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INVITED REVIEW

From sentinel cells to inflammatory culprits: cancer-associated fibroblasts in tumour-related inflammation

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Abstract

Inflammation is now established as a hallmark of cancer. Cancer-associated fibroblasts (CAFs) have been established as a key component of the crosstalk between tumour cells and their microenvironment. Central to the role of CAFs in facilitating tumour growth, invasion, and metastasis is their ability to orchestrate tumour-related inflammation. CAFs and their soluble mediators provide multiple complex regulatory signals that modulate the trafficking, differentiation status, and function of inflammatory cells in the tumour microenvironment. This review focuses on pathways by which CAFs mediate tumour-promoting inflammation and modify the components of the inflammatory microenvironment that facilitate tumour initiation, progression, and metastasis.

Keywords: cancer-associated fibroblasts; inflammation; cancer; metastasis

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Introduction

Inflammation is now established as an enabling characteristic of cancer [1,2]. Chronic inflammation, caused by bacterial and viral infections or by some autoimmune diseases, can predispose tissue to neoplasia by providing a fertile microenvironment that fosters both genetic instability and proliferative programming. Alternatively, inflammation can be a secondary event: a physiological response to aberrant proliferation and tissue remodelling caused by oncogenic mutations in cells. In both scenarios, an inflammatory milieu facilitates neoplastic progression [3,4]. Hallmarks of inflammation, such as the infiltration of tissue by innate and adaptive leukocytes, activated angiogenic vasculature, tissue remodelling, and increased levels of chemokines and cytokines, are found in all solid tumours, even those that are not aetiologically related to inflammation [5].

There has been extensive research in recent years focusing on the roles of various cells of innate and adaptive immunity that facilitate solid tumour growth by regulating angiogenesis, invasion, and metastasis [4–8], and more recently in regulating the response of tumours to cytotoxic therapy [9–11]. Notably lacking from these studies have been in-depth analyses focusing on the role of cancer-associated fibroblasts (CAFs) as inflammatory mediators.

Fibroblasts are a vastly heterogeneous multifunctional cellular component of connective tissue. They play a central role in providing structural scaffolding and growth regulatory elements, as well as significantly contributing to tissue remodelling that occurs during development, and in homeostatic tissues. In addition, fibroblasts serve as resident sentinel cells that initiate tissue repair, and modulate the immune response during wound repair [12,13]. Following tissue damage, fibroblasts exhibit an activated and contractile phenotype and have been referred to as myofibroblasts: these synthesize increased levels of various collagen types that provide provisional scaffolds to aid in wound repair [14], and function as important sources of many growth factors and cytokines that regulate wound healing responses [15].

CAFs are a heterogeneous population of fibroblastic cells found in the microenvironment of solid tumours. In some cancer types, including breast and pancreatic carcinomas, CAFs are the most prominent stromal cell type. CAFs include several subpopulations with diverse origins, including myofibroblasts [characterized by α-smooth muscle actin (SMA) expression], reprogrammed local tissue fibroblasts, and bone marrow-derived progenitor cells [16]. While the distinct functional characteristics of the various CAF subsets are poorly defined, their role in supporting tumour growth has been established: CAFs have been found to promote tumour growth by directly stimulating tumour cell proliferation via secreted growth factors, and by enhancing angiogenesis [17–19]. Enhancement of tumour angiogenesis by CAFs can be mediated

either directly, by secreting pro-angiogenic factors including interleukin (IL)-8/CXCL8, vascular endothelial growth factor (VEGF), and fibroblast growth factor (FGF)-2, or indirectly, by secreting extracellular matrix (ECM)-remodelling proteases, such as matrix metalloproteinase (MMP)-9, MMP-13, and MMP-14 that activate a multitude of latent soluble and insoluble factors with diverse activities [7,20–22]. In addition, CAFs foster tumour progression and metastasis by modifying the architecture of the ECM, by enhancing deposition of collagen, and by mediating increased cross-linking of collagen fibres, thus stiffening stroma. Such increased stiffness has been reported to be linked to enhanced tumour growth, motility, and invasion [23–25]. In some cancer types, in particular breast and pancreatic, activation of fibroblasts leads to substantial deposition of fibrotic ECM. Such aberrant fibrotic responses, termed desmoplasia, correlate with tumour progression and poor prognosis in some cancer types [23]. These non-inflammatory tumour-promoting effects of CAFs have been recently reviewed [22,26,27]. The focus of this review is on pathways by which CAFs mediate tumour-promoting inflammation and modulate the components of the inflammatory microenvironment that facilitate tumour initiation, progression, and metastasis.

