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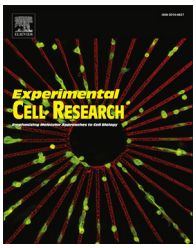

Review Article

An inflammatory vicious cycle: Fibroblasts and immune cell recruitment in cancer

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ABSTRACT

Cancer-associated fibroblasts (CAFs) have been established as a key component of the crosstalk between tumor cells and their microenvironment. The ability of CAFs to orchestrate tumor-promoting inflammation is central to their role in facilitating tumor growth, invasion, and metastasis. Here we review pathways by which CAFs and their soluble mediators provide multiple complex signals that modulate the recruitment, functional activation status, and retention of immune cells in the tumor microenvironment.`

Contents

Introduction

Inflammation is now accepted as an enabling characteristic of cancer [1,2]. Inflammatory mediators such as chemokines and cytokines as well as cells of the innate and adaptive immune systems are important constituents of the microenvironment in virtually all solid tumors, even those that are not etiologically related to inflammation [3]. Although the specific inflammatory

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molecular circuits and cellular components may be tumor type specific, the chronic recruitment and presence of activated leukocytes in tumors is a hallmark of the tumorigenic process and a predictor of aggressive disease [4]. Immune cells facilitate tumor growth and progression by regulating angiogenesis, invasion and metastasis [3,5–8]. More recently, leukocytes were also implicated in regulating the response of tumors to cytotoxic therapy [9–11].

There has been extensive research in recent years focusing on the recruitment of various immune cells to the microenvironments of primary tumors, as well as to sites of metastases. Tumor cells were shown to secrete chemokines and cytokines that facilitate the recruitment and functional activation status of myeloid cells as well as lymphocytes that facilitate tumor growth and metastasis [5,8]. Macrophages and T lymphocytes were also implicated in mutually affecting the recruitment and reprogramming of each other toward a tumor-promoting phenotype [12–14]. However, the role of cancer-associated fibroblasts (CAFs) as novel key players in mediating recruitment of immune cells into various cancer types is only recently emerging.

CAFs are a heterogeneous population of fibroblastic cells that constitute the most prominent stromal cell type in the microenvironment of many solid tumors, in particular breast and pancreatic carcinomas. CAFs comprise 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 [15]. While the distinct functional characteristics of the various CAF subsets are poorly defined, their role in supporting tumor growth has been established: CAFs have been found to promote tumor growth by directly stimulating tumor cell proliferation via secreted growth factors, and by enhancing angiogenesis [16–19]. In addition, CAFs foster tumor progression and metastasis by modifying the architecture and stiffness of the extracellular matrix (ECM), which has been linked to enhanced tumor growth, motility and invasion [20–22]. These tumor-promoting effects of CAFs have been recently reviewed [23–25].

One of the central mechanisms by which CAFs regulate tumor-promoting inflammation is by secreting cytokines and chemokines that recruit and modulate the function of innate and adaptive immune cells in the tumor microenvironment. This review focuses on the role of fibroblasts in recruiting leukocytes to tumors and in modulating their function, thus contributing to initiation and maintenance of cancer-related inflammation.

Recruitment and functional modulation of monocytes/macrophages by CAF-derived signaling

Cells of the monocyte/macrophage lineage are a common component of the microenvironment of solid tumors that have a crucial contribution to tumor progression and metastasis in multiple tumor types [13]. Diversity and plasticity are hallmarks of cells of the monocyte–macrophage lineage and multiple subsets of tumor-associated macrophages (TAMs) have been described. In response to environmental signals, macrophages may undergo “classical” M1 activation (stimulated by TLR ligands and IFN-γ) or “alternative” M2 activation (stimulated by IL-4/IL-13); these states correlate with the Th1–Th2 polarization of T cells, whereby Th2-type immunity is associated with pro-tumorigenic functions. TAMs are usually shifted toward an M2-like phenotype [26]. Recently, this binary M1/M2 polarization definition has come under question, and TAMs were proposed to be composed of a continuum of several functionally distinct populations that often share features of both types and support inflammation, angiogenesis, invasion and metastasis [13]. Studies in recent years reported on multiple molecular pathways by which CAFs contribute to the recruitment and functional modulation of myeloid cells, identified by various markers, into the tumor microenvironment.

