T_{reg} cells). A subset of immunosuppressive T cells that express CD4, CD25 and FOXP3, maintain immune tolerance to self-antigens and prevent activation of effector T cells.

M2 polarization of macrophages

The M2 macrophage is a subtype of tumour-associated macrophage that suppresses antitumour immunity and promotes cancer progression.

Extravasation
Leakage of blood cells from capillaries to the surrounding tissue.

Fibrocyte
A bone marrow-derived circulating cell that has features of both fibroblasts and monocytes.

Antibody–drug conjugates
Constructs that contain a small molecule drug linked to a monoclonal antibody that recognizes a tumour-associated antigen.

Immunocytokines
Cytokines that are fused to monoclonal antibodies that recognizes a tumour-associated antigen.