The biology and function of fibroblasts in cancer

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Abstract | Among all cells, fibroblasts could be considered the cockroaches of the human body. They survive severe stress that is usually lethal to all other cells, and they are the only normal cell type that can be live-cultured from post-mortem and decaying tissue. Their resilient adaptation may reside in their intrinsic survival programmes and cellular plasticity. Cancer is associated with fibroblasts at all stages of disease progression, including metastasis, and they are a considerable component of the general host response to tissue damage caused by cancer cells. Cancer-associated fibroblasts (CAFs) become synthetic machines that produce many different tumour components. CAFs have a role in creating extracellular matrix (ECM) structure and metabolic and immune reprogramming of the tumour microenvironment with an impact on adaptive resistance to chemotherapy. The pleiotropic actions of CAFs on tumour cells are probably reflective of them being a heterogeneous and plastic population with context-dependent influence on cancer.

Desmoplastic reaction, tumour stroma or tumour microenvironment
All components of the tumour other than cancer cells. These are generally the components of the host response towards cancer cells, including the immune response. The terms are used interchangeably.

Basement membrane
Organized and assembled extracellular matrix that interacts with all epithelial cells and is also associated with all blood vessels and capillaries.

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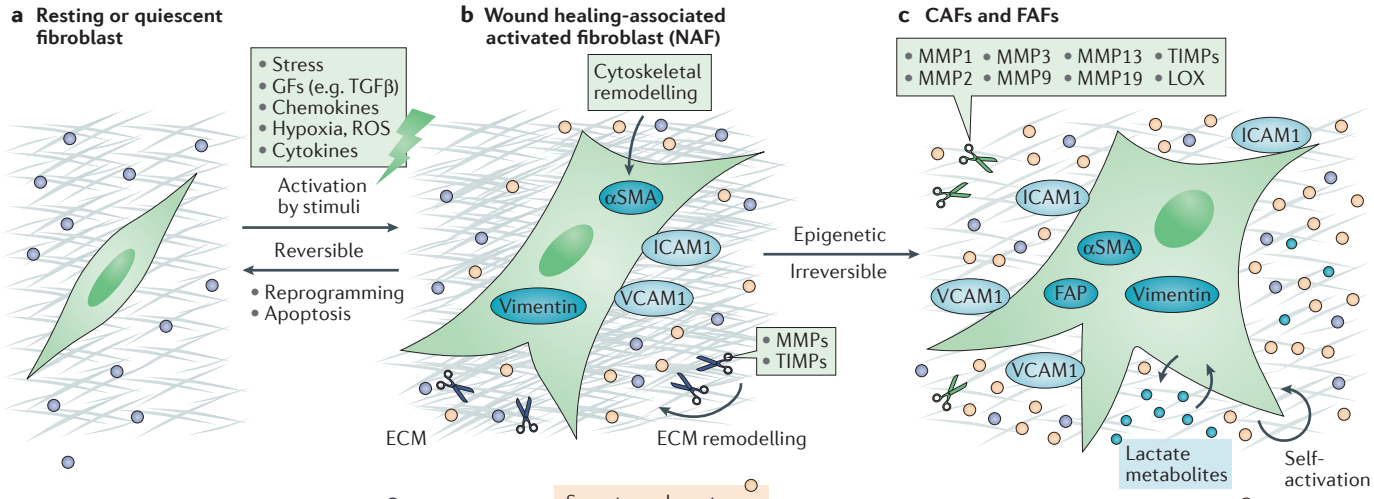
The host response to evolving cancer cells results in the generation of tumour tissue that contains components of normal organs[1–4]. Such robust host responses define complex heterotypic interactions of cancer cells with host cells and are known as desmoplastic reaction, tumour stroma or tumour microenvironment (TME)[4–7]. The immune cells, capillaries, basement membrane, activated fibroblasts and extracellular matrix (ECM) surrounding the cancer cells constitute the tumour stroma[2]. It is now clear that cancer progression and metastasis is controlled by the TME and does not depend solely on cancer cell-autonomous defects[4,8,9]. Immune cells, angiogenesis, oxygen tension, interstitial pressure, ECM remodelling and tumour metabolite components of the TME have received recent attention as important determinants of cancer cell behaviour and disease progression[1,5,10]. A dominant component of the tumour stroma is fibroblasts, and many studies over the years have suggested a prominent functional role for these cells in cancer progression and metastasis[11,12]. Fibroblasts associated with cancer have been termed cancer-associated fibroblasts (CAFs), tumour-associated fibroblasts (TAFs), activated fibroblasts or activated myofibroblasts, and could include cancer-associated mesenchymal stem cells (MSCs). In the past decade, CAFs have cemented themselves as key components of tumour progression, and evolving information suggests that they probably contribute to a wide range of fibrotic stromal programmes of many different tumours[13,14].

Fibroblasts are usually quiescent and become activated in a wound healing response. The governing principles of how quiescent, resting fibroblasts become 'activated' is still being unravelled, and exciting new information suggests that there might be two types of fibroblast activation profile: 'reversible' and 'irreversible', determined partly by epigenetic regulation[15,16] (FIG. 1). Activated fibroblasts were first described in the setting of wound healing and were identified predominantly by their expression of α-smooth muscle actin (αSMA; also known as ACTA2), a cytoskeletal protein associated with smooth muscle cells[17]. Owing to their expression of αSMA, fibroblasts are also called myofibroblasts[17–21]. Activated fibroblasts are also a major component of scars and chronic tissue wound healing response, also known as tissue or organ fibrosis[15,22–25]. A perpetual (or chronic) wound healing response is documented in organ fibrosis and tumour growth[26], which is different from acute wound healing[27,28].

Without question, fibroblasts are the most versatile and extensively studied cells *in vitro* owing to their ease of isolation and culture. They survive severe stress and can be live-cultured from human post-mortem tissue[29]. In the context of an unforgiving, highly dynamic and injurious tissue microenvironment, including damage induced by chemotherapy or radiotherapy, CAFs may represent a resistant stromal cell type that could participate in tumour relapse.

Despite being among the most studied cell-type in biology, the fibroblast remains the most enigmatic and mysterious. In recent years, much awaited genetics and engineering of new mouse models have begun to unravel the secrets embedded in the biology of fibroblasts. Furthermore, more information is emerging about their diversity and multipronged functions in cancer.



Contractility	–	+++	+
Proliferation	–	+	+++
Secretory phenotype	–	++	+++
Migration	–	++	++
Synthetic phenotype (ECM production)	–	+++	++

Figure 1 | **Multi-step activation of fibroblasts. a** | Quiescent or resting fibroblasts are inert and are identified as spindle-shaped single cells in the interstitial space embedded in physiological extracellular matrix (ECM). **b** | In response to tissue injury and the associated stimuli, the quiescent fibroblasts are reversibly activated to facilitate repair and regeneration. The normal activated fibroblasts (NAFs) gain expression of α-smooth muscle actin (αSMA) and vimentin and become stellate in shape. Together with enhanced ECM production, remodelling and cytoskeletal rearrangements, they gain contractile properties. The acquired synthetic functions are associated with secretory and migratory functions that amplify their activation, recruitment and proliferation. The reversibility of such activation may be mediated by reprogramming or apoptosis of the wound healing-associated activated fibroblasts when the repair process is complete. **c** | Activated fibroblasts may gain further secretory phenotypes, specialized ECM remodelling ability, and robust autocrine activation and dynamic immunomodulatory signalling functions. This process is associated with persisting and unabated injurious stimuli, such as the development of cancer lesions. Epigenetic regulation may limit the regression of such activated states. Such fibrosis-associated fibroblasts (FAFs) and cancer-associated fibroblasts (CAFs) gain enhanced proliferative properties and are a functionally diverse population, adding to the dynamic complexity of the evolving fibrotic and tumour microenvironment milieu. CCL5, C-C motif chemokine ligand 5 (also known as RANTES); CTGF, connective tissue growth factor; CXCL, C-X-C motif chemokine ligand; EDA-FN, extradomain A-fibronectin; EGF, epidermal growth factor; FAP, fibroblast activation protein; FGF, fibroblast growth factor; GFs, growth factors; HGF, hepatocyte growth factor; ICAM1, intercellular adhesion molecule 1; IFNγ, interferon-γ; IL, interleukin; LOX, lysyl oxidase; MMP, matrix metalloproteinase; NF-κB, nuclear factor-κB; PDGF, platelet-derived growth factor; PGE_2, prostaglandin E_2; ROS, reactive oxygen species; SDF1, stromal cell-derived factor 1; TGFβ, transforming growth factor-β; TIMPs, tissue inhibitors of metalloproteinases; TNF, tumour necrosis factor; VEGFA, vascular endothelial growth factor A; VCAM1, vascular adhesion molecule 1.

Extracellular matrix
(ECM). A collection of proteins that are present outside and between cells. ECM consists of large networks of glycoproteins that specialize in protein–protein interactions and serve as ligands of cell surface proteins such as integrins. ECM proteins can form insoluble bundles and generate networks using proteins such as collagens, laminins, entactins, fibronectins and many proteoglycans, such as perlecans.

Angiogenesis
Formation of new capillaries and blood vessels from pre-existing capillaries.

Cancer-associated fibroblasts
(CAFs). All fibroblasts associated with tumours.

Mesenchymal stem cells
(MSCs). Multi-potent stromal cells with the ability to generate connective tissue lineages.

Despite similarities based on obvious identical features at the cell biology level, functional differences, if any, between CAFs and fibrosis-associated fibroblasts (FAFs) are yet to be clearly defined at the molecular level. Moreover, our knowledge with respect to the plasticity of fibroblasts is increasing, offering further insights into how this might contribute to the complexity and functional malleability of the TME in cancer.

In this Review, the origins and functions of fibroblasts in physiological and pathological remodelling of adult parenchyma are summarized with a specific emphasis on their functional heterogeneity in health and disease.