Macrophage recruitment

CCL2, synthesized by both tumor cells and by CAFs, is a known chemoattractant of inflammatory monocytes, which express its receptor—CCR2 [27]. CCL2 was found to promote infiltration of blood monocytes into mammary CAF spheroids [28,29]. These observations were supported *in vivo* in a mouse model of transplantable mammary carcinoma, CAF-secreted CCL2 recruited macrophages into mammary tumors and specific ablation of CCL2 reduced tumor metastasis [30]. Pollard and colleagues had demonstrated the role of CCL2 in recruiting inflammatory monocytes at more advanced tumorigenic stages, thus facilitating tumor metastasis in a mammary carcinoma model of pulmonary metastasis, stromal-derived CCL2 facilitated the recruitment of $CD11b^{+}Ly6c^{+}$ inflammatory monocytes that supported lung metastasis [31]. CCR2-expressing monocytes were preferentially recruited early after breast tumor cell inoculation, while B and T lymphocytes were not recruited at that time point, thus demonstrating the significant role of recruited inflammatory monocytes at early stages of pulmonary metastasis. A recent study by Ren et al. further supported a role for the CCL2–CCR2 axis in CAF-mediated recruitment of monocytes/macrophages into tumors. In this study, tumor-educated stromal cells recruited $CD11b^{+}Ly6C^{+}$ monocytes, and $F4/80^{+}$ macrophages into spontaneous lymphoma and transplantable breast and melanoma tumors. This recruitment correlated with enhanced tumor growth and was shown to be CCR2-dependent [32].

Other CAF-derived chemokines were also reported to enhance macrophage recruitment to various tumors: Augsten et al. showed that prostate CAFs upregulate CXCL14 that functioned to promote $CD68^{+}$ macrophage migration into prostate cancer xenografts [33].

In a mouse model of skin carcinogenesis, $CD68^{+}$ macrophages are recruited into hyperplastic and dysplastic skin lesions by dermal CAFs that express a pro-inflammatory gene signature from the earliest pre-neoplastic stages. This pro-inflammatory gene signature was shown to be functionally dependent on NF-κB: inhibition of NF-κB signaling, driving expression of CXCL1 and CXCL2 in skin CAFs resulted in decreased macrophage infiltration into transplanted skin tumors, and reduced tumor growth, indicating a central role for CAFs in facilitating the trafficking of macrophages into tumors [34]. CAF-derived signaling that supports the formation of an inflammatory microenvironment in incipient tumors is supported also by studies in prostate cancer development: prostate fibroblasts in benign prostatic hyperplasia secrete cytokines and chemokines that foster an inflammatory proliferative microenvironment [35].

Fibroblasts contribute to myeloid cell recruitment not only through their expression of cytokine and chemokine secretion,

but also indirectly, by modifying ECM: Kobayashi et al. reported that macrophages preferentially traffic to stromal areas rich in hyaluronan (HA) in a transplanted model of mammary carcinoma. Disrupting the function of the HA synthase 2 (Has2) gene in stromal fibroblasts impaired macrophage trafficking, resulting in suppressed angiogenesis and lymphogenesis [36]. This mechanism of macrophage recruitment is likely mediated via Toll-Like Receptors (TLRs) on macrophages, that sense Damage Associated Molecular Patterns (DAMPs), including various ECM components produced by CAFs during tissue remodeling [37]. Notably, increased HA deposition in tumor stroma is associated with tumor aggressiveness and with poor clinical outcomes.

