A framework for advancing our understanding of cancer-associated fibroblasts

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Abstract | Cancer-associated fibroblasts (CAFs) are a key component of the tumour microenvironment with diverse functions, including matrix deposition and remodelling, extensive reciprocal signalling interactions with cancer cells and crosstalk with infiltrating leukocytes. As such, they are a potential target for optimizing therapeutic strategies against cancer. However, many challenges are present in ongoing attempts to modulate CAFs for therapeutic benefit. These include limitations in our understanding of the origin of CAFs and heterogeneity in CAF function, with it being desirable to retain some antitumorigenic functions. On the basis of a meeting of experts in the field of CAF biology, we summarize in this Consensus Statement our current knowledge and present a framework for advancing our understanding of this critical cell type within the tumour microenvironment.

Extracellular matrix (ECM). The structural network of secreted proteins and glycosaminoglycans that provides structure to tissue.

Angiogenesis The formation of new blood vessels.

Mesenchyme A type of tissue composed of loosely associated cells surrounded by extracellular matrix.

Mesoderm One of three fundamental layers of tissue formed early in development and the predominant source of fibroblastic lineages.

Cancer arises from mutations accruing within cancer cells, but both disease progression and responses to therapy are strongly modulated by non-mutant cells within the tumour microenvironment. The past few years have witnessed a great expansion in research into cancer-associated fibroblasts (CAFs). These cells modulate cancer metastasis through synthesis and remodelling of the extracellular matrix (ECM) and production of growth factors, and influence angiogenesis, tumour mechanics, drug access and therapy responses. More recently, there has been a growing appreciation of the ability of CAFs to modulate the immune system. Targeting CAFs, by altering their numbers, subtype or functionality, is being explored as an avenue to improve cancer therapies. However, research in this area faces numerous challenges — not least because CAFs can have both protumorigenic and antitumorigenic effects. This Consensus Statement follows a recent Banbury Center meeting at Cold Spring Harbor Laboratory (New York, USA) held in March 2019, which focused on CAF biology and therapeutic opportunities and included an open discussion to identify the challenges facing CAF research and suggest ways forward (BOX 1). On the basis of this, we, as an international group of cancer researchers and clinician scientists, herein present the current state of CAF research, summarize the challenges ahead and present both methodological advice and conceptual suggestions to provide the necessary framework to advance the field.

What is a fibroblast? The definition of a fibroblast is surprisingly tricky1,2. The embryonic origin of most fibroblasts is from the primitive mesenchyme that develops out of the mesoderm following gastrulation1, with a smaller subset of fibroblasts also derived from the neural crest, which is part of the ectoderm1. This embryonic origin is shared by other mesenchymal lineages, including adipocytes, chondrocytes and osteoblasts. The difficulty in defining fibroblasts results largely from the lack of unique markers that are not expressed in any other cell types. The result is that in practical terms, fibroblasts are often defined by a combination of their morphology, tissue position and lack of lineage markers for epithelial cells, endothelial cells and leukocytes. Vimentin and platelet-derived growth factor receptor-a (PDGFRa) are sometimes used but typically
Neural crest
Migratory mesenchymal cells derived from the neural tube and originally the ectoderm.

Adipocytes
Mesenchymal cells specialized for the storage of fat.

Pericytes
Mesenchymal cells that are located adjacent to smaller blood vessels and that support their function.

alongside other criteria such as cell shape and location. Markers for fibroblast subtypes also exist, including α-smooth muscle actin (αSMA; also known as ACTA2) and fibroblast activation protein (FAP)5,6, with the subset of fibroblasts expressing the latter playing roles in bone and fat homeostasis. Recent work is beginning to trace the lineage of fibroblasts from the earliest stage of mesenchyme specification through to the adult. This has already highlighted distinct subsets of dermal fibroblasts and is starting to provide more precise combinations of markers with which to define fibroblasts7,8. However, the links between lineage commitment in early development and the fibroblast subsets found in the adult remain, for the most part, to be determined.

In normal development and physiology, fibroblasts are the major producers of connective tissue ECM, with emerging data indicating that this function is modified with age9,10. They also play a key role in tissue repair and become activated following tissue damage11. During wound healing they can produce transforming growth factor-β (TGFβ) and acquire a highly contractile phenotype associated with the expression of αSMA12. In this state, fibroblasts are termed ‘myofibroblasts’. Both in normal homeostasis and following injury they participate in crosstalk with adjacent epithelia, with numerous studies documenting an ability to influence local epithelial stem cell behaviour13,14. They can also promote angiogenesis via the production of vascular endothelial growth factor A (VEGFA)15 and coordinate the function of the immune system via the production of chemokines and cytokines, although it should be noted that there is heterogeneity in the cytokines produced by different fibroblast subsets16–18. Fibroblasts also play a structural role within the immune system; fibroblastic reticular cells (FRCs) within lymph nodes generate ECM conduits for the transit of potential antigens and serve as migration ‘highways’ for leukocytes19. This allows effective immune surveillance. In addition, they promote immune tolerance by the expression and presentation of normally tissue-restricted antigens20. Emerging work is revealing complex crosstalk between fibroblastic cells and epithelial cells in exocrine organs. For example, stellate cells are a distinctive type of fibroblast found in the liver and pancreas that store lipid droplets and particular derivatives of retinoic acid. The balance between quiescence and activation of stellate cells is regulated by the vitamin D receptor, deletion of which leads to spontaneous liver and pancreas fibrosis21, with further work indicating that stellate cells play a broader role in metabolic homeostasis22–24. Thus, fibroblasts are not simply producers of ECM but play key roles in communicating with many other cell types during both normal tissue homeostasis and repair.

What is a CAF?
To first consider how CAFs are generated, it is important to try to define CAFs. Many of the same issues arising for normal fibroblasts also apply to CAFs. When analysing tissue biopsy samples, the simplest view is that cells negative for epithelial, endothelial and leukocyte markers with an elongated morphology and lacking the mutations found within cancer cells might be considered CAFs. The latter point is important because it excludes cancer cells that have undergone a profound epithelial to mesenchymal transition (EMT), although such cells are likely to be of considerable importance and warrant studying in their own right. In practice, lineage exclusion is typically combined with positivity for a mesenchymal marker, often vimentin; however, this may not be sufficient to exclude other mesenchymal lineages such as pericytes or adipocytes. Early experimental studies indicated that such cells cultured from tumours have distinctive properties compared with normal fibroblasts25. In practice, any mesenchymal cell cultured from a tumour that complies with the criteria described above is considered a CAF. Nevertheless, as discussed in the section entitled ‘Challenges and recommendations,’ how durable CAF subsets and phenotypes remain once fibroblasts are isolated and cultured warrants further investigation.
What is the origin of CAFs?
The lack of precision around fibroblast-specific markers poses a challenge when one is considering the origin of CAFs. When the markers of both normal tissue-resident fibroblasts and CAFs are ill-defined, it becomes very hard to propose hypotheses regarding the precise cell of origin of CAFs. To partially circumvent this problem, many studies have documented the changes in the fibroblastic component of carcinomas as they progress from hyperplasia, through adenoma or in situ lesion, to frank carcinoma in patients.