Definition and origin of fibroblasts

Virchow[30] originally (circa 1858), and later Duvall[31], used classic anatomy techniques and microscopy to describe cells residing in the connective tissues, and 'fibroblasts' were first defined as cells in the connective tissue that synthesized collagen. Fibroblasts in normal tissues are generally single cells present in the interstitial space or occasionally near a capillary, without any association with a basement membrane but embedded within fibrillar ECM of the interstitium. Fibroblasts are non-epithelial, non-immune cells with a likely mesenchymal lineage origin, and are part of the diverse connective

tissue components[32] (BOX 1). Fibroblasts exhibit classic spindle-shape morphology with a potential for planar polarity (FIG. 1). Fibroblasts in normal tissue are generally considered indolent with negligible metabolic and transcriptomic activity. As such, they are speculated to be in a hibernating, quiescent or resting state. Resting fibroblasts may share many features with MSC precursors (BOX 2).

The ability of quiescent fibroblasts to be activated and become synthetic (myofibroblasts) was first observed in the setting of wound healing[33], and later in conditions such as acute and chronic inflammation and tissue fibrosis[17,24,34]. There is no direct evidence to suggest that quiescent tissue fibroblasts in adults are synthetically active apart from their location within interstitial ECM.

Wound healing response
Injury to the functional parenchyma leads to a reversible host repair or regeneration response that involves myofibroblasts, among other components.

Quiescent, resting fibroblasts
Fibroblast-like cells that are in waiting to become activated when the need arises. They can be considered as resting fibroblasts.

Fibroblast activation
The cellular, biochemical and molecular programme that leads to the conversion of a resting fibroblast into an active fibroblast with a myriad of biological functions.

Scars
The pathologist's term for fibrosis. Fibrosis can be visualized by different dyes that histologically stain different tissue structures, including ECM band-like patterns, making them look like a scar.

Post-mortem
The status of a biological body or tissue after the death of an organism. Observations made in a body or tissue after death are considered post-mortem analyses.

Fibrosis-associated fibroblasts
(FAFs). Activated fibroblasts associated with chronic tissue fibrosis.

Interstitial space
This is the space between layers of functional parenchyma of an organ. It is generally where the support system for an organ exists.

Tissue fibrosis
Chronic wound healing response due to unabated injury to the functional parenchyma.

Mesoderm
The third germ layer, which is considered the precursor to all future mesenchymal cells in the mammalian body, including haematopoietic and connective tissues.

Box 1 | Quiescent versus activated fibroblasts

Primitive mesenchymal cells (primary mesenchyme) are first encountered when the epiblast undergoes epithelial to mesenchymal transition (EMT), a process by which vertebrate embryos disperse cells to generate new tissue structures, leading to the generation of the third germ layer or mesoderm. Such mesenchymal cells have been shown to be important for epithelial differentiation, lung development being a prime example of their role in orchestrating organ development and pathological remodelling[231]. Secondary epithelial cells can emerge from primary mesenchyme during development, and somitogenesis and the formation of mesoendodermal structures are examples of that process. Secondary epithelium can also undergo EMT following tissue injury owing to wounding, inflammation, fibrosis and cancer, to potentially generate fibroblast-like cells[232]. Carcinoma cells are documented to undergo EMT to generate cancer cells with properties that enable them to move and reach distant organs[232–234]. Despite such evidence for the emergence and functional role of mesenchymal cells in embryogenesis, the distinguishing features of mesenchymal cells found during embryogenesis and adult activated fibroblasts remain unknown.

Quiescent or resting fibroblasts are generally detected in the interstitial stromal areas between layers of functional parenchyma in adult tissues. They are detected as thin, elongated cells with front and back extensions and a spindle-like or fusiform shape[32]. Most tissues, particularly human skin and mouse ears and tail tips, can be digested and placed in tissue culture medium on a plastic surface to derive fibroblasts. A central premise behind this technique is the notion that other cell types cannot be cultured as easily, hence giving fibroblasts the widely held reputation that they are easy to culture on plastic. An absolute marker of quiescent tissue fibroblasts is still missing, but we postulate that fibroblast-specific protein 1 (FSP1) comes closest[91]. The working definition of a quiescent fibroblast is its ability to respond to growth factors to become activated, when it then exhibits properties of proliferation, migration and production of growth factors and extracellular matrix (ECM). From a molecular standpoint, quiescent fibroblasts still remain poorly defined. Activated fibroblasts exhibit prolific protein synthesis activity and contractile activity that is crucial for actions such as closure of wounds and production of connective tissue.

Activated fibroblasts can be highly heterogeneous, with distinct expression patterns depending on the different tissues from which they were isolated [235]. The number of culture passages and non-identical culture conditions could also explain such diversity. The homeobox (HOX) gene expression signature of fibroblasts is retained in culture[235], suggesting that fibroblast heterogeneity may reflect distinct lineages of site-specific differentiation. Careful examination of compiled evidence suggests that embryonic and developing tissues do not possess fibroblasts and are probably active mesenchymal cells. Once tissue development is completed, most of the active mesenchymal cells undergo apoptosis whereas a few, in every tissue, revert to a quiescent phenotype. Such cells were probably observed by Virchow[30] and later named fibroblasts. In tissues such as adult heart, many such quiescent fibroblasts remain. Therefore, as of today, all the properties attributed to fibroblasts are associated with their activated phenotype, mostly the myofibroblast subtype. The function of quiescent or resting fibroblasts remains unknown. One likely function of quiescent fibroblasts is their ability to differentiate into activated fibroblasts and subsequently, based on the appropriate stimuli, give rise to other mesenchymal lineages, including chondrocytes, adipocytes and endothelial cells. In this regard, a quiescent fibroblast can be considered an adult tissue-resident mesenchymal stem cell (rMSC). Conceivably, such stem cells are different from the bone marrow-derived MSCs (classic BM-MSCs). How these two types of MSC differ in their function remains to be unravelled.

Quiescent or resting fibroblasts	Activated fibroblasts
Morphologically bland (spindle shaped)	Morphologically active (cruciform or stellate shaped)
Metabolically indolent	Metabolically active
G0/G1 arrest or slow cycling self-renewal	Proliferative
Activated by growth factors	Further activated by growth factors
FSP1$^+$, α1β1 integrin$^+$	αSMA$^+$, PDGFRβ$^+$, FAP$^+$
Non-migratory	Migratory
No ECM production	ECM production and synthetic phenotype
No active secretome	Active and dynamic secretome
Epigenetically stable	Epigenetically modified (e.g. *RASAL1* hypermethylation)
Precursor for activated fibroblasts	Precursor for iPSCs, chondrocytes, adipocytes, myocytes and endothelial cells

αSMA, α-smooth muscle actin; ECM, extracellular matrix; iPSCs, induced pluripotent stem cells; PDGFRβ, platelet-derived growth factor receptor-β; *RASAL1*, RAS protein activator like 1.

Box 2 | **Fibroblasts and mesenchymal stem cells**

Fibroblasts from various tissues can be easily cultured on plastic under normal culture conditions in their activated phenotype, similar to what is reported for mesenchymal stem cells (MSCs)[236,237]. MSCs in culture exhibit fibroblast-like morphological features and properties, and become synthetically active and produce extracellular matrix (ECM) and growth factors, among other substrates. Cultured MSCs, just like activated fibroblasts, are considered to be multipotent with a capacity to undergo osteogenic, adipogenic, myogenic and chondrogenic differentiation to generate connective tissue, and with the ability to also generate haematopoietic lineages. Some studies suggest that neuron-like and endothelial-like cells can also be generated from cultured MSCs and fibroblasts. MSCs are purported to be self-renewing — as has also been observed for activated fibroblasts in culture — and share many molecular identifiers with activated fibroblasts. Both undergo senescence, with a diminished capacity to proliferate and differentiate after several passages and with enhanced rapidity when cultured from older tissues. Adipose tissue, umbilical cord and bone marrow are a major source of fibroblasts and MSCs. Moreover, it is well established that embryonic and umbilical cord-derived fibroblasts and MSCs exhibit enhanced survival and stem cell-like properties compared with those isolated from adult tissue. The collective evidence suggests that quiescent or resting fibroblasts are precursors of activated fibroblasts, which could also be called MSCs. Whether all activated fibroblasts are MSCs or only a subset that exhibits such properties remains to be determined and could depend on whether identical culture conditions are used. Nevertheless, one could argue that most of the clinical trials with MSCs can also be considered as clinical testing of cultured, activated fibroblasts.

In fact, a more accurate definition of a fibroblast is a resting mesenchymal cell with the potential to be activated by appropriate stimuli to become an MSC (BOX 2). Such resting mesenchymal cells are rare interstitial cells with the potential to proliferate when stimulated by a growth factor or growth factors, for example, transforming growth factor-β (TGFβ), platelet-derived growth factor (PDGF) and interleukin-6 (IL-6), among others[11,12] (BOX 3). With this new definition for fibroblasts in place, it is proposed that most properties assigned to fibroblasts are in fact properties associated with 'activated' fibroblasts, myofibroblasts and MSCs. Fibroblasts cultured from the site of a healing wound or from fibrotic tissue secrete higher levels of ECM constituents and proliferate more than their counterparts isolated from healthy organs[35,36]. Such increased activity is referred to as activation[35] (FIG. 1). Once activated, their functions include synthesizing ECM, generating cytokines and chemokines, recruiting immune cells and exerting physical forces to modify tissue architecture[37,38]

Although identification of fibroblasts in many tissues is undisputed at this point in time, their identity in embryonic tissue is still ambiguous. Generally, quiescent or resting fibroblasts do not exist in embryonic tissue and they are first encountered in differentiated tissues and organs. In adults, it remains unknown whether MSCs or monocyte precursor-derived mesenchymal cells (fibrocytes) contribute to the population of activated fibroblasts. Some evidence suggests that bone marrow-derived MSCs (BM-MSCs) can enhance the metastatic capabilities of breast cancer cells[39]. For example, BM-MSCs constitute a substantial proportion of CAFs with pro-tumorigenic functions in inflammation-induced gastric cancer[40]. Fibrocytes, or bone marrow-derived CD45$^+$ (CD45 is also known as PTPRC) collagen type I-producing cells, may contribute to fibrotic scarring through collagen deposition and production of a pro-inflammatory secretome[41]. One possibility remains — that MSCs might simply be resting fibroblasts that can be stimulated to generate activated fibroblasts or MSCs with multi-lineage potential. It is well known that activated fibroblasts in culture can become adipocytes, or endothelial or chondrocyte-like cells, and can be induced to become induced pluripotent stem cells (iPSCs)[42–45] (FIG. 2). It is important to remember that quiescent fibroblasts are not used for any such plasticity induction experiments.