From tumour suppressors to tumour promoters

Under homeostatic conditions, fibroblasts play an important role in regulating epithelial proliferation and thereby modulating the oncogenic potential of adjacent epithelium, activities that are in part regulated by transforming growth factor beta (TGF-β)-mediated signalling [18]. Ablation of TGF-β responsiveness in fibroblasts exacerbates neoplastic progression in several tissues, including prostate, stomach, and breast, [18,28,29]. Others have reported that stromal ablation of the tumour suppressor Pten during mammary carcinogenesis results in accelerated tumourigenesis, via Ets2 inactivation, suggesting multiple pathways by which fibroblasts can inhibit neoplastic growth [30,31]. *In vitro* co-culture studies using primary normal fibroblasts isolated from various human tissues, when cultured with human prostate, lung, and lymphoblastoid tumour cell lines, support a role for fibroblasts in restricting the proliferative potential of tumour cells in a contact-dependent manner [32]. Some of the growth-inhibiting activity provided by fibroblasts may be directly associated with their role as sentinel cells, capable of 'sensing' tissue damage, associated with aberrant epithelial proliferation: Experimental analysis using a mouse model of intestinal inflammation revealed that colonic fibroblasts express NLRP6 (NOD-like receptor family pyrin domain containing 6), a stress-associated molecular pattern recognition receptor. NLRP6-expressing fibroblasts suppressed carcinogenesis by regulating the regeneration of colonic mucosa and processes of epithelial proliferation and migration. Consistently, NLRP6-deficient mice were highly susceptible to experimental colitis and exhibited spontaneous intestinal hyperplasia and accelerated colitis-associated tumour growth [33,34]. Taken together, these studies indicate a potent and functional regulatory role for fibroblasts in maintaining epithelial tissue homeostasis and preventing initiation of neoplastic growth [35].

In contrast, fibroblasts in carcinomas exhibit pro-inflammatory tumour-promoting activities that partially resemble their established role in chronic inflammatory diseases and in wound healing [36]. Solid tumours, historically referred to as 'wounds that never heal' [37], share several similar characteristics with healing wounds, including a gene expression signature of fibroblasts from various anatomic sites, activated in wound healing, that was reminiscent of gene expression signatures found in breast, lung, and gastric carcinomas [38]. The immune response associated with tissue damage is an important component of tissue repair and wound healing programmes that are activated in tumours, notably orchestrated in part by CAFs. Indeed, CAFs were recently found to promote tumour growth by mediating tumour-promoting inflammation, which was apparent at the earliest pre-malignant stage. CAFs in skin, breast, and pancreatic cancers were found to express a pro-inflammatory gene signature including cyclooxygenase (COX)-2, osteopontin (OPN), CXCL1, CXCL2, IL-6, and IL-1β. In addition, dermal CAFs isolated from a transgenic mouse model of squamous cell carcinogenesis contribute to macrophage recruitment, angiogenesis, and enhanced tumour growth in a nuclear factor-kappa B (NF-κB)-dependent manner [39]. A similar pro-inflammatory gene signature was identified in a subtype of CAFs in inflammation-induced gastric cancer [40]. Quante *et al* reported that CAFs recruited into gastric tumours from bone marrow express a pro-inflammatory gene signature including CXCL1, CCL5, OPN, IL-6, IL-1β, stromal-derived factor (SDF-1α), and tumour necrosis factor alpha (TNF-α). COX-2 is induced in colorectal CAFs, and its up-regulation correlates with proliferation and invasiveness of colorectal tumour cells [41,42]. IL-1β, expressed by hepatic stellate cells, induces CXCL5 expression in cholangiocarcinoma tumour cells, resulting in neutrophil recruitment and increased tumour cell migration and invasion [43]. IL-6, expressed by mammary CAFs, contributes to a positive feedback cycle of growth and invasion between CAFs and mammary carcinoma cells, and to a tumour-promoting reciprocal relationship with mast cells in the tumour microenvironment [44]. Thus, CAFs emerge as novel key players in orchestrating tumour-promoting inflammation. These studies support the observation that pro-inflammatory signalling by fibroblasts is initiated during early carcinogenesis and is amplified as levels of recruited leukocytes increase, leading invariably to tumour-promoting inflammation in which fibroblasts play a central role.