These studies using human tissue observe progressive changes in the fibroblastic stroma. In many cases, the initial apparent expansion of fibroblasts precedes the conversion to malignancy, and fibroblasts are often observed circumscribing early or premalignant lesions. The gradual nature of the transitions observed has given rise to the view that the majority of stromal fibroblasts initially originate from local fibroblasts that experience some form of tissue dysfunction—perhaps due to changes in the microenvironment. This process has been termed ‘stromagenesis’, with the implication that it proceeds alongside and is coupled to tumorigenesis. Furthermore, experimental studies and observations of early lesions encircled by fibroblasts support the idea that the initial fibroblast response can be tumour suppressive, with subsequent events in stromagenesis generating protumorigenic fibroblasts.

To shed more light on the origin of CAFs, many researchers have turned to mouse models in which cells can be irreversibly labelled using transgenic techniques and well-characterized models of disease progression are available. These typically use tissue-restricted expression of the Cre recombinase in mice that also contain a reporter gene that becomes irreversibly active in cells that express Cre. Importantly, the active reporter will be inherited by all daughter cells and will continue to be expressed even if the Cre recombinase is not. However, the lack of fibroblast-restricted markers causes problems when one is selecting a promoter to drive the expression of the Cre recombinase. This is exemplified by the divergent phenotypes observed in a colitis model of stromal knockout of Ikkb (inhibitor of nuclear factor-kB (NF-kB) kinase subunit-β) depending on whether a collagen type 1 α2 chain (Col1a2) or collagen type V α1 chain (Col5a1) Cre driver was used. The most widely used fibroblast drivers and their caveats are detailed in the section entitled ‘Challenges and recommendations’. This approach can also be used to explore hypotheses including the conversion of adipocytes, pericytes, endothelial cells and bone marrow-derived mesenchymal stem cells (MSCs) into CAFs. The injection of bone marrow-derived MSCs into tumour-bearing mice has demonstrated that they can become CAFs, with more recent studies supporting the MSC origin of PDGFRα+ CAFs. Adipocyte conversion into CAFs has been reported by several groups, although it does not appear to be a universally applicable phenomenon across different tumour types.

How are CAFs generated?
The studies described in the previous section aimed to document which cells give rise to CAFs but do not provide a mechanism for their conversion. Given the prominent role fibroblasts play in coordinating the wound repair response in skin, it is plausible that key CAF traits correspond to the normal physiological role fibroblasts play. Well-established activating signals for fibroblasts include TGFβ family ligands and the lipid mediator...
lysophosphatidic acid\(^{[52,54]}\), which promote the activity of the SMAD transcription factors and serum response factor (SRF), respectively, and converge to drive expression of the activated fibroblast marker αSMA as well as increase the activity of the contractile cytoskeleton\(^5\) (Fig. 1). Contact between cancer cells and fibroblasts can promote the CAF phenotype in breast cancer through Notch signalling\(^6\); however, this mechanism is unlikely to be universal as loss of Notch signalling can promote CAF phenotypes in squamous cell carcinoma\(^7\). Various inflammatory modulators can promote CAF activation, with interleukin-1 (IL-1) acting through NF-kB and IL-6 acting primarily on signal transducer and activator of transcription (STAT) transcription factors\(^8,9\). Crosstalk and positive feedback involving Janus kinase (JAK)–STAT signalling, the contractile cytoskeleton and alterations in histone acetylation further promote CAF activation\(^10,11\). Physical changes in the ECM are also capable of activating CAFs\(^12,13,14\). In vitro studies have shown that fibroblast stretching, which may result from the hyperproliferation of transformed epithelial cells, can activate SRF-driven transcription and Yes-associated protein 1 (YAP1)–TEAD-driven transcription\(^15,16,17\). These transcription factors cooperate to drive the expression of a wide range of genes associated with CAFs, including the genes encoding connective tissue growth factor (CTGF; also known as CCN2) and cysteine-rich angiogenic inducer 61 (Cyr61; also known as CCN1)\(^18\). Furthermore, matricellular molecules, such as CTGF and Cyr61, and the contractile cytoskeleton cooperate to increase tissue stiffness, which further drives SRF-dependent and YAP1-dependent transcriptional programmes, locking CAFs into a self-sustaining positive-feedback loop\(^19\). Physiological stress is also another factor contributing to stromagenesis. Heat shock factor 1 (HSF1), which responds in part to protein misfolding, is required for the generation of CAFs\(^20,21\). Physiological and genomic stresses can also trigger changes in fibroblasts. Double-stranded DNA breaks can promote the production of IL-6 and the TGFβ family ligand activin A\(^22,23\). In some cases, these triggers cause fibroblasts to enter a non-proliferative state termed ‘senescence’\(^24\), which is distinct from the phenotype of an aged fibroblast. There is clear overlap between the secretome of senescent fibroblasts and CAFs, with high levels of IL-6 production being common to both, and senescent fibroblasts have been found in the microenvironment of some tumours\(^25\). The non-proliferative nature of senescent cells makes it unlikely that they are a major contributor to the abundant stromal fibroblasts observed in desmoplastic tumours\(^26\). Nonetheless, it remains possible that CAFs and senescent fibroblasts share some transcriptional regulatory mechanisms\(^27,28\). Furthermore, even if senescent fibroblasts are a minor component of the tumour microenvironment, experimental analysis suggests that their elimination can have substantial consequences for disease relapse\(^29\).

In addition to considering tumour cells as the direct source of cues that generate CAFs, signals from other cells within the tumour microenvironment may also instruct CAF function; for example, granulin produced by macrophages promotes the activation of a fibrotic environment in liver metastases\(^30,31\). Such mechanisms that do not directly depend on the presence of cancer may contribute to the protumorigenic environments found in inflammatory conditions that are linked to increased cancer risk. In addition, cancer therapies, including conventional chemotherapies, radiotherapy and targeted agents, can promote the generation of CAFs and modulate their functionality. These changes can aid the development of therapy resistance\(^32–36\) and contribute to undesirable side effects\(^37\). Being able to mitigate these events is another potential appeal of CAF-directed therapies.