Resting mesenchymal cell
Similar to a resting fibroblast, this is a fibroblast-like cell that is ready to be activated when the need arises. This can also be referred to as an adult tissue-resident mesenchymal stem cell (rMSC).

Induced pluripotent stem cells
(iPSCs). The use of differentiated cells such as fibroblasts to induce stem cell-like features by inserting new genes and/or subjecting cells to physical and biochemical pressures to induce a plastic phenotype.

Wound healing, fibrosis and cancer

Acute wound healing. Any injury to the functional parenchyma results in a host response. Injurious stimuli include mechanical trauma, radiation-induced or extreme temperature-induced damage, toxins, pathogens or metabolic impairments. Once a functional parenchymal cell is damaged, an injury response is unleashed to repair the cellular damage and restore tissue homeostasis[21,34,37,46,47]. Such wound healing occurs in response to diverse types of acute injury. The classic wound healing response recruits inflammation, immune cells and fibroblasts to promote angiogenesis and deposition of ECM[12,26,34]. Many of the constituents of the ECM and basement membranes — such as type I, type III, type IV and type V collagens, many different laminins and fibronectin — are produced by activated fibroblasts or myofibroblasts[37,48]. Myofibroblasts are also a major source of ECM-degrading proteases, including matrix metalloproteinases (MMPs), underscoring their crucial role in maintaining ECM homeostasis by regulation of ECM turnover[49]. Myofibroblasts were first identified in the healing wound of the skin, and contraction of the skin in this process was attributed to their action[32,37]. Myofibroblasts, induced by TGFβ-mediated signalling, proliferate and express vimentin and αSMA[17,18]. In addition, activated fibroblasts are important in maintaining the homeostasis of adjacent epithelia, by the secretion of growth factors and by direct mesenchymal–epithelial cell interactions[50] (BOX 1). Once the wound is repaired, the number of activated fibroblasts decreases significantly owing to apoptosis, and the resting phenotype is probably restored[37] (FIG. 1). Such reversibility is the hallmark feature of tissue repair associated with wound healing.

Tissue fibrosis. If the insult is perpetual, such as in the settings of chronic physical, toxic, metabolic or autoimmune insults, the repair response continues unabated and leads to a chronic wound healing condition that is also known as tissue fibrosis. Therefore, tissue fibrosis can be considered as wounds that never complete the healing process, exhibiting continuous repair activation. This is controlled partly by epigenetic mechanisms in the activated fibroblasts, enhancing anti-apoptotic pathways and initiating proliferation to generate hyper-activated fibroblasts. Pathological remodelling and fibrosis in different tissues can engage activation of FAFs with distinct origins, activation markers and functions[51–54].

Carcinoma *in situ* (CIS). A basement membrane-contained cancerous lesion. This type of lesion is the lesion that forms early in carcinogenesis. A basement membrane, presumably deposited by CAFs, separates such lesions from the outside interstitial environment.

Cancer fibrosis. The chronic tissue repair response also occurs in the setting of the genetic insult to the functional parenchyma encountered in cancer. In this regard, tumours are considered 'wounds that do not heal' (REF. 26). Persistent emergence and accumulation of cancer cells in a given tissue represents an ongoing tissue injury, initiating a chronic wound healing response towards the cancer cells. This results in a chronic host repair response in tumours that is known as cancer fibrosis or stroma. The term tumour fibrosis is interchangeable with desmoplastic reaction, tumour stroma and TME. Although the role of myofibroblasts in wound healing is well understood, their functional role in cancer progression and metastasis is emerging as being complex and bimodal, with both cancer-promoting and cancer-restraining actions[12,55,56]. In the 1970s, seminal studies led to the consideration that cancer cells may recruit activated fibroblasts similar to fibroblasts associated with wound healing[57–61]. The recruitment of stromal fibroblasts to the tumour is largely governed by the growth factors released by the cancer cells and the infiltrating immune cells. TGFβ, PDGF and fibroblast growth factor 2 (FGF2) are key mediators of fibroblast activation in acute and chronic tissue damage[62]. The recruitment of activated fibroblasts in many cancers is dependent on TGFβ[63,64]. Local CAF proliferation and invasion is stimulated by TGFβ present in the TME. One school of thought is that activation of fibroblasts reflects a host defence mechanism to restrain cancer progression and potentially eliminate cancer[11,56,62,65]. PDGF secreted by cancer cells and stromal cells (including fibroblasts) can activate and induce the proliferation of fibroblasts and correlates with cancer progression[66]. A detailed mechanism associated with the recruitment of tumour stroma or tumour fibrosis is still being unravelled.

At early stages of tumorigenesis in solid tumours, cancer cells form a neoplastic lesion that arises within the microenvironment of the epithelium[10,67]. Many studies have shown that carcinoma *in situ* (CIS) already involves 'reactive' tumour fibrosis[2,68]. There is an active debate with regard to the function of such reactive tumour stroma in cancer progression, with some arguing that it might provide protection from invasive or malignant conversion of CIS[69]. Generally, the action of the cancer stroma is viewed as promoting cancer initiation and progression. The precise cellular and molecular programmes that enable conversion of CIS into invasive cancer are still largely unknown, but most agree that tumour stroma is a likely contributor. In this regard, myoepithelial cells, which share some common features with myofibroblasts in the mammary ducts containing cancer cells, may have a regulatory role in cancer progression[70]. In the early stages of neoplasia, inflammatory cues, perhaps emerging from pathological tissue remodelling, may initiate pro-inflammatory and tumour-promoting functions in fibroblasts. IL-1β secretion by immune cells in early lesions emerges as a potential initiator of nuclear factor-κB (NF-κB) signalling in fibroblasts, instructing them to produce a pro-tumorigenic secretome[71].

Generating wounds in cancer-prone adult chickens (infected with Rous sarcoma virus) results in invasive carcinomas within the wounded tissue[72]. Transgenic mice expressing the *JUN* oncogene also linked wounding with tumour emergence[73]. Although the connection between wounding and cancer emergence remains unclear in mammals, many epidemiological and clinical studies have suggested that tissue fibrosis in some organs, such as liver, lung and pancreas, may increase the risk of emergence of carcinomas[74–76]. Emergence of hepatocellular carcinoma is implicitly connected with the previous presence of liver fibrosis[77,78]. Normal stroma in most organs contains a small number of quiescent or resting fibroblasts embedded in physiological ECM[2], whereas reactive tumour stroma or fibrosis generally presents with an increased number of activated fibroblasts that usually express αSMA or fibroblast activation protein (FAP) and exhibit increased deposition of collagens, fibrin and other ECM constituents compared with quiescent fibroblasts[6,11,13]. The recruitment of new capillaries and accumulation of immune cells within the TME are mediated by many different growth factors, such as vascular endothelial growth factor A (VEGFA), PDGF, epidermal growth factor (EGF), IL-6 and IL-8, among others. In addition to cancer cell production of VEGFA, a source of host-derived VEGFA is

Box 3 | **Regulatory molecules associated with cancer-associated fibroblast activation**

The exogenous signals leading to the activation of fibroblasts in the tumour microenvironment are numerous and probably distinct in different tumour types. A given set of growth factors and cytokines, specific to each tumour type, may activate the anti-invasive or pro-invasive functions of cancer-associated fibroblasts (CAFs). The cellular origin and rate of production of the CAF-regulatory molecules may also be dynamically modulated. Therefore, a snapshot assessment of their postulated functions on CAFs *in vivo* may not capture the complexity of their roles in tumour progression and metastatic dissemination. *In vitro* studies and associated measurements of fibroblast activation often rely on the commonly used fibroblast activation ligands such as those belonging to the transforming growth factor-β (TGFβ) superfamily and bone morphogenic proteins (BMPs), platelet-derived growth factors (PDGFs), epidermal growth factors (EGFs), fibroblast growth factors (FGFs) and sonic hedgehog (SHH). In this regard, leukaemia inhibitory factor (LIF) has been implicated in the contractile and invasive properties of CAFs[238]. In breast cancer, increased production of WNT7A by invasive cancer cells may enhance TGFβ receptor signalling associated with the invasive properties of CAFs[239]. In pancreatic ductal adenocarcinoma (PDAC), vitamin D receptor activation may suppress the tumour-promoting secretome of CAFs[225]. In early neoplastic lesions, immune cell-derived interleukin-1β (IL-1β) elicits nuclear factor-κB (NF-κB) signalling pathways in fibroblasts, potentially enhancing their pro-inflammatory and tumour-promoting functions[71]. A systems biology approach will probably help in elucidating the complex and rate-limiting functions of CAF-regulatory molecules and their crosstalk with cancer cells[240].

activated fibroblasts[79]. VEGFA, also known for its ability to increase vascular permeability, has a central role in generating vascular leaks, leading to reactive perivascular areas containing fibrin and platelets, which in turn promote accumulation of immune cells, proliferative endothelial cells and activated fibroblasts[80]. Tumour stroma becomes enriched with ECM proteins such as type I collagen and fibronectin, which initiate tumour angiogenesis[80–82].