Activation of CAF pro-inflammatory signalling

What are the signals that trigger pro-inflammatory signalling in fibroblasts?

While the detailed molecular mechanisms by which fibroblasts are activated or 'educated' to become pro-inflammatory CAFs in incipient neoplasias are largely unresolved and are likely context-dependent and tissue-specific, several studies provide putative mechanisms.

Activation by biomechanical forces

Fibroblasts are capable of 'sensing' physical changes in their local ECM environment and in turn converting this physical stimulation into chemical signals, thereby leading to selective changes in gene expression that foster neoplastic progression, invasion, and metastasis [45,46]. While there are few cancer-related studies that directly connect mechano-sensing by fibroblasts with pro-inflammatory signalling, several studies performed in other fields have reported that activation of fibroblasts by biomechanical forces induces pro-inflammatory signalling: For example, periodontal fibroblasts respond to compression forces by increased TNF-α production at the compression site, resulting in the activation of $CD4^+$ T cells and facilitating bone resorption [47]. Furthermore, application of mechanical forces induces fibroblasts to express several pro-inflammatory cytokines including IL-1α, IL-1β, and IL-6 [48]. Kook *et al* reported that application of tensile forces on fibroblasts stimulated mRNA expression of collagen I and MMP-1, typical of activated fibroblasts, in an ERK-NF-κB signalling-dependent manner [49], thus linking mechanical forces with inflammatory signalling. Moreover, a recent *in vivo* wound healing study linked mechanical forces to inflammatory activation of fibroblasts via focal adhesion kinase (FAK), a transducer of both inflammatory and physical signals: In a mouse model of hypertrophic scar formation, fibroblast-specific knockdown of FAK resulted in reduced inflammation and fibrosis, compared with control mice [50]. While not involving cancer, these studies indicate that changes in tissue architecture as a result of aberrant epithelial proliferation, an early neoplastic response, may result in activation of inflammatory signalling by stromal fibroblasts, a hypothesis supported by reports of inflammatory signalling by stromal fibroblasts in benign prostatic hyperplasia [50–53]. Taken together, it is reasonable to hypothesize that biomechanical forces applied by aberrant proliferation of transformed epithelial cells in incipient tumours may be one of the physiological signals that trigger pro-inflammatory signalling in resident tissue fibroblasts. Future studies performed in early stages of carcinogenesis will test this emergent hypothesis.

Activation by paracrine signalling

Pro-inflammatory signalling by CAFs is also induced by paracrine signalling derived from initiated epithelia and/or resident immune cells starting at early, pre-neoplastic tissues. Several recent studies revealed that paracrine signalling pathways resulted in activation of cytokine and chemokine expression in CAFs: In a mouse model of squamous cell carcinogenesis where humoral immunity fosters cancer development by Ig- and FcRγ-activation of recruited myeloid cells, IL-1β induced NF-κB-dependent pro-inflammatory signalling in dermal fibroblasts [39,54]. IL-1β was expressed by resident immune cells in hyperplastic skin and activated a pro-inflammatory gene signature in dermal CAFs; however, this signature was not induced in CAFs derived from either B-cell-deficient or FcRγ-deficient mice, thus indicating that CAF activation is downstream of humoral immune-mediated activation of inflammatory leukocytes in neoplastic tissue [55]. Thus, B cells produce antibodies that are deposited in neoplastic skin by leaky angiogenic vasculature; these in turn engage FcγR on resident immune cells and induce secretion of IL-1, which activates pro-inflammatory properties of CAFs [39,54,56].