Modulation of macrophage functional activation status

Secretion of chemokines and cytokines by CAFs contributes not only to macrophage recruitment, but also to modulating the functional differentiation/activation status of TAMs. In co-culture experiments, CAF-derived IL-6 was shown to induce differentiation of $CD14^{+}CD1a^{-}$ monocytes into macrophages rather than to antigen-presenting dendritic cells [38]. Another study supporting a functional role for CAFs in affecting the functional activation status of recruited myeloid cells was performed in a transplantable mouse model of breast cancer. In this study, CAFs were targeted by vaccination with an antibody against fibroblast activating protein (FAP). FAP is highly expressed in the tumor stroma and has been shown to enhance tumor growth *in vivo* [39]. FAP-directed CAF elimination markedly reduced the presence of $F4/80^{+}$ TAMs and resulted in enhancement of Th1-type immunity (as reflected by increased levels of IL-2 and IL-7) [40]. In addition, CAF elimination reduced the presence of Myeloid Derived Suppressor Cells (MDSCs). MDSCs are an immunosuppressive myeloid cell population, known to accumulate in tumors, commonly identified by expressing both the myeloid cell marker CD11b and the granulocyte marker Gr1 [13]. These results whereby targeting of CAFs suppressed multiple characteristics of Th2 immunity implicate CAFs in skewing the tumor microenvironment toward a tumor-promoting phenotype. Moreover, CAFs may also directly influence the polarization of macrophages toward an M2 phenotype: IL-4, one of the key cytokines driving M2-like polarization of macrophages, was shown to be secreted by CAFs [41], and fibroblasts-derived IL-6 and IL-8 were shown to support upregulation of S100A8/A9 in tumor-infiltrated myeloid cells and induce their differentiation into S100A8/9-expressing MDSCs or M2 macrophages in colorectal cancer [42]. Thus, macrophages can be recruited into tumors by CAFs and, in the tumor microenvironment, an M2 phenotype can be activated by fibroblast-derived IL-4, IL-6 and IL-8, reinforcing an immunosuppressive and tumor promoting microenvironment.

Taken together, these studies suggest that CAFs in the microenvironment of primary, as well as metastatic tumors contribute to monocytes/macrophages recruitment and differentiation via multiple independent mechanisms that collectively result in amplification of tumor-promoting inflammation.

CAFs and mast cells

Mast cells are a bone marrow-derived, heterogeneous cellular population that have key roles in parasitic infections, allergies and angiogenesis, as well as in innate and adaptive immune responses. Increasing evidence in recent years highlight a role for mast cells as important modulators of the tumor microenvironment. Indeed, mast cells accumulate in tumors and remodel the tumor microenvironment by either releasing various factors after activation or interacting with other cells within tumors and, as a result, facilitate tumor invasion and metastasis [43,44]. Although not much is known about the interaction between CAFs and mast cells, several studies suggest that fibroblasts may affect the recruitment and migration of mast cells. In co-culture experiments, fibroblasts conditioned-medium could induce migration of cultured mast cells [45]. Supporting this interaction, Hugo et al. demonstrated that IL-6 in mammary carcinoma, expressed by mammary CAFs as well as by mast cells, contributes to a positive feedback cycle of growth and invasion between CAFs and mammary carcinoma cells, and to a tumor-promoting reciprocal positive feedback cycle with mast cells in the tumor microenvironment [46]. Notably, IL-6 was implicated by others as a mast cell chemoattractant [47]. Another study reinforces IL-6 dependent interaction between fibroblasts and mast cells: Gyotoku et al. showed in a co-culture system that in the presence of fibroblasts, IL-6 enhanced the proliferation of mast cells [48].

Tumor necrosis factor (TNF) was also shown to be a potent mast cell chemoattractant. TNF induced a strong, dose-dependent migratory response of peritoneal mast cells [49]. Interestingly, the data from this study suggests that fibronectin facilitates this TNF-mediated mast cell migration. Fibronectin is an ECM protein secreted by activated fibroblasts, and is upregulated in the desmoplastic reaction found in the stroma of many tumors. Indeed, upregulation of fibronectin secreted by resident fibroblasts in pre-metastatic sites was previously shown to precede the homing of bone marrow derived cells [50]. Taken together, this suggests that ECM modulation as well as cytokine secretion by CAFs may affect mast cell recruitment into tumors.