**The expanding range of CAF functions**

The functions of CAFs have been determined using a variety of strategies, ranging from reductionist cell culture experiments and mouse models to correlative associations in large patient cohorts. These approaches have revealed a diverse array of functions (Fig. 2). The relative ease of culturing CAFs and matched normal fibroblasts from patient material has greatly facilitated mechanistic delineation of CAF functions. CAFs are perhaps the most effective cell within the tumour microenvironment at depositing and remodelling the ECM. This depends on RHO and RAB GTPase-mediated control of integrin-mediated adhesions and the actomyosin cytoskeleton\(^38–40\) and is linked to down-regulation of the transmembrane receptor CD36 (also known as platelet glycoprotein 4)\(^41\). CAFs also produce matrix-crosslinking enzymes and, together with force-mediated ECM remodelling (reviewed in detail\(^42,43\)), these contribute to the increased stiffness of tumour tissue\(^44–46\). Although chemical crosslinks are not readily reversed, the production of matrix proteases allows the tumour matrix to be remodelled, and this can lead to the generation of permissive tracks that allow cancer cell invasion\(^47\). Contact-mediated Eph–ephrin signalling further influences cancer cell migration\(^48\). In addition to promoting local invasion, CAFs are able to boost metastasis in experimental models, and this correlated with their ability to remodel the ECM\(^49–51\). Once cancer cells

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**Fig. 1 | Diverse mechanisms of cancer-associated fibroblast activation.** This schematic highlights the multiple mechanisms that can contribute to cancer-associated fibroblast (CAF) activation. FGF, fibroblast growth factor; PDGF, platelet-derived growth factor; ROS, reactive oxygen species; RTK, receptor tyrosine kinase; TGFβ, transforming growth factor-β; TNF, tumour necrosis factor.
Interference with metabolic resources.

A process of cellular 'self-eating' that serves to remove damaged organelles and provide metabolic resources.

ConSenSuS Statement

Fig. 2 | Summary of cancer-associated fibroblast functions and the mechanisms by which they are achieved.

Dark blue text boxes indicate the biological functions being regulated, with light blue, green, purple and grey text boxes indicating the processes and mechanisms leading to the control of function. Lines connect mechanisms to functions. Both matrix remodelling and the production of soluble factors contribute to increased tumour cell invasion. Soluble factors also contribute to changes in tumour growth and the immune microenvironment, which is also affected by the altered metabolic state of the tumour. CAF, cancer-associated fibroblast; CCL2, CC-chemokine ligand 2; CXCL12, CXC-chemokine ligand 12; IL-6, interleukin-6; GAS6, growth arrest-specific protein 6; HGF, hepatocyte growth factor; TGFβ, transforming growth factor-β; VEGF, vascular endothelial growth factor.

have disseminated, the de novo activation of fibroblasts at secondary sites favours the establishment of macro-metastases via multiple mechanisms, including the production of matrix components such as tenasin and peristin that provide supporting signals to the cancer cells. These molecules boost WNT signalling, which may link to the role of some fibroblasts in normal physiology in regulating stem cell niches that are rich in WNT ligands. More recently, changes in ECM organization have been shown to influence the migration of infiltrating leukocytes, which has implications for the immune surveillance of tumours.

The alterations in matrix production and tumour mechanics that result in a large part from the action of CAFs have complex consequences for tumours. Increased tissue stiffness triggers prosurvival and proproliferation signalling in cancer cells. Increased mechanical stress can collapse blood vessels, leading to hypoxia, thereby promoting more aggressive cancer phenotypes, and reducing drug delivery. Altered tissue mechanics are also likely to play a role in cancer development and premalignant disease; this is evidenced by the links between mammographic density and breast cancer incidence. Targeting the interplay between CAFs and the mechanical properties of tumours for patient benefit is currently being explored (see TABLE 1).

CAFs are also a substantial source of growth factors, cytokines and exosomes that can promote tumour growth and modulate therapy responses. The production of TGFβ, leukaemia inhibitory factor (LIF), growth arrest-specific protein 6 (GAS6), fibroblast growth factor 5 (FGF5), growth differentiation factor 15 (GDF15) and hepatocyte growth factor (HGF) promotes invasive and proliferative behaviour in cancer cells. In addition, HGF has been implicated in mediating resistance to BRAF-targeted therapies by providing an alternative BRAF-independent mechanism for ERK–MAPK activation.

The secretome of CAFs also influences other components of the tumour microenvironment. VEGF expression by stromal cells can drive angiogenesis. Numerous cytokines and chemokines are produced by CAFs, and these act on a range of leukocytes, including CD8+ T cells, regulatory T (Treg) cells and macrophages, with both immunosuppressive and immunopromoting consequences. However, the consensus is that the predominant effect of CAFs is immunosuppressive with IL-6, CXC-chemokine ligand 9 (CXCL9) and TGFβ having well-established roles in reducing T cell responses. More recently, antigen cross presentation by CAFs has been observed, and this may lead to CD4+ T cell activation and suppression of CD8+ T cells.

Clinical analysis further supports an inverse association between CAFs and CD8+ T cells. IL-6 may also promote immunosuppression via systemic effects on metabolism. Interference with the action of CXCL12 produced by CAFs promotes T cell-mediated tumour control and targeting focal adhesion kinase (FAK) in cancer cells concomitantly reduces stromal fibroblast activation and the development of an immunosuppressive environment. However, the situation with tumour necrosis factor (TNF) produced by CAFs is more nuanced; the tumour-promoting immunosuppressive activity of FAP+ fibroblasts is associated with suppression of TNF signalling, yet TNF is also able to drive fibroblast activation in certain contexts.

The exchange of metabolites and amino acids between cancer cells and CAFs is an additional avenue by which stromal fibroblasts interact with tumour cells. Autophagy in stromal fibroblasts can generate...
alanine, which is subsequently used by pancreatic ductal adenocarcinoma (PDAC) cells to fuel the tricarboxylic acid (TCA) cycle. Furthermore, metabolic dysregulation of CAFs may also be coupled to altered immunoregulation, possibly through IL-6 production or depletion of immunomodulating amino acids.