As tumours grow and become invasive, the stromal content also increases. Tumour stroma in advanced stages of cancer contains increased amounts of various types of collagens, laminins, fibronectins, proteoglycans, periostin and tenascin C, among others[5,83]. Periostin and tenascin C, produced in part by activated fibroblasts, are generally absent in normal adult mammary tissue, but they become expressed in breast cancer[2,84–87]. Many recent studies continue to suggest that activated fibroblasts regulate cancer progression via their active secretome, which includes growth factors and ECM (FIG. 3).

Fibroblast function and heterogeneity

It is now clear that many different markers can identify activated fibroblasts. Such markers include fibroblast-specific protein 1 (FSP1; also known as S100A4), vimentin, αSMA, FAP, PDGF receptor-α (PDGFRα), PDGFRβ, desmin and discoidin domain-containing receptor 2 (DDR2)[4]. Of note, PDGF receptors (PDGFRα and PDGFRβ) and the associated downstream signalling can be pharmacologically targeted[88–90]. FSP1 is a reliable marker to detect quiescent, non-proliferating ($Ki67^-$) fibroblasts, in the interstitium[91]. It is important to emphasize that none of these markers is specific for fibroblasts or activated fibroblasts. For example, FSP1 also identifies macrophages and possibly other immune cells and is expressed by some cancer cells[92,93]. FAP is also present in a subset of $CD45^+$ immune cells[94]. Desmin and PDGFRβ are also expressed in perivascular cells[95]. Therefore, when using these markers, context, morphology and spatial distribution should be taken into consideration to identify cells as resting or activated fibroblasts. Also, it is likely that many functionally activated fibroblasts may not express all of these putative markers at the same time[96,97], creating another degree of heterogeneity (FIG. 4). Whether each subset of activated fibroblasts, as defined by several overlapping or non-overlapping markers, performs unique functions remains unknown. CAF markers may also be associated with cells with diverse, and possibly opposing, functions in the context of specific TMEs. Loss of caveolin 1 (CAV1) expression in breast tumour CAFs may define metabolically altered fibroblasts with pro-tumorigenic functions[98,99], yet high expression of CAV1 in CAFs could also facilitate tumour invasion via ECM remodelling[100]. It is conceivable that, just like T cell differentiation, resting fibroblasts might be capable of differentiating into distinct subsets of functional fibroblasts, which, one may speculate, could possess diverse activities (FIG. 5). To identify such subsets, one may have to use multiple cell surface markers for detection. Once isolated, functional studies could be conducted to unravel specialized activities.

Stellate cells
Particular mesenchymal cells that are characterized by their vitamin A stores and are found in the liver and pancreas, among other organs. Upon stimulation, they can become activated fibroblasts or myofibroblasts.

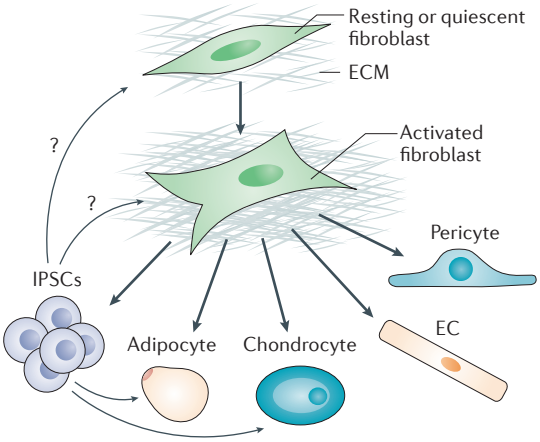


Figure 2 | **Fibroblasts are highly plastic and exhibit multipotency.** Activated fibroblasts readily transdifferentiate into chondrocytes, myocytes, adipocytes and endothelial cells (ECs), among others[42–45]. Activated fibroblasts may adapt perivascular and vascular functions. Induced pluripotent stem cells (iPSCs) were first engineered using activated fibroblasts, and these cells remain the most efficient cellular source for iPSC reprogramming[42]. The plastic nature of fibroblasts may also contribute to their functional heterogeneity. ECM, extracellular matrix.

The heterogeneity of fibroblasts, in particular those that are activated from their quiescent state, could also depend on the origin of the precursor fibroblasts. Activated fibroblasts are reported to arise from bone marrow-derived precursors, MSCs, endothelial cells, liver and pancreas stellate cells, resting tissue fibroblasts and possibly from some types of epithelial cell[4,11,22,40]. Depending on their origin, the function of such activated fibroblasts could be diverse and unique. It is likely that future studies will tackle these exciting questions.

Epigenetic regulation of fibroblasts. In most carcinomas, activated fibroblasts are thought to be master regulators of many diverse stromal programmes and cancer cell signalling pathways[20]. The signals that mediate the transition of resting fibroblasts into CAFs in tumours are probably complex, but in culture some of the phenotypic features associated with CAFs can be induced by TGFβ[18]. Nevertheless, emerging data suggest that the irreversible activation of fibroblasts might be driven by epigenetic alterations[15,16,101,102]. Unlike wound healing, but similar to organ fibrosis, the fibroblasts in the tumour remain perpetually activated. In recent years it has been proposed that in acute settings, growth factor-induced activation of fibroblasts is reversible. But in the chronic setting of tissue fibrosis and tumour stroma, the activated fibroblasts acquire unique properties that are not observed in fibroblasts associated with wound healing or acute tissue injury (FIG. 1). Studies with human breast tumours show that stromal cells (presumably fibroblasts) have unique epigenetic changes that are not observed in fibroblasts from normal mammary tissue[103].

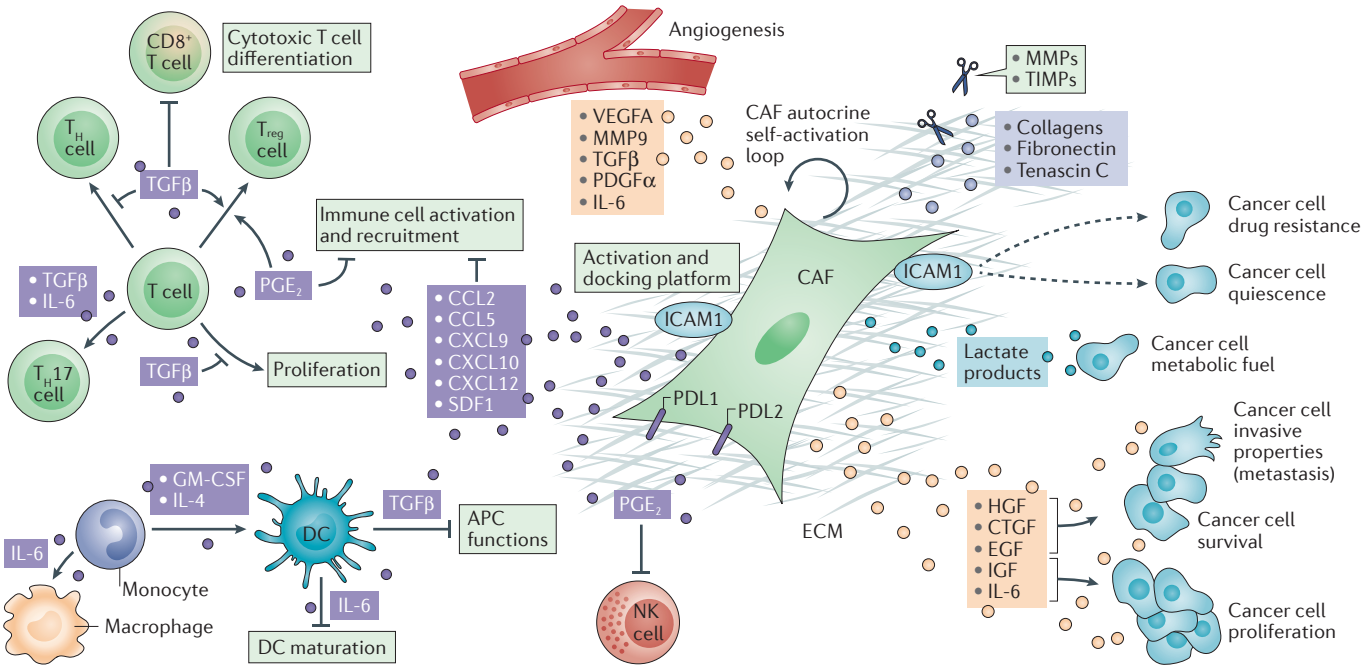


Figure 3 | **CAFs and their secretome remodel the tumour stroma.** The extracellular matrix (ECM), together with cellular components of the tumour microenvironment (TME), are actively remodelled and reprogrammed by cancer-associated fibroblasts (CAFs). Secretory functions mediate immune reprogramming (left) and self-sustained activation (middle), and engage cancer cells (right), promoting or restraining their growth, survival or resistance to therapy. Metabolic reprogramming in CAFs may also fuel the TME and enhance the adaptation of cancer cells to a growing tumour. CAFs also strongly engage tumour angiogenesis, which indirectly affects immune cell recruitment and activation, and cancer cell migratory and invasive properties. Intercellular adhesion molecule 1 (ICAM1), which is expressed on CAFs, may serve as a docking site for the activation or repression of immune cells, and programmed cell death protein 1 ligand 1 (PDL1) and PDL2 expression on CAFs may mediate immunosuppressive functions. The CAF secretome exerts potent remodelling effects on tumour immunity, affecting innate immune cell recruitment and activation, and polarizing the adaptive immune response. APC, antigen-presenting cell; CCL, C-C motif chemokine ligand; CTGF, connective tissue growth factor; CXCL, C-X-C motif chemokine ligand; DC, dendritic cell; EGF, epidermal growth factor; GM-CSF, granulocyte–macrophage colony-stimulating factor; HGF, hepatocyte growth factor; IGF, insulin-like growth factor; IL, interleukin; MMP, matrix metalloproteinase; NK, natural killer; PDGFα, platelet-derived growth factor-α; PGE_2, prostaglandin E_2; SDF1, stromal cell-derived factor 1; TGFβ, transforming growth factor-β; T_H, T helper; TIMPs, tissue inhibitors of metalloproteinases; T_{reg} cell, regulatory T cell; VEGFA, vascular endothelial growth factor A.