Other studies also support a role for inflammatory mediators in activating fibroblasts derived from other tumour types including TNF-α-induced expression of the pro-angiogenic chemokine CXCL8/IL-8 in liver fibroblasts, also in an NF-κB-dependent manner [57]. Similarly, TNF-α induces IL-6 and the chemokine CCL2 in CAFs derived from colorectal liver metastases [58]. However, the physiological origin of TNF-α in these studies was not defined. While it is conceivable that such paracrine signalling mechanisms that activate fibroblasts *in vivo* could be derived from immune cells, there is also evidence indicating direct regulation by neoplastic cells: co-culture of fibroblasts with either melanoma cells or oral squamous cell carcinoma cells induces pro-inflammatory gene expression in fibroblasts that includes CXCL1 and CXCL2 [59,60]. Activation of CAFs can also be accelerated by autocrine signalling: Kojima *et al* reported that autocrine TGF-β and SDF-1α/CXCL12 chemokine signalling result in activation of mammary CAFs, but the *in vivo* molecular signals that trigger these inflammatory pathways in CAFs remain unknown [61]. TGF-β and SDF-1α/CXCL12 may have an additional role in the recruitment of pro-inflammatory CAFs from bone marrow: In a model of induced gastric cancer, Quante *et al* reported that CAFs recruited into gastric tumours from bone marrow express a pro-inflammatory gene signature that includes CXCL1, CCL5, OPN, IL-6, IL-1β, CXCL12, and TNF-α [40]. TGF-β was hypothesized to induce this recruitment, in part through up-regulation of CXCL12/SDF-1α. Although the signals that recruit, 'educate', and activate CAFs to mediate tumour-promoting inflammation are complex and most likely tumour/organ type-specific, these studies collectively reveal that CAFs generating an inflammatory microenvironment originate from resident or recruited fibroblasts that are activated by paracrine signalling from initiated epithelia and/or immune cells.

CAFs modulate leukocyte recruitment and function in tumours

One of the central mechanisms by which CAFs regulate tumour-promoting inflammation is by secreting cytokines and chemokines that recruit and modulate the function of innate and adaptive immune cells in the tumour microenvironment. While CAFs most likely exert complex interactions with multiple immune cell types, studies in recent years have largely reported on molecular pathways by which CAFs interact with myeloid cells and T lymphocytes in the tumour microenvironment.

CAF signalling recruits myeloid cells

CCL2, a known macrophage chemoattractant [62], was found to promote infiltration of blood monocytes into mammary CAF spheroids [63,64]. These observations were supported by *in vivo* data: in a mouse model of transplantable mammary carcinoma, CAF-secreted CCL2 recruited macrophages into mammary tumours and specific ablation of CCL2 reduced tumour metastasis [65]. Other CAF-derived chemokines were also reported to enhance macrophage recruitment to various tumours: Augsten *et al* reported that prostate CAFs up-regulate CXCL14 that functioned to promote macrophage migration into prostate cancer xenografts [66]. Inhibition of NF-κB signalling, driving expression of CXCL1 and CXCL2 in skin CAFs, resulted in decreased macrophage infiltration into transplanted skin tumours and reduced tumour growth, indicating a central role for CAFs in facilitating the trafficking of macrophages into tumours [39].

Interestingly, fibroblasts can recruit macrophages into tumours not only via cytokine and chemokine secretion, but also by modifying the ECM: Kobayashi *et al* reported that macrophages infiltrate hyaluronan (HA)-rich tumour microenvironments in a transplanted model of mammary carcinoma. Disrupting the function of the HA synthase 2 (*Has2*) gene in stromal fibroblasts impaired macrophage trafficking [67]. This mechanism of leukocyte recruitment is likely mediated via toll-like receptors (TLRs) on macrophages, which sense damage-associated molecular patterns (DAMPs), including various ECM products produced by CAFs during tissue remodelling [68].

CAF-mediated recruitment of macrophages to tumours is operative at various tumourigenic stages: macrophages are recruited into hyperplastic skin lesions in a mouse model of skin carcinogenesis by dermal CAFs that express a pro-inflammatory gene signature from the earliest pre-neoplastic stages [39]. Similarly, prostate fibroblasts in benign prostatic hyperplasia secrete cytokines and chemokines that support an inflammatory proliferative microenvironment [52]. At more advanced tumourigenic stages, CAFs facilitate trafficking of myeloid cells into metastasizing tumours: in a mammary carcinoma model of pulmonary metastasis, stromal-derived CCL2 recruits $CD11b^+Gr1^+Ly6c^+$ inflammatory monocytes that support pulmonary metastasis [69].

CAFs modulate recruitment and activation of lymphocytes

Through the production of a diverse array of chemokines, cytokines, and extracellular matrix molecules, fibroblasts alter the trafficking and activation status of T lymphocytes [70]. Although tumour-infiltrating lymphocytes (TILs) are prevalent in many tumours, they are usually incapable of eradicating tumours. Numerous studies have described immunosuppressive mechanisms that underlie this hypo-responsiveness of lymphocytes and escape mechanisms mediated by tumour cells and these have been reviewed elsewhere [2,71]. However, several studies have revealed that CAFs are also capable of providing multiple complex regulatory signals with the potential to enhance or suppress T-cell function in the tumour microenvironment.