**CAF heterogeneity and plasticity**

The large array of functions attributed to CAFs in a range of model systems poses the question of whether a single type of CAF simultaneously performs all these functions or whether there is subspecialization of CAFs and possibly switching between distinct functional states. Overwhelming evidence now points to a degree of specialization among CAFs, which may reflect the increasingly appreciated specialization of normal fibroblasts. This is informed by the increasing array of functional assays combined with the emergence of single-cell technologies, including single-cell RNA sequencing. New analyses are being reported at an impressive rate, and the field is in a state of flux. Nonetheless, there is a recurrent observation of distinct CAFs exhibiting either a matrix-producing contractile phenotype or an immunomodulating secretome — often termed 'myoCAFs' and 'iCAFs', with the prefixes alluding to a myofibroblast phenotype and regulation of inflammation, respectively. In pancreatic cancer, CAFs most proximal to the cancer cells exhibit a myoCAF phenotype, with high TGFβ-driven αSMA expression and a contractile phenotype. More distal CAFs express higher levels of IL-6 and are labelled iCAFs. The apparent exclusivity of the two phenotypes can be explained by TGFβ-mediated suppression of the IL-1 receptor, which is responsible for driving NF-κB signalling and subsequent IL-6 expression. Breast cancer also shows divergent CAF phenotypes, with the primary discriminating marker being FAP. FAP-high fibroblasts are correlated with Treg cell-mediated immune suppression and a poor outcome, which is broadly consistent with the tumour rejection observed following the ablation of FAP+ fibroblasts in experimental systems.

| Target | Name | Drug or biologic | Mechanism | Current status |
|--------|------|----------------|-----------|---------------|
| **Interference with CAF activation** | | | | |
| FGFR | JNJ-42756493 | Small-molecule inhibitor | Prevents CAF activation | Phase I and phase II trials under way |
| Hedgehog | IPI-926 (saridegib) and vismodegib | Small-molecule inhibitor | Reduces CAF activation | Clinical trials ongoing; some reported lack of efficacy |
| **Interference with CAF activation and CAF action** | | | | |
| TGFβ | Various, including galunisertib | Both blocking Abs and small-molecule receptor inhibitors | Prevents CAF activation and immunosuppression | Phase I, phase II and phase III trials under way |
| Angiotensin receptor | Losartan | Small-molecule inhibitor | Reduces collagen and hyaluronan levels | Phase II trial completed; randomized trial ongoing |
| **Interference with CAF action** | | | | |
| CXCR4 | AMD3100 | Small-molecule inhibitor | Prevents signalling from CAFs to immune cells | Clinical trials ongoing |
| ROCK | AT13148 | Small-molecule inhibitor | Reduces contractility | Phase I trial completed |
| FAK | Defactinib (VS-6063, PF-04554878) | Small-molecule inhibitor | Reduces signalling downstream of integrins | Clinical trials ongoing |
| LOXL2 | Sintuzumab (GS 6624) | Blocking Ab | Anticrosslinking | Preclinical and fibrosis trials |
| CTGF | FG-3019 | Blocking Ab | Blocks binding to receptors, including integrins | Early-phase clinical trials ongoing |
| Hyaluronic acid | PEGPH20 (PVHA) | Pegylated enzyme | ECM degradation to increase the access and efficacy of cytotoxic therapies and immunotherapies | Phase III trial complete, awaiting final analysis |
| FAP-expressing cells | Various, including PT630 and RO6874281 | Blocking Abs (sibrotuzumab (REF.15)), molecular radiotherapy, inhibitors (PT630) or an Ab–IL-2 fusion (RO6874281) | Blocks FAP+ CAF function, promoting T cell function | Phase I and phase II trials under way |
| **CAF normalization** | | | | |
| Vitamin A metabolism | ATRA | Vitamin A metabolite | ‘Normalizes’ stellate cells | Clinical trials ongoing |
| Vitamin D receptor | Paricalcitol | Small-molecule agonist | ‘Normalizes’ stellate cells | Clinical trial started |

Ab, antibody; ATRA, all-trans retinoic acid; CAF, cancer-associated fibroblast; CTGF, connective tissue growth factor; CXCR4, CXC-chemokine receptor 4; ECM, extracellular matrix; FAK, focal adhesion kinase; FAP, fibroblast activation protein; FGFR, fibroblast growth factor receptor; IL-2, interleukin-2; LOXL2, lysyl oxidase-like 2; ROCK, RHO kinase; TGFβ, transforming growth factor-β.
Idiopathic pulmonary fibrosis
A life-limiting progressive condition involving persistent activation of fibroblasts and inflammation in the lungs.

not be viewed as solely immune modulating, as their targeting with chimeric antigen receptor (CAR) T cells leads to reduced matrix deposition78. Another study reported an NF-kB-driven subset of CAFs expressing GPR77 and CD10, which promote ‘stemness’ and chemoresistance within breast cancer cells89. In the long term, it will be important for researchers to coalesce around a consensus for CAF subtypes and nomenclature (discussed in more detail later). Improvements in multiplexed immunohistochemistry that allow the analysis of multiple markers simultaneously and more quantitative methods for determining relative degrees of marker expression should aid reproducible evaluation of CAF subtypes.

The issue of CAF heterogeneity raises additional questions; including whether CAF subtypes might interconvert or whether they are more stable, possibly because they are instructed by oncogenic or tumour suppressor mutations within cancer cells. Knowledge in this area is currently emerging. Work in PDAC has shown how KRAS mutation or different p53 mutational status can influence CAFs111,136. Mutant p53 drives TNF production by cancer cells, leading to enhanced matrix remodelling and perlecan expression by CAFs136. However, such studies do not preclude additional non-genetic factors influencing CAF subtype. Indeed, CAFs isolated from mouse PDAC can be switched from the αSMA-high and IL-6-producing states through manipulation of TGFβ and IL-1 signalling, arguing for considerable plasticity in fibroblast states135. Furthermore, the responsiveness of matrix production by fibroblasts and the αSMA promoter to a range of extracellular cues, including substrate stiffness, supports the idea that the αSMA-high, matrix producing-high state is reversible63,64,137–139. Once again, irreversible lineage marking approaches in mouse models should be informative in addressing the interconvertibility of different CAF subtypes, and improved understanding of the epigenetic regulation of CAF states should shed further light on the stability of CAFs.

Targeting CAFs for clinical benefit
Many patient studies have documented how either CAF number or CAF function is linked to outcome140–142, and thus being able to target CAFs would represent an appealing addition to the suite of anticancer therapies. Further targeting mechanisms, such as TGFβ signalling, that activate CAFs or emanate from CAFs to modulate the tumour phenotype are being intensively explored143,144. There is already much activity in the area of CAF targeting — summarized in BOX 2, TABLE 1 and detailed reviews145,146. However, the breadth of CAF functions and possible interconvertibility of subtypes poses a challenge for the field, with preclinical studies suggesting that the non-specific targeting or deletion of stromal fibroblasts may not enhance tumour control35,36. Thus, patient benefit might require targeting of CAF subtypes or reprogramming of CAFs to either a normal fibroblast or an antitumorigenic CAF phenotype. This highlights the importance of defining CAF subtypes and their inter-relationships. One appealing strategy is to make CAFs more ‘normal’. An example of this approach is provided by the targeting of the vitamin D receptor in pancreatic cancer. Treatment with a vitamin D receptor ligand caused activated stellate cells to revert to a more quiescent state and reduced disease aggressiveness35,147 (see TABLE 1). Therefore, it is important to delineate whether individual fibroblast populations represent ‘states’ and are therefore interconvertible or whether distinct ‘lineage-restricted’ effects exist as this may dictate a different therapeutic approach. The functional contribution of CAFs to tumour biology is also typically assumed to be preserved across tumour types, but this remains to be demonstrated, and care will be needed when one is extrapolating between different tumour types.