CAFs mediate lymphocyte recruitment and activation

Tumor-infiltrating lymphocytes are present in many types of solid tumors. Nevertheless, they are usually incapable of eliminating the tumor due to immunosuppressive mechanisms that result in hypo-responsiveness of lymphocytes as well as escape mechanisms mediated by tumor cells. Studies from recent years suggest that fibroblasts in tumor microenvironments provide multiple signals that affect the trafficking and activation status of T lymphocytes via diverse mechanisms that promote an immunosuppressive and pro-inflammatory lymphocyte milieu.

CAF-mediated recruitment of T lymphocytes is facilitated by secretion of chemokines and cytokines that promote the recruitment of tumor-promoting T cells, including CXCL9, CXCL10, and CXCL12 (SDF-1α) [51]. CAF-mediated recruitment of T lymphocytes to tumors and metastatic sites is facilitated also by secretion of S100A4/FSP1, a member of the S100 family of Ca^{2+}-binding proteins. S100A4/FSP1 is expressed by fibroblasts as well as by tumor cells and by immune cells. While its normal physiologic function is not well defined, multiple studies suggest a role for S100A4 in promoting tumor metastasis [52,53]. Grum-Schwensen and colleagues reported that tumor 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 (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 presence of S100A4(+/+), but not S100A4(−/−) fibroblasts significantly stimulated attraction of T lymphocytes to sites of growing tumors. Moreover, S100A4 not only attracted T lymphocytes but also activated them, which was manifested by cytokine production: S100A4-stimulated T cells produced increased levels of distinct cytokines and growth factors including CCL5/RANTES, CXCL16, IL-4, G-CSF and MIP [54].

In addition to promoting the recruitment of T lymphocytes into tumors, CAFs orchestrate an inflammatory microenvironment in tumors by swaying the balance of tumor-promoting lymphocytes, such as T regulatory cells and the T helper subtypes Th2 and Th17, versus cytotoxic T cells and tumor suppressing T helper (Th1) cells. In a murine transplantable breast cancer model, Liao et al. reported that CAFs promote tumor growth and metastasis in part through modulating the tumor immune microenvironment by inducing a switch from Th1 to Th2 type immunity. *In vivo* elimination of CAFs via pFAP vaccination significantly suppressed the recruitment of $CD8^+$ cytotoxic T lymphocytes, and correlated with an increase in Th1 cytokine expression in the tumor microenvironment [40]. In agreement with this capacity of CAFs to affect T cell polarization, it was recently reported that CAF-derived thymic stromal lymphopoietin (TSLP), which favors a Th2-type cell polarization, is associated with reduced patient survival in pancreatic cancer [55]. The exact signals exerted by CAFs which favor the induction of Th2 immunity response are currently unknown. Future studies that will further decipher the molecular mechanisms underlying CAF modulation of pro-tumorigenic immunity are warranted.

In line with their capacity to preferentially recruit and activate specific tumor-promoting T helper cell populations, CAFs were shown to recruit and expand Th17 cells in tumors. 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 [51]. Su et al. reported that CCL5/RANTES and CCL2 secreted by CAFs isolated from human melanoma, breast and colon cancers mediated recruitment of Th17 cells from peripheral blood [56]. In addition, CAFs produce a pro-inflammatory cytokine milieu that facilitates generation and expansion of Th17 cells, including IL-1, IL-6, IL-23, and TGF-β, key cytokines for human Th17 generation and differentiation [51,56,57].