CAFs express high levels of the immunosuppressive cytokine TGF-β1, which suppresses both the acquisition and the expression of T-cell effector functions [72]. Furthermore, TGF-β inhibits the function of natural killer (NK) cells and $CD8^+$ cytotoxic T lymphocytes (CTLs), thus blocking anti-tumour cytotoxic activities [73]. Balsamo *et al* provided experimental evidence that CAFs inhibit NK cell activity: in co-culture experiments, melanoma-derived fibroblasts isolated from human metastatic melanoma interfered with the induction of NK cell effector functions, including expression of NK surface receptors and cell-mediated killing of melanoma target cells. This effect of CAFs was mediated by their secretion of prostaglandin E2 (PGE2) [74]. In agreement with this study, fibroblasts derived from hepatocellular carcinoma patients induced deactivation of human NK cells, characterized by low expression of cytotoxic molecules and surface markers for cell activation, impaired production of cytokines, and decreased cytotoxic activity *in vitro* with a leukaemia cell line. These effects were partially mediated by CAF-derived PGE2 and indoleamine 2,3-dioxygenase (IDO) [75]. While these observations are intriguing, *in vivo* studies are required to confirm that CAFs can indeed function to suppress NK cytotoxic effector activity and thereby contribute to immune escape and foster tumour progression.

CAF-induced immunosuppression has also been suggested to be mediated by fibroblast secretion of ECM molecules, including tenascin-C: CAF-derived tenascin-C effects the migration and activation of T lymphocytes via binding to fibronectin, as well as by down-regulation of IL-2 receptor, and inhibition of CD2 and CD28 co-stimulation [76]. However, co-culture experiments of autologous tumour-derived CAFs and T lymphocytes demonstrate that different subpopulations of fibroblasts either suppress or enhance the activity of tumour-associated T cells, further highlighting the heterogeneous nature of fibroblastic cells [77].

In addition to their ability to suppress cytotoxic T lymphocytes, CAFs secrete chemokines and cytokines that promote the recruitment of tumour-promoting T cells, including CXCL9, CXCL10, and CXCL12 (SDF-1α) [70]. This role of fibroblasts in T-cell recruitment is supported by reports from Grum-Schwensen *et al*, who reported that tumour infiltration of T lymphocytes is mediated in part through CAF-secreted S100A4/FSP-1: genetic depletion of S100A4 significantly reduced the metastatic burden in lungs of transgenic mice predisposed to mammary carcinogenesis (eg MMTV-PyMT mice), and this was associated with a significant suppression of T-cell infiltration. While some of the secreted S100A4 may be produced by myeloid cells, this study demonstrated that the presence of S100A4(+/+), but not S100A4(−/−), fibroblasts significantly stimulated attraction of T lymphocytes to sites of growing tumours [78].

In yet another manifestation of their capacity to orchestrate an inflammatory microenvironment in tumours, fibroblasts have been found to influence the balance between tumour-promoting lymphocytes, such as T regulatory cells and the T helper subtypes Th2 and Th17, versus cytotoxic T cells and tumour-suppressing T helper (Th1) cells. In a murine transplantable breast cancer model, Liao *et al* reported that CAFs promote tumour growth and metastasis in part by modulating the tumour immune microenvironment by inducing a switch from Th1- to Th2-type immunity. *In vivo* elimination of CAFs markedly suppressed the recruitment of $CD8^+$ cytotoxic T lymphocytes and correlated with an increase in Th1 cytokine expression in the tumour microenvironment [79]. In agreement with this capacity of CAFs to affect T-cell polarization, it was recently reported that CAF-derived thymic stromal lymphopoietin (TSLP), which favours a Th2-type cell polarization, is associated with reduced patient survival in pancreatic cancer [80].

Th17 is a newly defined T-helper cell population that expresses IL-17. These T helper cells regulate leukocyte recruitment and activation and play important roles in the pathogenesis of autoimmune diseases and inflammation [70]. While their functional role in human tumour immunology is still under debate [81], several studies indicate that CAFs can recruit, generate, and expand Th17 cells in tumours: Su *et al* reported that CCL5/RANTES and CCL2 secreted by CAFs isolated from human melanoma and breast and colon cancers mediated the recruitment of Th17 cells from peripheral blood [82]. In addition to Th17 recruitment, CAFs also produce a pro-inflammatory cytokine milieu that facilitates the generation and expansion of Th17 cells, including IL-1, IL-6, IL-23, and TGF-β, key cytokines for human Th17 generation and differentiation [70,82,83].