In practice, achieving clinical benefit may not necessarily require elimination or reprogramming of CAFs, but could be achieved by blocking signals coming from the CAFs. For example, targeting CXCL12 signalling could be considered to be targeting CAFs as they are the major source of the chemokin in many tumours11. Similarly, targeting ECM components and downstream signalling represents a means of interfering with CAF–cancer cell communication. Indeed, many existing therapies influence CAF–cancer cell communication and already modulate how CAFs affect cancer cells. As mentioned earlier, BRAF inhibitors can activate stromal fibroblasts and thereby promote a compensatory mechanism for activating ERK–MAPK in cancer cells12. Many of the expanding range of receptor tyrosine kinase inhibitors have some activity on FGF and PDGF receptors that can drive fibroblast function106,108. This is exemplified by the repurposing of nintedanib, which was originally developed with oncology in mind, for treatment of idiopathic pulmonary fibrosis13. Finally, both conventional DNA damaging chemotherapy and radiotherapy...
can trigger changes in CAF biology, with fibrosis being a common late side effect of radiotherapy\(^\text{15}\). These data argue that more studies to assess the extent to which responses to therapies might be influenced by altered CAF biology are warranted.

**Challenges and recommendations**

**An emerging framework for nomenclature.** A key challenge now facing researchers of CAF biology is nomenclature (BOX 3). Ideally a system should be simple enough to allow it to be used by the wider cancer and stromal biology communities but not so dogmatic and constrictive that it masks subtle variations in function and markers. In addition, it must have flexibility to incorporate fibroblast subtypes that are only currently being revealed by single-cell transcriptomics and mass cytometry methods. Although our view was that it is too soon for definitive nomenclature to be established, the consensus was that the main determinant of CAF categorization should be function, informed primarily by direct experimental evidence and, in some cases, robust clinical correlation analyses. These categories should then be linked to markers, ideally cell surface markers, so that they can be further interrogated in analyses that might not be compatible with functional testing. A sensible starting point for such a classification would be the reiteration that activated fibroblasts can adopt a high matrix producing and remodelling state — analogous to the myofibroblast in other pathologies. This is linked to high levels of TGFβ signalling and αSMA expression\(^\text{16}\). It will also be necessary to include immunomodulation into CAF categories. Although most studies have suggested an immunosuppressive role of CAFs\(^\text{17}\), it should be left open that CAFs could promote immune-mediated tumour surveillance. Indeed, the function of FRCs in lymph nodes is to make possible effective T cell-mediated immune response\(^\text{18}\). Scope should also be left for antigen presentation, the metabolic state of fibroblasts, and their lineage history to be incorporated into any nomenclature.

**Robust and standardized methods for detecting CAFs in tissue.** Progress in translational studies will require accurate recording of CAF numbers and subtype within clinical samples. Clinical studies that target either CAFs or CAF-associated functions must include measurement of CAFs in their design. More generally, our consensus was that CAF metrics should be recorded even in studies that do not have CAFs as their focus, for example in immuno-oncology trials. This will depend on high-quality antibodies against CAF marker proteins, which in many cases are lacking. While reliable αSMA antibodies are available, antibodies against putative CAF subtype markers often require painstaking optimization, and this hampers their adoption in clinical pathology laboratories. The technology around multiplexed mRNA probes is developing rapidly and, in the long term, this might provide a better and more flexible solution than antibody-based methods. Furthermore, researchers are aware of caveats in studies that involve dissociation of tumour tissue, for example those using cytometry by time of flight (CyTOF) and single-cell RNA sequencing.

Detaching fibroblasts from tissue typically requires more aggressive methods than for leukocytes, and there is risk that fibroblasts are substantially under-represented in studies optimized for leukocyte biology\(^\text{19}\).

**Measuring CAF functions in vitro and in vivo.** The diversity of CAF function is reflected in the wide range of assays used to assess CAF function. While the breadth of assays is necessary, it presents a challenge when one is interpreting the literature. In this subsection, we review the main assays used and highlight key points regarding interpretation of their results.

The function of CAFs can be directly investigated in vitro. Given the ability of both serum and stiff substrates to activate fibroblasts, attention should be paid to the culture conditions used, with lower serum concentrations and matrices with more physiological mechanical properties being preferable. Furthermore, it is important to consider whether the CAFs being tested are early passage primary cells or have been in culture for several passages and even immortalized. Although certain CAF characteristics are stably maintained in culture, such as their increased ability to remodel the ECM\(^\text{20}\), it is highly likely that some traits are not. Detailed characterization of how CAF properties change on isolation and longer-term culture will help to clarify which functional assays necessitate early passage primary CAFs and which work equally well after longer periods of cell culture.

Matrix production and remodelling can be easily measured. CAFs will produce ECM in culture, and this can be assayed for its composition and quantity using western blotting, quantitative immunofluorescence and mass spectrometry methods\(^\text{15}\). The organization of this matrix can be determined by immunofluorescence, frequently staining for fibronectin, and its mechanical properties can be determined by either atomic force microscopy or shear rheology. Similar techniques can be applied in vivo, with collagen second-harmonic imaging frequently used to assess matrix organization. Multiparametric magnetic resonance imaging (MRI) and magnetic resonance elastography (MRE) can also be
used to infer tissue mechanics, with the advantage that these techniques can be translated to clinical imaging and used in clinical trials\textsuperscript{154,159}. Histochemical stains to distinguish collagen, including Masson’s trichrome and picrosirius red, provide similar information and the use of crossed-polarizing filters during imaging of picrosirius red-stained sections provides a measure of collagen crosslinking\textsuperscript{160}. However, most methods for the analysis of pattern lack universal quantitative metrics; in the future, the implementation of methods from network topology analysis and the use of spatial statistics will aid comparison between studies\textsuperscript{157,158}.