It has long been recognized that FAFs and CAFs can be cultured with ease from tumour tissue and they proliferate at much higher rates than activated fibroblasts cultured from normal tissue or from tissue with acute injury or healing wounds. When FAFs are treated with the demethylating agent 5-azacytidine, the proliferation rate is reduced and collagen I synthesis and αSMA expression is decreased[104]. Hypermethylation of RAS protein activator like 1 (*RASAL1*) and downregulation of RASAL1 protein lead to *de novo* activation of RAS in kidney FAFs but not in fibroblasts isolated from normal tissue or fibroblasts associated with acute kidney injury[104]. This study provides the first evidence for causative epigenetic alterations in irreversible activation of activated FAFs. In lung fibrosis, epigenetic modifications may confer FAFs with resistance to FAS (also known as TNFRSF6)-mediated apoptosis[105] and myofibroblast differentiation[106,107]. Prostate stromal cells with overexpression of the epigenetic regulator high-mobility group AT-hook 2 (HMGA2) were sufficient to induce prostatic intraepithelial neoplasia[108]. An epigenetic switch implicating the regulation of leukaemia inhibitory factor (*LIF*) may result in the sustained and pro-invasive functions of CAFs, via enhanced Janus kinase 1 (JAK1)–signal transducer and activator of transcription 3 (STAT3) activation. Dual inhibition of DNA methyltransferase (DNMT) and JAK activity restores the non-invasive phenotype of CAFs[109]. Future studies are likely to unravel many more such epigenetic control nodes in the generation of CAFs.

CAFs: positive regulators of cancer

CAFs in tumorigenesis. Activated fibroblasts isolated from various human tumours exhibit many distinct properties when compared with fibroblasts cultured from normal organs[32] (FIG. 1). It is important to remember that fibroblast activation programmes can be induced *de novo* merely by culture conditions. Nevertheless, some fundamental differences exist between the cultured normal fibroblasts and fibroblasts cultured from organs with tissue fibrosis or from tumour tissue. FAFs and CAFs proliferate robustly when compared with

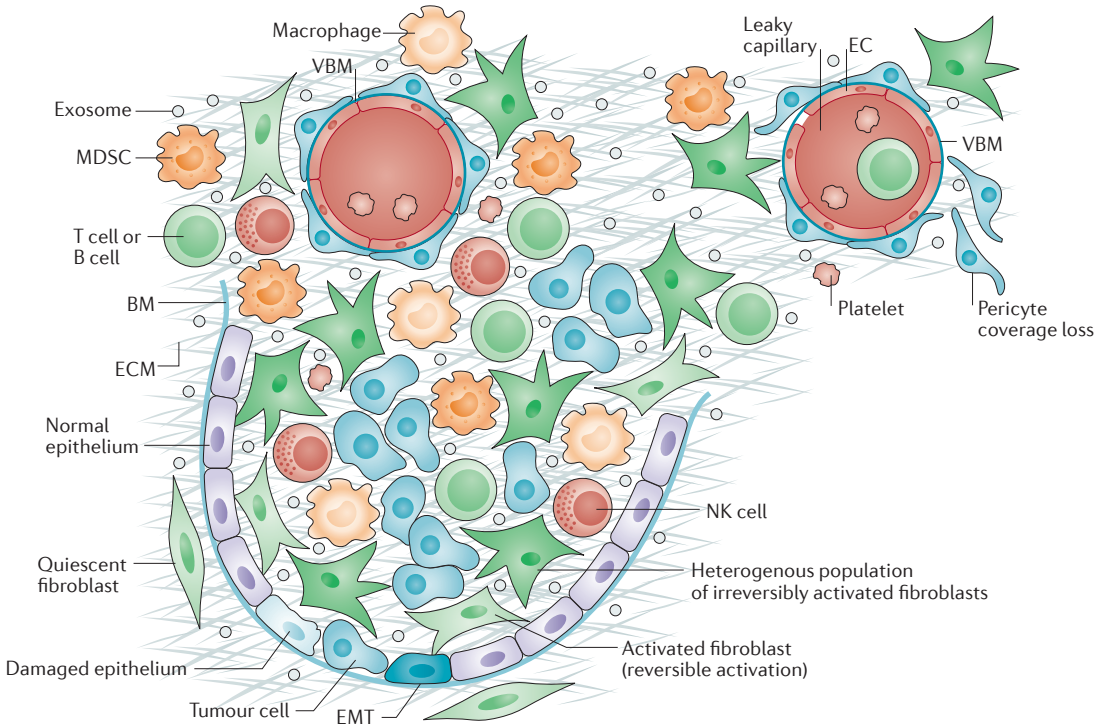


Figure 4 | **Fibroblasts are a heterogeneous population of the tumour microenvironment.** Fibroblasts constitute a dominant cellular component of the tumour microenvironment (TME). The TME is composed of immune cell infiltrates, normal and injured epithelium, neoplastic epithelial cells and blood vessels, which include endothelial cells, pericytes and vascular basement membrane (VBM). Cancer-associated fibroblasts (CAFs) are a heterogeneous population of irreversibly activated fibroblasts with distinct functions. BM, basement membrane; EC, endothelial cell; EMT, epithelial to mesenchymal transition; MDSC, myeloid-derived suppressor cell; NK, natural killer.

normal activated fibroblasts (NAFs)[104,110]. CAFs and FAFs exhibit enhanced migratory capacity, autocrine growth factor-induced signalling and increased levels of secretory molecules that include growth factors and chemokines (FIG. 1). Such differences could be due to epigenetic changes that are found, possibly as a consequence of the milieu that promotes their activation, in FAFs and CAFs but not in NAFs.

In many different co-culture experiments, CAFs enhance tumorigenesis of cancer cells when compared with NAFs[111,112]. Initially it was shown that when Simian virus 40 (SV40)-transformed prostate epithelial cells were mixed with NAFs or CAFs, and the mixtures were inoculated into mice, the CAFs but not the NAFs, led to the formation of tumours resembling prostatic intraepithelial neoplasia[112]. CAFs also induce invasion by non-invasive cancer cells[113]. Subsequently, such studies were reproduced in other cancer systems[111]. The ability of CAFs to influence tumour growth was partly dependent on their ability to induce angiogenesis by CAF-derived stromal cell-derived factor 1 (SDF1; also known as CXCL12) and recruitment of bone marrow-derived endothelial cells[111]. Several secreted molecular regulators of CAFs have a pro-tumorigenic role (BOX 3). Upregulation of heat shock factor 1 (HSF1) in CAFs may complement the HSF1-driven pro-tumorigenic programme in cancer cells, supporting a pro-cancer influence of the TME[114], and Yes-associated protein 1 (YAP1) activation in CAFs enhances ECM stiffening and cancer cell invasion[115]. Deregulation of Notch and p53 signalling pathways in CAFs further enhances their proliferation[116]; more work is needed to understand how these pathways become deregulated in CAFs.

Activated fibroblasts produce ECM-degrading proteases such as the MMPs[117–119]. Motility and invasion of cancer cells are facilitated by MMPs. Stromelysin 1 (also known as MMP3), is produced robustly by activated fibroblasts and cleaves E-cadherin, prompting epithelial to mesenchymal transition (EMT) and invasiveness in adjacent cancer cells[120]. Additionally, activated fibroblasts produce MMP1, which also induces invasiveness[117]. Further, when TGFβ receptor type II (*Tgfbr2*) is deleted in FSP1$^+$ fibroblasts, it leads to invasive squamous cell carcinoma of the forestomach and prostatic intraepithelial neoplasia that is dependent on fibroblast-produced hepatocyte growth factor (HGF)[8]. Fibroblast-derived exosomes have also emerged as positive mediators of cancer progression and stromal remodelling, by their regulation of fibroblast activity and chemoresistance[121–124]. Fibroblasts lacking four members of the tissue inhibitor of metalloproteinases (TIMP) family generate exosomes with enhanced MMP expression and disintegrin and metalloproteinase domain-containing protein 10 (ADAM10) activity, associated with enhanced cancer cell motility, metabolic reprogramming and induction of cancer stem cell features[125].

Normal activated fibroblasts
(NAFs). Fibroblasts cultured from normal organs.

Epithelial to mesenchymal transition
(EMT). Acquisition of mesenchymal expression programme by epithelial cells.

Exosomes
Extracellular vesicles, approximately 40–150 nm in size, released by all cell types and of multivesicular endosomal origin. They carry proteins and nucleic acids.

Cancer stem cell
Conceptually, a cancer cell that is able to give rise to malignant cancer cells indefinitely and generate tumours; operationally, cancer stem cells are a subset of cancer cells that can, in mice, initiate tumour formation in limiting dilution assay.