Regulatory T cells (Tregs), typically identified by CD4, CD25, and FOXP3 expression, have the ability to suppress the activity of T cells through cell–cell contact-dependent mechanisms, which have not yet been fully defined. Consequently, Treg deficiency is associated with severe autoimmunity and allergies [84]. In many types of human solid tumours, Tregs accumulate and act to promote tumour escape from cytotoxic immune responses. CAFs can contribute to this accumulation of $CD4^+FOXP3^+$ Tregs in tumour microenvironments in part via their production of TGF-β, which induces the expression of Foxp3 and differentiation of Treg cells [73]. Recruitment of Tregs may also be mediated by chemokine secretion by CAFs: in a breast cancer model, Tan *et al* suggested that CAFs may be the stromal source of the T-cell-attracting chemokine CCL5/RANTES, recruiting Treg cells into primary mammary tumours, which in turn stimulate metastatic progression [85]. Collectively, these findings illustrate an important regulatory role for CAFs in modulating both the content and the functional activation status of the leukocyte milieu in tumour microenvironments.

The senescence-associated secretory phenotype of CAFs promotes tumour growth

Fifty years ago, Hayflick and Moorhead reported that human diploid fibroblasts have a limited replication capacity in culture [86]. Mammalian cells can respond to replicative exhaustion, DNA damage or stress by entering a state of arrested proliferation and altered function known as cellular senescence. This state of permanent cell cycle arrest therefore constitutes a potent tumour-suppressive mechanism. On the other hand, senescence within the stromal compartment is believed to be an important tumour-promoting mechanism. Campisi and co-workers have reported that senescent human fibroblasts stimulate pre-malignant and malignant skin and mammary human epithelial cells to proliferate in culture and to form tumours in mice, due, at least in part, to soluble factors secreted by senescent fibroblasts [87]. Subsequent studies established that senescent cells change their expression profile to a senescence-associated secretory phenotype (SASP) [88]. SASP is characterized by expression of cytokines and chemokines leading to an inflammatory response that promotes leukocyte infiltration that is immunosuppressive [89–91]. Among others, senescent fibroblasts secrete the canonical pro-inflammatory factors IL-6, IL-1, IL-8, CXCL1, and CXCL2 [88]. NF-κB signalling is the major signalling pathway stimulating the induction of some of the major components of SASP, partially via the induction of a DNA damage response that activates NF-κB [92–94]. The matricellular protein OPN, known to have a role in tumour progression and metastasis, was also found to be secreted by senescent fibroblasts in skin and to promote preneoplastic keratinocyte cellular proliferation and cell survival through activation of the MAPK pathway [95].

While SASP-induced recruitment of leukocytes may have evolved to facilitate clearance of senescent cells by cells of the immune system, the accumulation

of senescent fibroblasts in the microenvironment of tumours is likely an additional mechanism contributing to the vicious cycle of tumour-promoting inflammation within tumours. Accumulation of senescent fibroblasts in tumours may be a result of the accelerated proliferation of CAFs or a response to extrinsic genotoxic stress caused by oxidative stress and high glucose in the tumour microenvironment [88]. In addition, increased presence of senescent fibroblasts in tumours may also be part of normal ageing of the tissue where tumourigenesis occurs [52]. While these senescent fibroblasts may be a minority in the tumour milieu, the acquisition of a senescence-associated secretory phenotype is another mechanism that turns fibroblasts into pro-inflammatory cells that have the ability to promote tumour progression.

CAFs modulate the metastatic niche

Mortality from cancer is almost exclusively a result of tumour metastasis. In many tumour types, there is a temporal lag (months to decades) between when malignant cells arrive in ectopic locations and when proliferative capabilities allowing organ colonization are acquired [96,97], implying that in addition to activation of cell-intrinsic survival programmes at the metastatic site, disseminated malignant cells must acquire additional capabilities enabling them to activate regulatory programmes in non-neoplastic local and recruited cells at the metastatic location, critical determinants for successful metastatic progression [8]. Whether the secondary site is primed systemically before the arrival of metastasizing cells or by early-disseminated cells is the subject of ongoing debate. Be that as it may, the successful engraftment and growth to clinically relevant macro-metastases is dependent on the formation of a permissive microenvironment, including activation of resident fibroblasts at the metastatic site. While little is known about the role of CAF-mediated inflammation in facilitating metastasis, there has been progress in appreciating their role.