CAFs were also suggested to contribute to accumulation of $CD4^+CD25^+FOXP3^+$ Regulatory T cells (Tregs) in tumors. Tregs, that have the ability to suppress the activity of T cells through partially defined mechanisms, accumulate in many types of human solid tumors and act to promote tumor escape from cytotoxic immune responses. CAFs can contribute to this accumulation of Tregs in tumor microenvironments in part by producing high levels of the immunosuppressive cytokine TGF-β1, that induces expression of Foxp3 and differentiation of Treg cells [58]. Fibroblasts found in the chronic inflammatory milieu of rheumatoid arthritis were shown to promote the stimulation of Tregs via secretion of IL-15 [59]. It is conceivable that this mechanism may also be operative in cancer-associated chronic inflammation. Recruitment of Tregs can also be mediated by chemokine secretion by CAFs: in a mouse model of breast cancer, 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 tumors, which in turn stimulate metastatic progression [60].

Collectively, these studies illustrate an important regulatory role for CAFs in modifying both the recruitment and the functional activation status of the lymphocytic milieu in tumor microenvironments.

CAFs contribute to maintenance of chronic inflammation in the tumor microenvironment

Acute inflammation is a physiologic process accompanying tissue damage and repair. This process is intrinsically self-resolving once the initiating stimulus is removed. During normal physiological processes of wound healing, inflammatory cells attracted to the injured site enhance tissue repair through various mechanisms and inflammation subsides once the tissue is repaired. The resolution of inflammatory leukocyte infiltrates, indicating the end of the inflammatory process is regulated by multiple regulatory pathways including the balance between cell recruitment, emigration, proliferation, and cell death [61]. In contrast, during all stages of tumorigenesis, these inflammatory processes deviate from their well-defined course, escape the normal regulatory circuits and become unrestrained and continuous, contributing to tumor formation, progression and metastatic spread [6]. Hence, the inflammation accompanying tumorigenesis possesses chronic, non-resolving characteristics.

Stromal fibroblasts were shown to dictate the type and duration of leukocyte infiltrates in non-cancerous inflammatory responses [62]. At the resolution of an acute inflammatory response, fibroblastic cells contribute to the withdrawal of survival signals and to normalization of chemokine gradients, thus enabling the infiltrating immune cells to undergo apoptosis or exit the site through the lymphatic system [61]. Once these regulatory circuits are damaged, inflammation becomes persistent and chronic [63].

As reviewed herein, CAFs contribute to the inflammatory process in tumors both directly, by recruiting cells of the innate and adaptive immune systems that respond to their cytokine and chemokine secretion, and indirectly by modifying the ECM to become favorable for different immune cell populations [64]. An additional possible mechanism by which CAFs promote cancer-related inflammation may be by *maintaining* the chronic nature of the inflammation accompanying tumors. In order to explore such a possibility, one can compare CAFs to the population of activated fibroblasts participating in pathologic inflammatory processes—e.g. activated fibroblasts in chronic inflammatory diseases such as Rheumatoid Arthritis (RA) and Osteoarthritis (OA).

Fibroblasts isolated from diseased tissues (for example, synovial fibroblasts derived from patients with RA, lung fibroblasts from experimentally-induced lung granulomas) display unique properties and secrete a distinct pattern of cytokines compared with fibroblasts isolated from non-inflamed normal tissues at the same anatomical site. Interestingly, this distinctive phenotype is stable in the absence of any external stimulation over many passages in culture, implying an epigenetic imprinted alteration in cell function. These phenotypic changes are associated with dramatic functional differences between normal and chronic-inflammation-derived fibroblasts in their ability