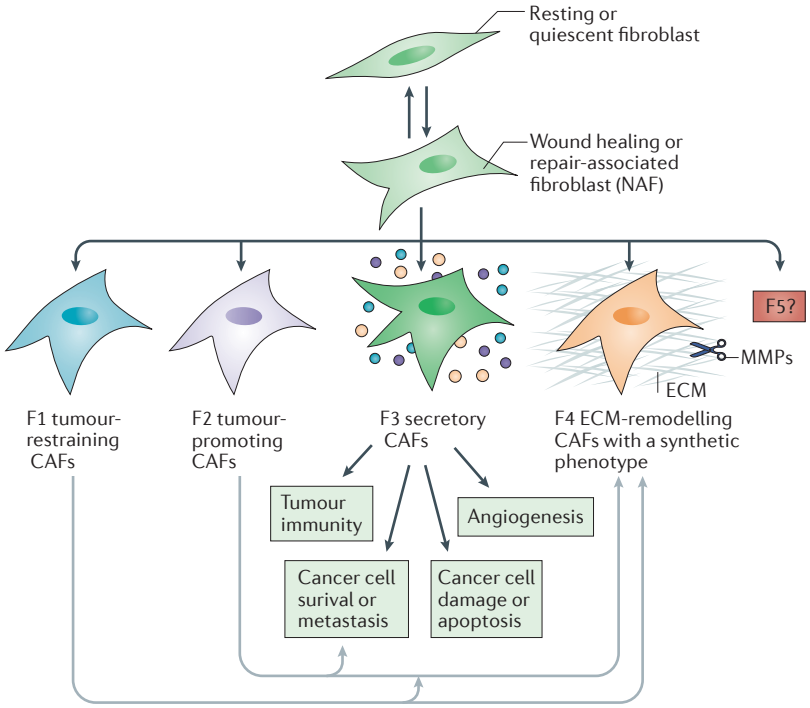


Figure 5 | **A proposed classification of subtypes of fibroblasts on the basis of function.** Following the activation of quiescent fibroblasts, the wound healing- or repair-associated fibroblasts (normal activated fibroblasts; NAFs) may further differentiate in step-wise activation, based on context-dependent signalling, into distinct functional subtypes. The tumour-restraining (F1 subtype) and the tumour-promoting (F2 subtype) cancer-associated fibroblasts (CAFs) could be distinct, context-dependent populations but with the potential to interchange. We speculate that an F3 subtype may constitute specialized CAFs with enhanced growth factor secretome activity that influences tumour immunity, angiogenesis and cancer cell proliferation or dormancy. The F4 subtype could define an extracellular matrix (ECM)-producing CAF with the ability to remodel the ECM content of the tumour microenvironment (TME). It is conceivable that more defined subtypes will be identified. The subtypes may acquire specific epigenetic marks to define their function. Characterization of other functions of CAFs (speculatively, an F5 subtype?) and precise molecular definitions of the putative F1–F4 subtypes may offer additional insights into TME biology and inform clinical opportunities for new cancer therapies.

ECM remodelling by fibroblasts may also participate in the generation and maintenance of the cancer stem cell niche. Fibroblasts can be co-opted by cancer stem cells to induce a milieu that could promote cancer stem cell maintenance. Periostin (POSTN) expression by fibroblasts is probably rate limiting for stromal remodelling, WNT ligand recruitment and WNT signalling-mediated cancer stem cell niche maintenance[126]. Elevated WNT signalling is found in colon cancer stem cells that are proximal to CAFs, possibly implicating CAF-derived HGF in regulating cancer stem cell niche formation[127]. In lung cancer, the cancer stem cells or cancer-initiating cells are strong activators of fibroblasts via thrombospondin 2 (THBS2) expression, enhancing metastatic growth[128]. Paracrine signalling between cancer stem cells and fibroblasts engages fibroblast-derived insulin-like growth factor II (IGF2) and IGF1 receptor (IGF1R) signalling in cancer stem cells, inducing Nanog expression and stemness-like phenotypes in cancer cells[129].

Warburg effect
The metabolic phenotype of cells operating predominantly on glycolysis and lactate production despite the bioavailability of oxygen to run oxidative phosphorylation. This is a feature of rapidly proliferating cells, including cancer cells.

CAFs in metastasis. Activated fibroblasts are important mediators of secondary tumour growth at the metastatic site. At the primary site, CAFs may enhance metastasis by releasing growth factors and cytokines into the circulation to stimulate, indirectly or directly, the growth and invasive features of cancer cells at a distant site[114,130,131]. CAFs may also affect ECM stiffness at primary tumours, enhancing cancer cell invasion[132], and they may generate ECM tracks to guide cancer cell invasion[133]. Metastasis-associated fibroblasts (MAFs) that express tenascin C and VEGFA are key mediators of breast cancer metastasis to the lung[134]. Melanoma metastasis to the liver relies on the activation of stellate cells (which are liver-resident fibroblasts) to support angiogenesis[135]. In colorectal cancer, TGFβ1-stimulated CAFs secrete IL-11 to enhance the survival of colorectal cancer cells and increase the efficiency of organ colonization[136]. PDGF-stimulated CAFs were also reported to enhance colorectal cancer cell intravasation and formation of distant metastases via the secretion of stanniocalcin 1 (STC1)[137]. Lung microenvironment-associated fibronectin and VEGF receptor 1 (VEGFR1)$^+$ cells from the bone marrow also support metastasis of melanoma cells[138]. Loss of *Fsp1* in mice (*Fsp1*-knockout mice) leads to impaired motility of fibroblasts and is associated with reduced metastasis[139]. MAFs may be recruited to or activated at the metastatic site as a result of metastatic cancer cell seeding and inflammatory responses. They may emerge from other tumour sites or from the bone marrow and be recruited to metastases, or they may be activated tissue-resident fibroblasts. Their multiple origins may also contribute to their functional heterogeneity.

CAFs and cancer metabolism

The cellular metabolism of CAFs mimics that of highly proliferating cells, which rely on aerobic glycolysis[140]. The drivers of metabolic shifts in the activation of fibroblasts may include TGFβ, PDGF, hypoxia, hypoxia-inducible factor 1α (HIF1α) and reactive oxygen species (ROS)-mediated suppression of CAV1 (REFS 98,141,142). Metabolic adaptation may reflect the survival response of CAFs to intratumoural hypoxia, possibly to sustain their acquired proliferative programme (FIG. 6). The increased Warburg effect in CAFs seems also to be coupled with increased catabolic activity and autophagy[98,141,143]. These metabolic adaptations have been posited to have a pivotal role in repurposing nutrients for other cells of the TME and cancer cells. Specifically, the enhanced lactate production in *Cav1*-knockout fibroblasts, as well as increased levels of ketone bodies, fatty acids and glutamine, emerge as possible fuel sources for mitochondrial respiration in anabolic cancer cells[141,144]. CAFs may play a part in the anabolic and catabolic balance of cancer cells in the regionally diverse TME milieu. The enhanced reliance on anabolic metabolism and mitochondrial respiration by cancer cells may promote the emergence of therapeutic resistance pathways and metastasis[145–147].

Not only may the metabolic reprogramming of CAFs result from paracrine signalling from cancer cells but direct intercellular contacts between CAFs and cancer

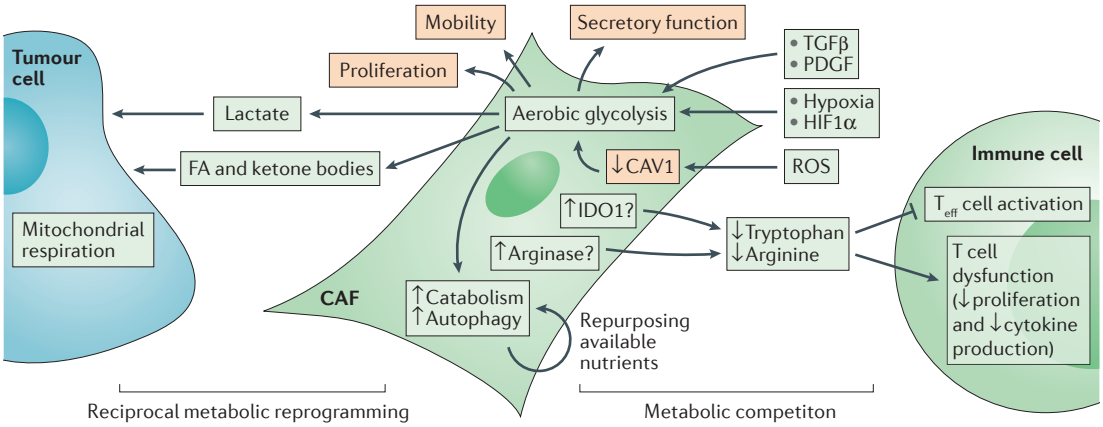


Figure 6 | **CAFs and metabolic reprogramming of the tumour microenvironment.** Fibroblast activation includes metabolic alterations to achieve functional conversion to the activated state and metabolic conversion to sustain activated secretome functions, proliferation and cellular mobility. The increased reliance of cancer-associated fibroblasts (CAFs) on aerobic glycolysis may be mediated in part by decreased oxygen availability in growing tumours, hypoxia-inducible factor 1α (HIF1α) stabilization, transforming growth factor-β (TGFβ) and platelet-derived growth factor (PDGF) signalling and reactive oxygen species (ROS)-mediated loss of caveolin 1 (CAV1). Increased production of lactate, fatty acid (FA) and ketone bodies may support cancer cell mitochondrial respiration and associated invasive and resistance properties. This reciprocal metabolic reprogramming between CAFs and cancer cells differs from the metabolic relationship of CAFs with other tumour microenvironment (TME) components. Increased catabolic and autophagic pathways in CAFs may regulate the bioavailability of metabolites to immune cells, and such metabolic competition may impair tumour immunity. Elevated indoleamine 2,3 dioxygenase 1 (IDO1)) and arginase (ARG1 and ARG2) in CAFs could deplete tryptophan and arginine bioavailability, decreasing T effector (T_{eff}) cell activation and proliferation.

cells may stimulate and reinforce mutual metabolic reprogramming[148]. Global gene expression profiling studies of CAFs implicated suppression of isocitrate dehydrogenase 3A (IDH3A), HIF1α stabilization and induction of pro-glycolytic genes in normoxic conditions[142]. Activation of p62 in CAFs impairs cellular redox and ROS-mediated increase in IL-6 production[149]. Although the specific molecular underpinnings that initiate CAF metabolic reprogramming require further study, the metabolic shift of CAFs, once established, may be self-sustaining, possibly in part via epigenetic remodelling. Conversely, the metabolic symbiosis of CAFs and cancer cells may dynamically evolve in response to oxygen, extracellular metabolite availability and chemokine or cytokine signalling.