The secretome of CAFs is typically measured using enzyme-linked immunosorbent assay (ELISA) and cytokine array tools, with a range of standardized commercial reagents available. Exosomes can be analysed following their purification by high-speed centrifugation with clear guidelines on optimal protocols\textsuperscript{159}. Crosstalk with cancer cells is usually evaluated in terms of changes in growth and invasion. Cells can be directly co-cultured, with either genetic labels or staining for markers used to distinguish the cancer cells and fibroblasts, indirectly co-cultured (that is, separated by a filter) or conditioned media can be exchanged between separate cultures. Cell number is the most common growth metric, and migration either into a 2D ‘wound’ or across a Transwell are most common invasion metrics. Advances in 3D co-cultures, including the use of organoid cultures and reconstituted matrices, are allowing in vitro assays to more closely mimic the in vivo tissue architecture. In these assays, it should be noted that basement membrane preparations often contain growth factors in addition to matrix components, leading to the possible confounding of matrix and growth factor influences on CAF biology. Pepsinized preparations of collagen I lacking the telopeptide cannot be crosslinked, which leads to altered dependencies on matrix metalloproteinases (MMPs) for cancer invasion\textsuperscript{160}. Co-cultures with other cell types from the tumour microenvironment can also be informative. For example, fibroblasts can boost angiogenesis in vitro assays, with exciting advances in the development of microfluidic angiogenesis models, and an increasing number of studies have shown how they can alter T cell functionality\textsuperscript{156,161,162}.

Two main methods are used to explore CAF functions in vivo: transgenic manipulations and injection methods. The latter are simpler to perform as they avoid the need for complex mouse crosses. However, there are some notable caveats. The most challenging is that as tumours grow they will contain a mixture of the co-injected CAFs and fibroblasts derived from the host mouse and, for reasons that are not fully understood, host-derived fibroblasts outgrow co-injected CAFs. In practice, this favours the early evaluation of CAF biology. Pepsinized preparations of collagen I lacking the telopeptide cannot be crosslinked, which leads to altered dependencies on matrix metalloproteinases (MMPs) for cancer invasion\textsuperscript{160}. Co-cultures with other cell types from the tumour microenvironment can also be informative.

An awareness of the caveats of the assays described above and subsequent improvements to the methods will aid further progress. The use of CAFs that have been established in culture allows molecular perturbations, such as CRISPR gene editing, and the easy repetition of experiments. In the future it will be desirable to determine primary culture conditions that most accurately preserve the in vivo phenotype of CAFs; this is likely to involve considering both the medium and the substrate, with several studies showing how culture in 3D conditions can return fibroblasts to their original phenotype within tissue\textsuperscript{156,162}. Combining this with ongoing improvements in the ability to manipulate primary cells will allow assays with human cells that more closely mimic the tumour context. For analysis of interplay with T cells, it will be desirable to isolate cancer cells, CAFs and tumour-infiltrating lymphocytes from the same patient. Improvements in tumour tissue slice culture methods should also be considered for the analysis of CAF biology.

**Reporting CAF metadata.** As with all experimental science, the issue of reproducibility is crucial. Research into CAFs is greatly made possible by their ability to be cultured in vitro, but the process of cell culture and the exact conditions can influence cell behaviours. Increased reporting of CAF metadata will improve standardization.
and robustness in the field. We recommend that studies involving CAFs document the following:

• An absence of the mutations that drive the tumour from which they originate. CAFs may accrue mutations, but it is necessary to exclude that they are simply cancer cells that have undergone EMT. Cancer cells that have undergone a profound EMT clearly warrant detailed study and comparison with CAFs, but these cells should be considered distinct from CAFs.

• The spatial position within the tumour from which the biopsy was taken — central versus margin. If ‘normal’ fibroblasts are isolated at the same time from non-cancerous tissue, then the distance of this tissue from the margin should be recorded.

• Key clinical (stage, grade, prior treatment regimen and driver mutations (if known)) and histological features of the tumour from which the CAFs originate, including ideally staining for CAF markers and the age of the patient or mouse.

• Short tandem repeat profiles of cultured CAFs to allow unambiguous identification of CAFs in subsequent studies. This will mitigate against inadvertent cross-contamination of cultures.

• The passage number of cultured CAFs and the immortalization method used, if any. Details of the culture medium should also be recorded, in particular, serum percentage, addition of exogenous TGFβ, culture substrate or matrix (including type of Matrigel and whether collagen I is telopeptide intact).

Conclusions

Research into CAFs is at an exciting and critical stage. Accumulating functional analyses in preclinical models and supporting correlative analyses of patient material indicate that improved treatment strategies should be possible by targeting CAFs. Indeed, several clinical trials are under way. However, targeting aspects of the tumour microenvironment has a chequered history, with failures in the area of MMP inhibition, mixed results in targeting angiogenesis and transformative results with inhibition of T cell immune checkpoints in some cancers. Therefore, translating the optimism in the CAF field into real clinical benefits will require careful attention to trial design and tumour sample analysis. Attention needs to be paid to nomenclature and the correct description of different CAF subtypes. This is more than just a semantic issue as the greatest success is likely to come from either targeting specific CAF subsets or interconverting CAF subtypes. Related to this, a better understanding is needed of the relationship between CAFs observed in preclinical models, which often grow very rapidly in young adult mice, as opposed to those in patients, which progress more slowly in an older population. Improved assay standardization and reporting of CAF metadata will assist this endeavour. There are also opportunities to incorporate analysis and reporting of CAF numbers and types in clinical studies that are not primarily focused on fibroblast biology, such as immuno-oncology and targeted therapy trials. This will help to build a more complete picture of the relationship between CAFs and therapy responses and highlight new areas in which combining a CAF-targeted agent with existing therapies could yield greater benefit. With these things in place, we are confident that CAF-targeted therapy will take its place in the toolkit of the oncologist within the next 10 years.

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1. Kalluri, R. & Zeisberg, M. Fibroblasts in cancer. Nat. Rev. Cancer 6, 392–401 (2006).

2. Kalluri, R. The biology and function of fibroblasts in cancer. Nat. Rev. Cancer 16, 582–598 (2016).

3. Hay, E. D. An overview of epithelial-mesenchymal transformation. Acta Anat. 154, 8–20 (1995).

4. Sharpe, P. T. Neural crest and tooth morphogenesis. Roberts, E. W. et al. Depletion of stromal cells.

5. Ecker, B. L. et al. Age-related changes in HAPLN1.

6. Kaur, A. et al. sFRP2 in the aged microenvironment.

7. Brown, R. A. Myofibroblasts and mechano-regulation of connective tissue remodelling. Nat. Rev. Mol. Cell Biol. 3, 349–363 (2002).

8. Drikska, R. R. et al. Distinct fibroblast lineages determine dermal architecture in skin development and repair. Nature 504, 277–281 (2013).