Secreted from the primary tumour, systemic inflammatory factors instigate the pre-metastatic niche: Tumour-secreted inflammatory cytokines such as SDF-1α, TNF-α, TGF-β, VEGF-A, OPN [98–100], and placenta growth factor (PlGF) [101] influence the recruitment of myeloid cells to pre-metastatic sites. Several recent studies implicated fibroblasts in facilitating formation of the metastatic niche: O'Connell *et al* reported that resident FSP-1$^+$ fibroblasts express VEGF-A and tenascin-C that correlated with increased metastasis in a mouse model of transplantable mammary carcinoma – depletion of fibroblasts or genetic ablation of VEGF-A and tenascin-C resulted in decreased metastatic capacity [102]. These results are supported by the findings of two other groups, demonstrating that expression of the ECM proteins tenascin-C and periostin by stromal fibroblasts in the lungs supports the formation of a metastatic niche that facilitates metastatic colonization of mammary tumour cells, by enhancing WNT signalling [103–105]. Expression of periostin by pancreatic stellate cells was also suggested to be in correlation with aggressive behaviour and worse prognosis in pancreatic cancer [106]. Whether CAF-derived ECM components such as periostin and tenascin-C elicit some of their metastatic niche formation role via facilitating the recruitment/retention of BM-derived cells remains to be determined.

More evidence supporting the crucial importance of ECM composition at metastatic sites was uncovered by Erler *et al*, who reported an important role for lysyl oxidase (LOX). LOX is secreted from the primary tumour to cross-link collagen IV in fibronectin-rich areas of the target organ, thus creating a permissive ECM to support the proliferation and survival of metastatic cells [107]. Fibroblasts are likely an important player in this metastasis-promoting matrix remodelling; Fibroblast-derived fibronectin is an essential co-factor for regulating LOX activity [108]. Myofibroblasts also express LOX, as well as lysyl oxidase-like-2 (LOXL2), in human invasive ductal breast carcinoma, gastrointestinal, and ovarian tumour specimens [109,110]. The relative contribution of CAF-derived versus tumour-derived soluble factors to the initiation of a permissive metastatic microenvironment is still unresolved but will provide valuable knowledge to metastasis biology. Notably, some of the early signalling that contributes to pre-metastatic niche formation may originate from passenger fibroblasts, accompanying the disseminating tumour cells to the secondary site. Support for this comes from two recent reports demonstrating that metastatic Lewis lung carcinoma cells and human pancreatic cancer cells provide their own stromal components, including activated fibroblasts, from primary sites to the metastatic organs [111].

While the studies detailed above did not directly implicate fibroblasts in pro-inflammatory signalling at the metastatic microenvironment, changes in gene expression by resident stromal cells at the metastatic site were found to promote the recruitment of immune cells to pre-metastatic niches. Resident fibroblasts at the secondary site respond to systemic signals originating from the primary tumour by depositing fibronectin at the pre-metastatic organ, thereby priming it for the homing of myeloid cells that support subsequent metastatic settlement [112]. This up-regulation of fibronectin, enabling myeloid cell recruitment, is STAT3 (signal transducer and activator of transcription 3)-dependent: activation of STAT3 in lung fibroblasts via paracrine signalling from melanoma cells contributes to activation of fibroblasts at the metastatic niche and results in the up-regulation of fibronectin and the recruitment of CD11b$^+$ myeloid cells [113]. Recruitment of bone marrow-derived CD11b$^+$ myeloid cells, known to be crucial for the formation of hospitable conditions for colonization of tumour cells, is