Haploinsufficiency of the oxygen sensor and HIF1α regulator prolyl-hydroxylase 2 (PHD2; also known as EGLN1) in MMTV-PyMT (mouse mammary tumour virus-polyoma middle T antigen) mice suppresses CAF activation *in vivo* and is associated with decreased pulmonary metastases, likely owing to decreased cancer cell-derived TGFβ1 production and reprogramming of CAFs[150]. Hypoxia may also have a direct role in CAF activation[151]. These observations suggest that oxygen sensing and metabolic reprogramming of CAFs may affect their function (or functions) in growing tumours. Finally, CAF metabolism may adapt further in response to cancer therapy regimens (discussed below).

The impact of the changes in the metabolic programmes of CAFs has thus far been studied mostly in the context of their influence on cancer cell growth, proliferation and invasion. CAF metabolism may also induce pro-tumorigenic circuits by altering metabolite availability. Tryptophan and arginine starvation are crucial for T cell activation and lymphocyte function, and metabolic adaptation of CAFs may regulate the bioavailability of these inflammatory metabolites[152–155]. Cytotoxic $CD8^+$ T cells exhibit enhanced glycolytic metabolism, and the role of CAFs in controlling nutrient bioavailability for immune cell responses also supports the immunomodulatory functions of CAFs. In this regard, metabolic competition between stromal cells may result in T cell hyporesponsiveness in tumours[152].

Epithelial carcinogenesis may instruct metabolic reprogramming of CAFs and their glycolytic and catabolic adaptation to promote a reciprocal metabolic symbiosis between CAFs and cancer cells[156]. Whether inadvertently or specifically directed, the symbiotic relationship between CAFs and cancer cells creates a tumour metabolic ecosystem that could be a potential target for cancer therapy. Whether such a symbiotic relationship operates to facilitate metastatic growth of cancer remains largely unknown.

CAFs and tumour immunity

The pleiotropic immunomodulatory functions of CAFs may be direct or indirect. CAFs may adopt a secretory phenotype, enabling the synthesis of ECM proteins, expression of ECM-remodelling enzymes and the production of a plethora of cytokines and chemokines. The secretome of CAFs not only contributes to their sustained activated state during tumour progression, but may also dynamically evolve during cancer progression, thus potentially affecting tumour immunity differently at different stages of cancer progression. The CAF secretome has also been implicated in directly regulating tumour immunity (FIG. 3). Although recent secretome analyses

Reciprocal metabolic symbiosis
Whereby cancer-associated fibroblasts provide metabolic support to cancer cells in tumours, and vice versa.

have reinforced the notion that CAF-specific secretomes may modulate tumour immune cell recruitment and activation, such profiling studies are limited to cells expanded *in vitro*[157–160], and it is therefore challenging to firmly define the crucial functions of CAF secretomes in conferring immunomodulatory responses *in vivo*. Nevertheless, these studies provide information on fibroblast secretome heterogeneity[157,159], and its differential impact on tumour biology with or without chemotherapy[160]. Future studies using fibroblast-specific deletion of crucial cytokines and chemokines in preclinical tumour models may offer a more precise, functional list of CAF-derived specific immunomodulatory cytokines and chemokines.

Generally, CAFs are considered to promote an immunosuppressive TME[161,162]. This may, however, be context dependent rather than a specific feature of CAFs. In the hypoxic TME, CAFs, cancer cells, endothelial cells and immune cells interact dynamically, and this could enhance the complexity of their paracrine signalling responses. Furthermore, most of the studies directly linking CAF immunomodulatory secretomes and immune responses are based largely on *in vitro* measurements or *in vivo* studies involving admixing of CAFs that were expanded *in vitro* before injection with cancer cells *in vivo*. Secretion of cytokines, chemokines and pro-angiogenic factors by CAFs in established tumours, including, but not limited to, IL-6, IL-4, IL-8, IL-10, tumour necrosis factor (TNF), TGFβ, C-C motif chemokine ligand 2 (CCL2), CCL5 (also known as RANTES), C-X-C motif chemokine ligand 9 (CXCL9), CXCL10, SDF1, prostaglandin E_2 (PGE_2), nitric oxide (NO), HGF and human leukocyte antigen G (HLAG) may have direct and/or indirect implications for tumour immunity[163–165].

Although IL-6 signalling has been implicated in restricting the maturation of dendritic cells (DCs), disabling T cell activation and inducing T cell anergy[166], fibroblast-derived IL-6 also redirects monocytes towards differentiation into a macrophage lineage rather than DC differentiation[167], and recruits and activates mast cells[168]. It is still unclear whether CAF-derived IL-6 is rate limiting for the observed immunological reactions or whether other sources of IL-6 in the TME and additional growth factors, such as TGFβ, are also required and synergistic. TGFβ regulates a myriad of mainly immunosuppressive responses[169], and recent studies have also implicated TGFβ in T helper 17 cell (T_H17 cell) differentiation[170], adding additional contextual complexity to CAF-derived TGFβ immunomodulatory functions[171]. On the basis of *in vitro* evaluations, CAF production of IL-4, IL-6 and IL-8 may induce immunosuppressive myeloid cell differentiation[19,172]. CAF-derived CXCL14 affects tumour immunity, in particular affecting macrophage recruitment to the tumour[173]. These studies highlight paracrine CAF–immune cell signalling that places their interaction at a crucial node of control for neoplasia and malignancy[174,175]. CCL2 also has a role in breast cancer progression in preclinical models[176], but the evidence for CAF-derived CCL2 in mediating these effects is restricted to *in vitro* analyses[176,177]. CCL2 is also produced by macrophages and DCs, and the CAF secretome may overlap with that of other stromal cells. Such secretome overlap may enhance the influence of CAFs on the TME. It has also been implied that T cell recruitment involves cytokines that are found in the CAF secretome, such as CXCL9, CXCL10 and SDF1 (REF. 178). Cultured fibroblasts from normal human colons have been reported to express negative co-regulatory immune signals (for example, programmed cell death 1 ligand 1 (PDL1) and PDL2) with a potential impact on T cell activation[179]. In a subset of CAFs derived from patients with lung cancer, expression of PDL1 and PDL2 may convey an immune-suppressive effect on T cell activation *ex vivo*[180]. Whether CAFs also contribute to immunosuppressive adaptive responses in solid tumours through gain of expression of co-regulatory signals *in vivo* remains unknown.

CAFs may also modulate tumour immunity indirectly, via their impact on tumour angiogenesis (by regulating trans-endothelial migration of immune cells), and through their acquisition of adhesion molecules (for example, intercellular adhesion molecule 1 (ICAM1)), which would offer immune cells a docking platform for specific reactions leading to their activation, repression or polarization[21]. CAFs are also influenced by immune cells via paracrine signalling, although the dynamic network of responses and potential reverse signalling between CAFs and immune cells remain largely unknown. Polarization of CAF phenotypes[181] during tumour progression may also offer a more dynamic and heterogeneous influence on tumour immunity (FIG. 5).

CAFs and cancer cells construct ECM protein networks that are presumed to restrict access of immune cells to cancer cells, inferring that CAFs and their generated ECM serve as a physical barrier to tumour infiltration by immune cells. Alternatively, ECM remodelling, which is in part mediated by CAFs, may promote T cell contact with cancer cells[182]. ECM remodelling releases pro-inflammatory growth factors and cytokines, and unmasks cryptic binding sites that could promote immune cell adhesion[5,183]. In genetically engineered animal models and studies of orthotopic tumour grafts in immunocompetent mice, targeting FAP^+ CAFs showed antitumour effects via intratumoural recruitment of $CD8^+$ T cells and $CD8^+$ T cell-mediated cancer cell killing[184,185]. Targeting FAP^+ CAFs with a DNA vaccine or treatment of mice with tranilast to suppress collagen synthesis (albeit not specifically targeting CAFs) resulted in recruitment and activation of $CD8^+$ T cells and immune control of tumour growth[186,187].

The dominant view of the pro-tumorigenic role of CAFs in cancer progression has been challenged and CAFs may restrain cancer progression[96,188] (discussed further below). Specific depletion of αSMA^+ CAFs in transgenic mice expressing viral thymidine kinase under the control of the *Acta2* (which encodes αSMA) promoter and enabling ganciclovir-mediated specific targeting of proliferating αSMA^+ cells, led to invasive tumours associated with an immunosuppressive adaptive response, with increased regulatory T cell (T_{reg} cell) infiltration[96]. Although the dominant views of CAFs as tumour promoting are largely driven by co-culture admixture experiments, ongoing and future studies may refine the functional role of CAFs in tumour progression and modulation of tumour immunity.

Immunosuppressive TME
A tumour microenvironment (TME) that contains cells and other components that interfere with tumour immunity and surveillance.

Dendritic cells
(DCs). Also known as accessory cells, they are antigen-presenting cells in the adaptive immune response.

T cell anergy
Following antigen presentation, T cells may become functionally inactivated, in a hyporesponsive state, to induce immune tolerance.

T helper 17 cell
(T_H17 cell). Interleukin-17 (IL-17)-producing T helper cells.

Migration
Movement of cells dependent on motility-inducing molecular signals.

Regulatory T cell
(T_{reg} cell). Regulatory T cells or suppressor T cells maintain immune tolerance to self-antigens and prevent unrestricted effector T cell expansion.

Drug resistance
The adaptive or evasive programmes launched by tumours after treatment with a drug.