9. Rinkevich, Y. et al. Skin fibrosis: Identification and isolation of a dermal lineage with intrinsic fibrogenic potential. Science 348, aaa2151 (2015).

10. Ecker, B. L. et al. Age-related changes in HAPLN1 increase lysymatic permeability and affect routes of melanoma metastasis. Cancer Discov. 9, 82–95 (2019).

11. Kaur, A. et al. FSP2 in the aged microenvironment drives melanoma metastasis and therapy resistance. Nature 532, 250–254 (2016).

12. Gabbiani, G. The myofibroblast in wound healing and fibrocontractive diseases. J. Pathol. 200, 500–503 (2003).

13. Rockey, D. C., Weymouth, N. & Shi, Z. Smooth muscle alpha actin (Acta2) and myofibroblast function during hepatic wound healing. PLoS One 8, e77166 (2013).

14. Bruzi, M. F., Tarone, G. & Deițiipp, P. Extracellular matrix, integrins, and growth factors as tailors of the stem cell niche. Curr. Opin. Cell Biol. 24, 645–651 (2012).

15. Le Guen, L., Marchal, S., Faure, S. & de Santa Barbara, P. Mesenchymal-epithelial interactions during digestive tract development and epithelial stem cell regeneration. Cell Mol. Life Sci. 72, 3883–3896 (2015).

16. Fujikura, D. et al. Tumor induction of VEGF promoter activity in stromal cells. Cell 94, 715–725 (1998).

17. Kraman, M. et al. Suppression of antitumor immunity by stromal cells expressing fibroblast activation protein-alpha. Science 330, 827–830 (2010).

18. Buechler, M. B. & Turley, S. J. A short field guide to fibroblast function in immunity. Semin. Immunol. 35, 48–58 (2018).

19. Wang, L. C. et al. Targeting fibroblast activation protein in tumor stroma with chimeric antigen receptor T cells can inhibit tumor growth and augment host immunity without severe toxicity. Cancer Immunol. Res. 2, 154–166 (2014).

20. Philipspe, C. et al. Spatial and single-cell transcriptional profiling identifies functionally distinct human dermal fibroblast subpopulations. J. Invest. Dermatol. 138, 811–825 (2018).

21. Biffi, G. et al. IL1-induced JAK/STAT signaling is inhibited by TGFβ. Immunity 43, 735–747 (2014).

22. Fletcher, A. L., Malhotra, D. & Turley, S. J. Fibroblastic reticular cells: a specialized lipid droplet for retinoid storage. Hepatology 54, 183–198 (2011).

23. Lockwood, D. S. et al. Tumor progression in hepatocellular carcinoma: relationship with tumor stroma and parenchymal disease. J. Gastroenterol. Hepatol. 18, 666–672 (2003).

24. Albadia, O. & Mahalingam, M. Desmoplakia: not always a bad thing. Histopathology 58, 643–659 (2011).

25. Blaner, W. S. et al. Hepatic stellate cell lipid droplets: a specialized lipid droplet for retinoid storage. Biochim. Biophys. Acta 1791, 467–473 (2009).

26. Sherman, M. H. et al. Stromal cues regulate the pancreatic cancer epigenome and metabolome. Proc. Natl Acad. Sci. USA 114, 1129–1134 (2017).

27. Olumi, A. F. et al. Carcinoma-associated fibroblasts direct tumor progression of initiated human prostatic epithelium. Cancer Res. 59, 5002–5011 (1999).

28. Paulsson, J. & Miele, P. Prognostic relevance of cancer-associated fibroblasts in human cancer. Semin. Cancer Biol. 25, 61–68 (2014).

29. Lockwood, D. S. et al. Tumor progression in hepatocellular carcinoma: relationship with tumor stroma and parenchymal disease. J. Gastroenterol. Hepatol. 18, 666–672 (2003).

30. Albadia, O. & Mahalingam, M. Desmoplakia: not always a bad thing. Histopathology 58, 643–659 (2011).

31. Kretzschmar, K., Weber, C., Driskell, R. R., Calonje, E. & Watt, F. M. Compartmentalized epidermal activation of β-catenin differentially affects lineage reprogramming and underlies tumor heterogeneity. Cell Rep. 14, 269–281 (2016).

32. Arina, A. et al. Tumor-associated fibroblasts predominantly come from local and not circulating precursors. Proc. Natl Acad. Sci. USA 113, 7551–7556 (2016).

33. Ohlund, D. et al. Distinct populations of inflammatory fibroblasts and myofibroblasts in pancreatic cancer. J. Exp. Med. 214, 1163–1179 (2017).

34. Beacham, D. A. & Cukierman, E. Stromagenesis: the changing face of fibroblastic microenvironments during tumor progression. Semin. Cancer Biol. 15, 329–341 (2005).

35. Rhim, A. D. et al. Stromal elements act to restrain, rather than support, pancreatic ductal adenocarcinoma. Cancer Cell 25, 735–747 (2014).

36. Ozdemir, B. C. et al. Depletion of carcinoma-associated fibroblasts and fibrosis induces immunosuppression
and accelerates pancreas cancer with reduced survival. Cancer Cell 25, 719–734 (2014).

37. Alcoleta, M. P. & Jones, P. H. Tracking cells in their native habitat: lineage tracing in epithelial neoplasia. Nat. Rev. Mol. Cell Biol. 14, 170–171 (2013).

38. Pallangyo, C. K., Ziegler, P. K. & Greeten, F. R. KIkBa acts as a tumor suppressor in cancer-associated fibroblasts by regulating tumor growth. J. Exp. Med. 212, 2255–2266 (2015).

39. Koliara, V., Pasparakis, M. & Kollaris, G. KIkBa in epithelial tumors promotes initiation of colitis-associated cancer. J. Exp. Med. 212, 2235–2251 (2015).

40. Karnoub, A. E. et al. Mesenchymal stem cells within tumor stroma promote breast cancer metastasis. Nature 457, 557–563 (2009).

41. Croft, A. P. et al. Distinct fibroblast subsets drive tumor progression in mouse models. Cancer Res. 69, 5273–5282 (2009).

42. Bochet, L. et al. Adipocyte-derived fibroblasts act as a tumor suppressor in cancer-associated fibroblasts. J. Exp. Med. 213, 2075–2087 (2014).

43. Dirat, B. et al. Cancer- associated adipocytes exhibit an activated phenotype and accelerate pancreas cancer with reduced survival. J. Exp. Med. 212, 2291–2301 (2015).

44. Albrengues, J. et al. Epigenetic switch drives the Warburg phenotype in tumor cells and stroma. Cancer Cell 22, 772–774 (2012).