to influence leukocyte survival, differentiation and accumulation within inflamed tissues [62]. Similarly, CAFs isolated from several types of cancers, e.g. skin, breast and colorectal cancer express a pro-inflammatory gene signature that is consistent even when fibroblasts are propagated *ex-vivo*, suggesting stable epigenetic changes [34,65,66]. Indeed, Jiang et al. reported global hypomethylation of genomic DNA, consistent with epigenetic alterations in cancer-associated myofibroblasts from mouse and human gastric cancers [67]. Thus, since fibroblasts are relatively long-lived cells, it is conceivable that following their reprogramming to become pro-inflammatory CAFs, they contribute not only to recruitment of leukocytes but also, via their incessant signaling, to the retention of infiltrated immune cells and consequently to maintenance of chronic inflammation in tumors. Fibroblasts are reprogrammed into CAFs by paracrine signaling from tumor cells as well as by biomechanical forces in the tumor microenvironment [64]. Data from studies of chronic inflammatory diseases suggest that prolonged tissue hypoxia may be another mechanism by which inflammatory signaling by CAFs is induced and maintained. Human inflammatory synovial fibroblasts isolated from osteoarthritis and rheumatoid arthritis patients induced a significant enhancement in myeloid cell infiltration and angiogenesis in immunodeficient mice. This enhancement was associated with hypoxia-induced constitutive increased expression of vascular endothelial growth factor (VEGF) by inflammatory fibroblasts [68]. VEGF and CXCL12 antagonists significantly reduced myeloid cell infiltration and angiogenesis. VEGF and CXCL12 are both hypoxia inducible factor 1α (HIF-1α) responsive factors that can synergize in the recruitment and retention of myeloid cells [69]. Targeting HIF-1α transcriptional activity or expression in RA fibroblasts significantly reduced myeloid cell recruitment, suggesting that HIF-1α plays a role in fibroblast-mediated recruitment of myeloid cells [68]. Thus, the hypoxic conditions in chronic synovial inflammation induce stable changes in fibroblasts, that enhance inflammatory cell recruitment and angiogenesis, both of which are processes relevant to the perpetuation of chronic inflammation. The inflammatory stroma in chronic RA resembles cancer-associated stroma in several characteristics, including severely reduced oxygen concentrations. It can be speculated that similarly to the stable changes that occur in fibroblasts from chronic synovial inflammation, CAF-mediated recruitment of immune cells into tumors may also be amplified by tissue hypoxia.

Therefore, as a result of continuous signaling by cancer cells and immune cells, combined with biomechanical forces, oxygen concentration and other biochemical modifications in the tumor