CAFs and drug resistance

A relentless clinical challenge for cancer therapy is the development of resistance, which re-enables cancer dissemination and metastasis despite therapeutic efforts. Cancer therapy resistance is defined as progression of a cancer lesion concurrent with or secondary to an initial response to therapeutic intervention. Early studies pointed to the role of organ-specific microenvironments for drug resistance[189], and CAFs have emerged as key players in promoting cancer cell evasion of anticancer therapies. A stroma-associated gene signature is associated with chemoresistance (to 5-fluorouracil, epirubicin and cyclophosphamide) in breast cancer, with a predictive value for response to chemotherapy in the neoadjuvant setting[190]. Although intriguing and robust, such studies linking reactive stroma signatures with poor response to chemotherapy implicates CAFs as promoters of resistance to therapy only in a guilt-by-association manner rather than by establishing a causal association. Continuing efforts to establish such mechanistic connections are ongoing and are naturally of great interest to better harness the therapeutic value of anticancer therapies.

Mechanisms of resistance involving the stroma include the modulation of pathways involving cancer cell–ECM interactions, CAF–ECM adhesion and cytokine- or chemokine-mediated signalling pathways[191,192]. CAFs may also participate in increased intratumoural interstitial fluid pressure, thus indirectly inhibiting uptake of anticancer drugs[193]. Other studies have suggested that CAF-mediated immune modulation, pro-angiogenic actions and metabolic reprogramming of the TME might aid in cancer cell survival and facilitate escape from therapy-induced cancer control[12,194,195]. Although most studies on this topic rely on *in vitro* analyses and xenograft models of cancer progression, studies using genetically engineered mouse models (GEMMs) and clinical specimens may shed further light on unforeseen actions of CAFs as inhibitors of anticancer therapies.

Enhanced adhesion of cancer cells to ECM may offer a signalling platform that enhances pro-survival mechanisms[196–198]. Such pro-survival responses may engage a dormancy phenotype, via β1-integrin-mediated cell cycle arrest[198,199]. CAFs in BRAF-mutant melanomas may participate in resistance to BRAF inhibition by generating a fibronectin-rich ECM that enhances β1-integrin-induced focal adhesion kinase (FAK)–SRC-mediated ERK activation, which compensates for BRAF inhibition in cancer cells[200]. Crucially, such CAF-mediated programmes may not necessarily be facilitating resistance, but may instead be facilitating new mechanisms of cancer development, for example, by promoting the outgrowth of resistant clones. Of note, the adhesion of cancer cells directly to CAFs may also confer drug resistance, possibly via N-cadherin homotypic binding and increased AKT pro-survival signalling in the tumour cell[201,202]. Interestingly, the gain of adhesive properties of cancer cells to CAF-remodelled ECM can induce EMT in the cancer cells, leading to therapeutic resistance[203–205]. The EMT programme may confer chemoresistance of cancer cells by inducing cell cycle arrest[96,145] or by altering the expression of cellular transporters enabling chemotherapy uptake[206].

CAFs may also confer resistance to anticancer drug therapy on cancer cells by means of soluble factors[191]. In this context, TGFβ, IL-6 and HGF produced by CAFs have emerged as potential drug resistance mediators. TGFβ may induce mesenchymal programmes in cancer cells, enabling their enhanced adhesion to ECM, and CAF-produced IL-6 induces well-studied pro-survival signalling cascades[207]. HGF is also a key modulator of CAF-mediated resistance to receptor tyrosine kinase inhibitors[208]. CAF-derived HGF was shown to promote resistance in preclinical cancer models treated with BRAF-V600E[209] or epidermal growth factor receptor (EGFR)[210] inhibitors. Cancer cells showing survival advantages after targeted therapy may thus emerge via co-opting CAF-derived autocrine and paracrine signalling within the TME. These observations provide a rationale for co-targeting of CAF and cancer cells, possibly harnessing stroma-induced synthetic lethality pathways[211]. However, the proposed targeting of Hedgehog (Hh) signalling to suppress CAF pro-tumorigenic functions in pancreatic ductal adenocarcinoma (PDAC)[212] resulted in a failed phase II clinical trial[213]. Follow-up studies, with long-term assessment of preclinical models and specific genetic targeting strategies, supported the antitumorigenic functions of CAFs and Hh signalling in GEMMs of PDAC[96,188]. A careful auditing of the CAF secretome in conferring cancer cell drug resistance will be necessary to better predict the value of targeting CAFs for clinical intervention.

Several combinatorial strategies are being tested in the clinic to overcome CAF-mediated drug resistance[191], and careful analyses of the stromal responses and adaptation to therapy will inform on the convergent response of CAF–cancer cell signalling during therapy. One strategy includes enzymatic breakdown of CAF-deposited ECM. Degradation of hyaluronic acid[214,215] or anti-angiogenic therapies[216,217] may reform and normalize tumour vessels, enable more efficient chemotherapeutic delivery to cancer cells in solid tumours or promote immune-mediated antitumour benefit. Although promising, these approaches are fundamentally designed on the premise of an absolute pro-tumorigenic function of CAFs, a concept that is being challenged and needs more focused studies to clarify this issue.

CAFs: negative regulators of cancer

There is an overwhelming abundance of literature that supports a tumour-promoting role of CAFs. Most likely the pro-cancer action of fibroblasts can best be defined as inadvertent collateral damage resulting from their participation in host stromal responses to tissue injury. In this regard, NAFs may inhibit tumour growth by reversing the growth-promoting effect of TGFβ and HGF produced by CAFs[218]. Deregulation of TGFβ signalling in NAFs can induce prostatic intraepithelial neoplasia, implying that NAFs suppress tumour emergence[8]. How NAFs prevent tumorigenesis remains largely unknown[8,218,219]. Some studies also suggest

that elimination of senescent dermal fibroblasts via dermabrasion-induced wounding leads to increased IGF1 expression and corrects the inappropriate response to ultraviolet-B (UVB) radiation and tumorigenesis found in aged skin[220]. Such actions are likely to protect aged keratinocytes from UVB-induced squamous cell carcinoma[220]. It is likely that NAFs are tumour restraining compared with age-activated fibroblasts. Recent studies have suggested that CAFs can restrain PDAC by reducing fibrosis and hypoxia[188]. Another study shows that direct depletion of αSMA$^+$ CAFs reduces fibrosis and survival of mice with PDAC[96].

Activated fibroblasts may influence host defence through modulation of innate and adaptive tumour immunity. Many studies have shown that CAFs produce immunomodulatory cytokines, such as IL-10, TGFβ, TNF, IFNγ and IL-6, and help to recruit and polarize macrophages, T lymphocytes and natural killer cells[162–165,171,175,181]. Studies using mouse models that enable specific ablation of activated fibroblasts are required to address the specific role of CAFs in the modulation of tumour immunity. In this regard, clinical studies that correlate expression of collagen I and markers of CAFs, including αSMA and FAP, with disease outcome show that patients with high desmoplasia can have improved prognosis and overall survival in PDAC, and breast and lung cancer[221,222]. In breast cancer, a stroma-derived gene expression signature offers prognostic information[223]. The composition of the desmoplastic stromal cells may also be specifically associated with clinical correlates. For example, CAV1^{lo} CAFs[99] or PDGFRβ^{hi} CAFs[224] are independently associated with poor prognosis in breast cancer. Such studies will undoubtedly expand in the future to further our understanding of the potential restraining role of tumour stroma and CAFs in cancer progression.

Accumulating evidence continues to suggest that it is conceivable to therapeutically target CAFs to promote antitumour responses. However, finding an agent that specifically targets CAFs remains elusive. CAF-directed therapy designed to either eliminate them or potentially reprogramme them back to their normal resting phenotype is showing some promise. In this regard, recent studies have demonstrated that calcitrol can reprogramme pancreatic stellate cell-derived CAFs in PDAC, probably by reprogramming them to become normal stellate cells with restored retinoic acid content[225]. Such preclinical efforts can easily be translated into clinical studies.

Recent approval of pirfenidone for treatment of idiopathic pulmonary fibrosis relies on targeting FAF activation and secretory functions[226]. This opens the possibility of combining this drug with standard chemotherapy to target both CAFs and cancer cells. CAFs can also be targeted by antibodies that inactivate FAP (for example, sibrotuzumab)[227] and advanced clinical testing is under way. Targeting proliferating cancer cells might also target proliferating CAFs, and chemotherapy and radiation therapy may induce pro-tumour or antitumour effects on CAFs. Although targeting CAFs may offer powerful new tools for anticancer treatment, their functional heterogeneity and dynamic polarization may require precise targeting efforts and more detailed understanding of response to anti-CAF therapy.

Conclusions and future considerations

It is becoming clear that quiescent fibroblasts probably give rise to a heterogeneous population of activated fibroblasts. The differences in the cell of origin for activated fibroblasts or myofibroblasts could also promote a heterogeneous population in tumours. We speculate that activated fibroblasts could represent subtypes such as F1, F2 and so on, similar to the classification of macrophages and other immune cells[228] (FIG. 5). Studies in developmental biology identifying subsets of mesenchymal cells with distinct functions in different tissues[51,229,230] may also offer insights towards the definition of functional heterogeneity in various pathologies, including cancer.

For several decades now, activated fibroblasts have been considered co-conspirators of cancer cells in furthering tumour growth. However, fibroblasts are likely inadvertent partners of cancer cells and can function as positive or negative regulators of tumour growth. Whether this property can be assigned to different subtypes of activated fibroblasts, or the same fibroblast population working in a context-dependent manner and at different stages of tumour progression, remains unknown. Nevertheless, the primary function of fibroblasts is to respond to tissue injury and facilitate regenerative repair. In response to stimuli released by damaged organs and emerging inflammation, quiescent fibroblasts expand by becoming activated and generate growth factors and ECM to self-regulate their expansion and also regulate inflammation and immunity. In the milieu of such actions, it is conceivable that cancer cells consequently derive advantageous growth, migratory and survival properties from the released growth-promoting factors. Therefore, fibroblasts may indirectly promote cancer progression. Our initial host response to cancer is not to help but to control tissue damage.

The next 10 years warrants to be an exciting time for unravelling more hidden secrets of fibroblasts.

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Competing interests statement
The author declares competing interests: see Web version for details.