45. Bochet, L., Bochet, B. & Plummett, J. Tumor-promoting stromal fibroblasts cooperate to control actomyosin contractility in fibroblast- driven cancer. Cancer Cell 23, 983–995 (2012).

46. Sanz- Moreno, V. et al. ROCK and JAK1 signaling drives mesenchymal stem cell plasticity overcomes chemoresistance. Semin Cancer Biol. 100.0019 (2020).

47. Herrero, N. et al. Dickkopf3 links HSF1 and VAP-TAZ signalling to control aggressive behaviours in cancer-associated fibroblasts. Nat. Commun. 10, 1903 (2019).

48. Forduce, C. A. et al. Cell-extrinsic consequences of epithelial stress: activation of tumorigenic tissue phenotypes. Breast Cancer Res. 14, R155 (2012).

49. Cui, Y. et al. Cyclic stretching of soft substrates induces mechanotransduction and the emergence of endogenous adipose tissue contribute to pericytes and adipocytes that populate the tumor microenvironment. Cancer Res. 72, 5198–5208 (2012).

50. Ferrao, Y. et al. White adipose tissue cells are recruited by experimental tumors and promote cancer progression in mouse models. Cancer Res. 69, 5283–5292 (2009).

51. Dirat, B. et al. Cancer- associated adipocytes exhibit an activated phenotype and contribute to breast cancer invasion. Cancer Res. 71, 2455–2465 (2011).

52. Chen, H. et al. Bone marrow- derived fibroblasts are a functional cell population in breast cancer. J. Exp. Med. 205, 3075–3095 (2008).

53. Yang, Z. et al. Stromal progenitor cells from endogenous adipose tissue contribute to pericytes and adipocytes that populate the tumor microenvironment. Cancer Res. 72, 5198–5208 (2012).

54. Albrengues, J. et al. Competition amongst Eph receptors regulates contact inhibition of locomotion and invasiveness in prostate cancer cells. Nat. Cell. Biol. 12, 1194–1206 (2010).

55. Dumont, N. et al. Breast fibroblasts modulate early dissemination, tumorigenesis, and metastasis through alteration of extracellular matrix characteristics. Neoplasia 15, 249–262 (2013).

56. Madsen, C. D. et al. Hypoxia and loss of PHD2 inactivate stromal fibroblasts to decrease tumour stiffness and metastasis. EMBO Rep. 16, 1594–1608 (2015).

57. Calon, C. et al. Dependency of colorectal cancer on a TGF-beta-driven program in stromal cells for metastasis initiation. Cancer Cell 22, 571–586 (2012).

58. Malarini, I. et al. Interactions between cancer stem cells and their niche govern metastatic colonization. Nature 481, 85–89 (2011).

59. Oskarsson, T. et al. Breast cancer cells produce tenascin C as a metastatic factor that colonizes the lungs. Nat. Med. 17, 867–874 (2011).

60. Nabhan, A. N., Brownfield, D. G., Harbury, P. B., Krahn, M. A. & Devi, N. Signaling niches maintain stemness of alveolar type 2 cells. Science 359, 1118–1123 (2018).

61. Vermeulen, L. et al. Filamenous fibroblast cell stem is and is regulated by the microenvironment. Nat. Cell. Biol. 12, 468–476 (2010).

62. Kaur, A. et al. Remodeling of the collagen matrix in aging skin promotes melanoma metastases and affects immune cell motility. Cancer Discov. 9, 64–81 (2019).

63. Paszek, M. J. et al. Tensional homeostasis and the malignant phenotype. Cancer Cell 22, 241–254 (2005).

64. Doop-Frimpomp, B., Chauhan, V. P., Krane, S., Bourchier, Y. & Jain, R. K. Losartan inhibits collagen I synthesis and improves the distribution and efficacy of nanothrombocytes in tumors. Proc. Natl Acad. Sci. USA 108, 2909–2914 (2011).

65. Chauhan, V. P. et al. Angiotensin inhibition enhances drug delivery and potentiates chemotherapeutic drug by decompressing tumour blood vessels. Nat. Commun. 4, 2156 (2013).

66. Jain, R. K., Martin, J. D. & Stylianopoulos, T. The role of mechanical forces in tumor growth and therapy. Annu. Rev. Biomed. Eng. 12, 387–408 (2010).

67. Provenzano, P. P. et al. Engineered targeting of the stroma ablates physical barriers to treatment of pancreatic ductal adenocarcinoma. Cancer Cell 21, 418–429 (2012).

68. DuFort, C. C. et al. Intestinal pressure in pancreatic ductal adenocarcinoma: a role for the fluid phase. Biophys. J. 110, 2106–2119 (2015).

69. DuFort, C. C., DelGorno, K. E. & Hingorani, S. R. Mounting pressure in the microenvironment: fluids, solids, and cells in pancreatic ductal adenocarcinoma. Gastroenterology 150, 1545–1557 e1542 (2016).

70. Bhome, R. et al. Exosomal microRNAs derived from cancer- associated fibroblasts: role in driving cancer progression. Aging 9, 2666–2694 (2017).

71. Bhome, R. et al. Profiling the microRNA payload of exosomes derived from ex vivo primary colorectal fibroblasts. Methods Mol. Biol. 1509, 115–122 (2017).

72. Luga, V. et al. Exosomes mediate stromal mobilization of autocrine Wnt-PCP signaling in breast cancer cell migration. Cell 151, 155–163 (2013).

73. Shi, Y. et al. Targeting LIF- mediated stromal- related interaction for pancreatic cancer therapy and monitoring. Nature 565, 151–155 (2019).

74. Cazet, A. et al. Targeting 3D remodeling and cancer stem cell plasticity overcomes chemoresistance in triple negative breast cancer. Nat. Commun. 9, 1200–1209 (2018).

75. Tape, C. J. et al. Oncogenic KRAS regulates tumor cell signaling via stromal reciprocation. Cell 165, 320–330 (2016).

76. Bruzese, F. et al. Local and systemic protumorigenic effects of cancer- associated fibroblast- derived CDF15. Cancer Res. 74, 3408–3414 (2014).

77. Nguyen, E. V. et al. Proteomic profiling of human prostate cancer- associated fibroblasts (CAF) reveals LOL2-dependent regulation of the tumor microenvironment. Mol. Cell. Proteomics 18, 1419–1427 (2019).

78. Tang, X. et al. Stromal miR-200s contribute to breast cancer cell invasion through CAF activation and ECM remodeling. Cell Rep. 51, 152–164 (2016).

79. Mohammad, H. & Sahai, E. Mechanisms and impact of altered tumour mechanics. Nat. Cell. Biol. 20, 766–774 (2018).