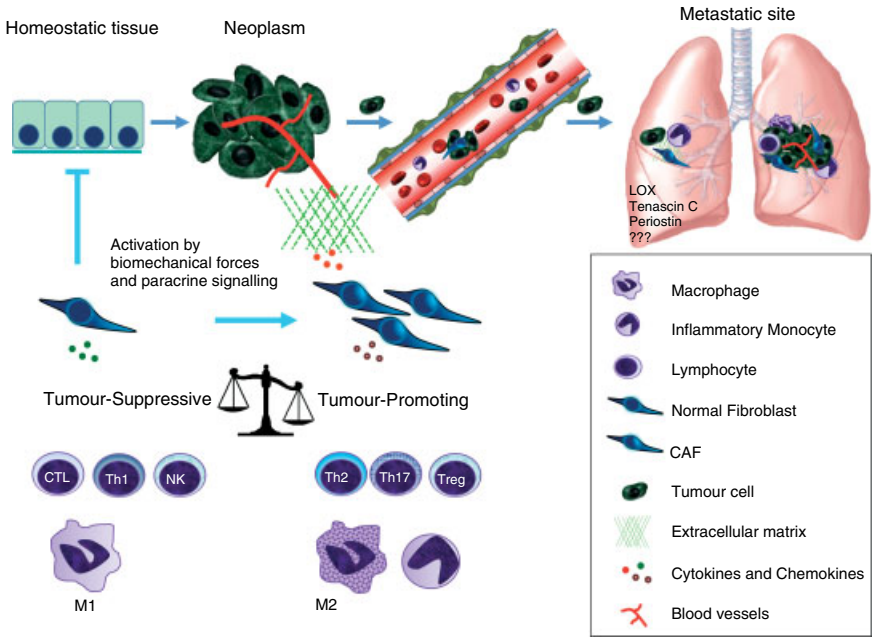

Figure 1. Tumour-promoting inflammation, mediated by cancer-associated fibroblasts, facilitates tumour growth, progression, and metastasis. In homeostatic tissues, fibroblasts regulate epithelial proliferation and inhibit neoplastic transformation of adjacent epithelium. In neoplastic tissues, reprogrammed CAFs secrete pro-inflammatory cytokines and chemokines and modify ECM components that facilitate tumour growth. Pro-inflammatory signalling by CAFs mediates the recruitment of inflammatory cells to tumour microenvironments and fosters the differentiation of T lymphocytes to pro-tumourigenic phenotypes. Colonization of distal organs is enabled by disseminated CAFs from the primary tumour and/or by activated local fibroblasts at the metastatic site that provides a fertile soil to facilitate the formation of metastases.

also mediated in part by up-regulation of the pro-inflammatory chemoattractants S100A8 and S100A9 in lung stromal cells [114]. Secretion of S100A8 and S100A9 in pre-metastatic lungs creates a chemotactic gradient that guides melanoma cell migration [115]. Whether fibroblasts are a stromal source of these pro-inflammatory factors remains to be determined. Recently, signalling by tumour cell-derived exosomes was found to contribute to the crosstalk between the primary tumour and bone marrow-derived cells, leading to the homing of both cell types to sites of metastasis [116]. Tumour-secreted exosome-mediated signalling may also be relevant for activation of fibroblasts at metastatic sites.

Knowledge accumulated in recent years has established that the formation of a pro-inflammatory microenvironment in metastatic organs, whether prior to or at the time of malignant cell arrival, enhances survival and proliferative possibilities for metastatic cells [117]. The role of local fibroblasts at the metastatic site in mediating metastasis-promoting inflammation is still largely unresolved. Future studies will reveal whether the knowledge accumulated on primary CAF biology can be extrapolated to CAFs at metastatic organs.

Concluding remarks

CAFs have been established as a key component of the crosstalk between malignant tumour cells and their microenvironment. Central to their role in facilitating tumour growth, invasion, and metastasis is their ability to orchestrate tumour-promoting inflammation (Figure 1). Due to the vast heterogeneity of fibroblasts, as well as their different origins, much of the accumulating knowledge on the functional roles and activation pathways of fibroblasts may be tumour type- and tumour stage-specific. Nevertheless, clinical oncology is progressing increasingly towards a new era of integrative cancer therapy, based on personalized diagnostics that takes into account the individual complexities of tumours, including cells, pathways, and molecular mediators in the tumour microenvironment [118]. As a result, cancer therapeutics is moving progressively towards combinatorial approaches that act synergistically by targeting intrinsic pathways in neoplastic cells, as well as extrinsic tumour-enabling pathways in the tumour microenvironment. Future studies will decipher in detail the molecular pathways underlying the activation/recruitment of fibroblasts to become pro-inflammatory CAFs supporting early carcinogenic progress, as well as CAF functions that enable the formation of metastases, and will hopefully result in innovative therapeutic strategies allowing the co-targeting of immune cells and CAFs to maximize treatment efficacy and prevent evasive resistance.

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Author contribution statement

CS participated in writing of the manuscript and prepared the figure. NE wrote the manuscript.

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