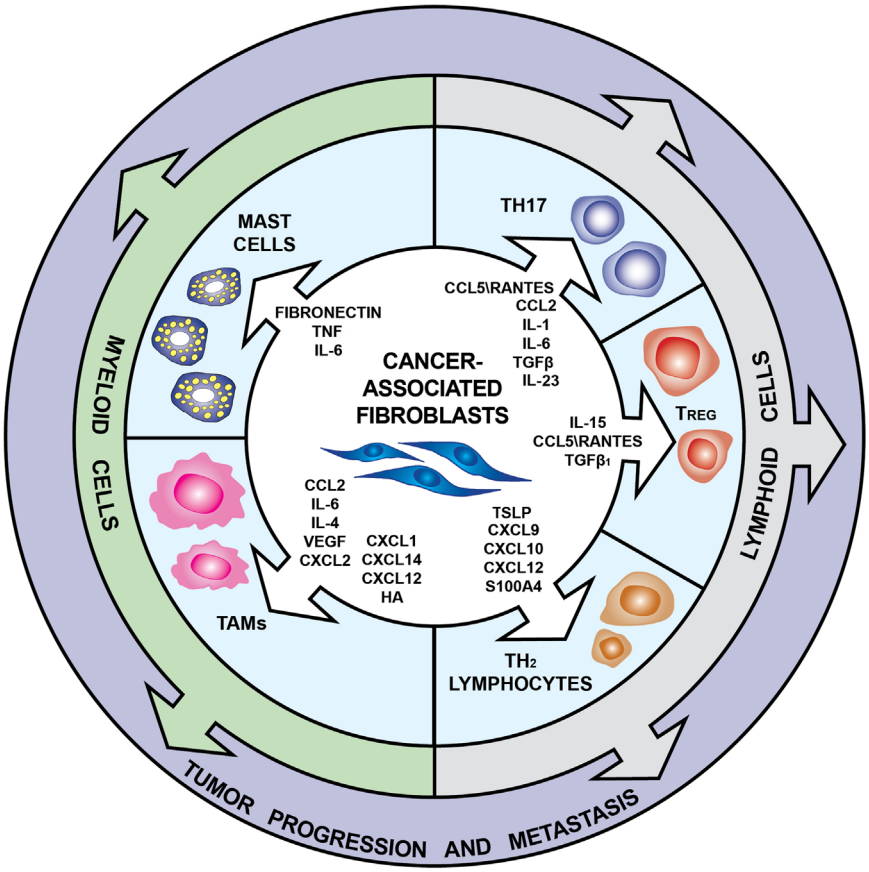

Fig. 1 – Cancer-associated fibroblasts (CAFs) exert multiple effects on various immune cell populations to promote a pro-inflammatory, immunosuppressive microenvironment. Chemokines and cytokines, as well as ECM components secreted by CAFs facilitate the recruitment and functional activation status of both myeloid and lymphoid cells into the tumor microenvironment at all stages of tumor growth. By enhancing tumor-promoting inflammation CAFs support tumor progression and metastasis. For simplicity, although many of the interactions are reciprocal, only the effects of CAFs on immune cells are shown. Abbreviations: TNF: tumor necrosis factor; IL: interleukin; VEGF: vascular endothelial growth factor; HA: hyaluronan; TSLP: thymic stromal lymphopoietin; TGFβ: transforming growth factor β; TREG: regulatory T cells; TH: T helper cells; TAMs: tumor associated macrophages.

microenvironment, CAFs are persistently activated. Pro-inflammatory signaling by CAFs contributes to recruitment, survival and retention of leukocytes in tumor microenvironments, and facilitates the maintenance of chronic inflammation.

Therapeutic implications and perspectives

The ability of CAFs to orchestrate tumor-promoting inflammation is central to their role in facilitating tumor growth, invasion and metastasis (Fig. 1). Compelling evidence from recent years demonstrating the crucial role CAFs play in the crosstalk between malignant cells and their microenvironment makes them an attractive therapeutic target. Several strategies have been suggested and, in some cases, clinically evaluated to inactivate or eliminate CAFs. Imatinib (Glivec) inhibits the receptor for platelet-derived growth factor (PDGF). The PDGF family of growth factors are well-established regulators of CAFs and pre-clinical targeting of PDGFR in experimental tumors had growth inhibiting effects in cervix and colorectal cancers [65]. Several clinically approved targeted drugs, including Sunitinib and Sorafenib incorporate PDGF receptor-inhibitory action and their therapeutic effects may partially result from their blockade of stromal signaling. Members of the TGFβ family are also known activators of fibroblasts and several TGFβ inhibitors are currently under development or in clinical trials (e.g., Lerdelimumab, Metelimumab) [70,71]. However, the complex and context-dependent activities of TGFβ on fibroblasts in human cancers should be taken into account when therapeutically targeting its activity [72]. CAF targeting with FAP vaccination has documented therapeutic effects in mice [65], and a humanized monoclonal antibody targeting FAP (Sibrotuzumab) had some efficacy in a phase I clinical trial [73]. In addition, several cytokine blockade therapeutics with anti-inflammatory activity that have been approved for treatment of chronic inflammatory diseases such as rheumatoid arthritis, are also in clinical trials for cancer. For example: Anakinra, targeting IL-1 signaling [74,75], and neutralizing anti-IL-6 antibodies (e.g: Sirukumab) [76]. These cytokine blocking therapeutics may exert some of their effects by blunting the pro-inflammatory activities of CAFs.

Clinical oncology progresses increasingly toward combinatorial approaches that act synergistically by targeting intrinsic pathways in cancer cells, as well as extrinsic tumor-enhancing pathways in the tumor microenvironment. Future studies will decipher in detail the molecular pathways underlying pro-inflammatory signaling by CAFs that support carcinogenic progression and metastasis, and will hopefully result in innovative therapeutic strategies allowing co-targeting of immune cells and CAFs to maximize treatment efficacy and prevent evasive resistance.

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