Cancer-Associated Fibroblasts in Inflammation and Antitumor Immunity

Kilian B. Kennel[1,2], Müge Bozlar[1,2], Adalbert F. De Valk[1,2], and Florian R. Greten[1,2,3]

ABSTRACT

Tumor-associated inflammation (TAI) is a feature of essentially all cancers and can confer both tumor-promoting and -suppressive functions. Cancer-associated fibroblasts (CAF) comprise one very heterogeneous cellular component of the tumor microenvironment characterized by a high degree of plasticity. Recent single-cell sequencing analyses revealed distinct CAF populations in various human cancers and helped to define key CAF subtypes, such as myofibroblastic, inflammatory, and antigen-presenting CAFs, with the first two being present in virtually all tumors. Importantly, these three CAF populations are involved in and modulate the positive and negative consequences of TAI. The remarkable plasticity of CAFs allows them to shift phenotypically and functionally in response to environmental changes. In this review, we describe how CAFs nurture tumor-promoting inflammation and suppress adaptive immunity. We also summarize the recently emerging evidence pertaining to tumor-suppressive CAF functions in the context of TAI. Finally, we summarize therapeutic concepts that aim at modulating CAF functions or depleting immunosuppressive CAFs to synergize with immunotherapy.

Introduction

Tumor-associated inflammation (TAI) is an important hallmark of all cancers and involves all cells of the so-called "tumor microenvironment" (TME), with the latter term describing the entirety of cancer, immune and endothelial cells as well as fibroblasts within a tumor (1). Despite its ubiquitous presence in cancers, the consequences of TAI are highly context-dependent and double-edged: for instance, longstanding inflammation of the liver or colon increases the risk of developing hepatocellular or colorectal carcinoma, respectively, and also in established cancers, TAI promotes tumorigenesis (1). On the other hand, TAI is necessary for tumoral T-cell infiltration, which positively correlates with prognosis in numerous cancer entities (2, 3). Moreover, T cells are the cellular mediators of immune checkpoint blockade (ICB) which implies that a certain degree of TAI is needed for ICB to be effective (3).

Fibroblasts are the main constituent of connective tissues and fulfill multiple functions under homeostatic conditions such as extracellular matrix (ECM) remodeling, angiogenesis, growth factor secretion as well as orchestration of the immune system (4). Furthermore, fibroblasts play a paramount role in wound healing where they secrete ECM molecules and contract wound edges, thereby leaving fibrotic (i.e., scar) tissue and terminating inflammatory processes that follow tissue damage. Intriguingly, tumors display several features that can also be found in wounded tissues, one of which is the elicitation of an inflammatory reaction (i.e., TAI) in response to a damaging event (i.e., malignant cells). And comparable with wounds, this inflammatory reaction is aimed at eliminating the harmful stimulus. However, in contrast to benign cells, tumor cells hijack several mechanisms to evade antitumor immunity which enables them to continuously grow, invade their surroundings and sustain TAI. As part of this perpetual process, tissue-resident fibroblasts become activated and turn into so-called "cancer-associated fibroblasts" (CAF). In addition to inflammatory signals such as IL1, IL6, and TNF, stimuli including TGFβ, Notch signaling, reactive oxygen species and cytotoxic cancer therapies mediate CAF activation and shape their phenotypes within the TME (4). In turn, CAFs secrete a variety of inflammatory cytokines and chemokines (e.g., IL1, IL6, CXCL1, -2, -5, -12, CCL2, -3), growth factors (e.g., TGFβ, hepatocyte growth factor, and vascular endothelial growth factor) and perform ECM remodeling, thereby interfering with key aspects of tumor biology including tumor cell proliferation, invasion, metastasis, angiogenesis, and, importantly, TAI (4, 5). So far, most studies described pro-tumorigenic functions of CAFs in the context of TAI and, generally speaking, a high CAF content is associated with a negative outcome in various cancers (6). In fact, in colorectal cancer, the fibroblast-rich CMS4 (= "Consensus molecular subtype 4") subtype is associated with the worst prognosis (7). However, there is accumulating evidence suggesting that CAFs also fulfil tumor-suppressive tasks during TAI (6–9). This ambiguity can be in part attributed to the fact that CAFs do not represent a single, homogenous population but rather a group of heterogeneous TME cells exhibiting different activation patterns (10). Single-cell sequencing technologies permitted investigations into CAFs at an unprecedented resolution and greatly advanced our understanding of CAF heterogeneity in the TME. Importantly, these analyses yielded several CAF subtypes which have been implied in TAI, including the key CAF populations myofibroblastic (my)CAFs, inflammatory (i)CAFs and antigen-presenting (ap)CAFs, as well as numerous others (11–14). However, many of these analyses remained descriptive and correlative and studies that try to functionally dissect the several CAF subtypes defined by single-cell sequencing in preclinical models are just emerging (9, 14–16). **Figure 1** explains some of the origins, marker genes and functions of CAF and the key CAF subsets, which we

[1]Institute for Tumor Biology and Experimental Therapy, Georg-Speyer-Haus, Frankfurt/Main, Germany. [2]Frankfurt Cancer Institute, Goethe University Frankfurt, Frankfurt/Main, Germany. [3]German Cancer Consortium (DKTK) and German Cancer Research Center (DKFZ), Heidelberg, Germany.

Corresponding Author: Florian R. Greten, Institute for Tumor Biology and Experimental Therapy, Georg-Speyer-Haus, Paul-Ehrlich-Straße 42-44, Frankfurt/Main 60596, Germany. E-mail: greten@gsh.uni-frankfurt.de

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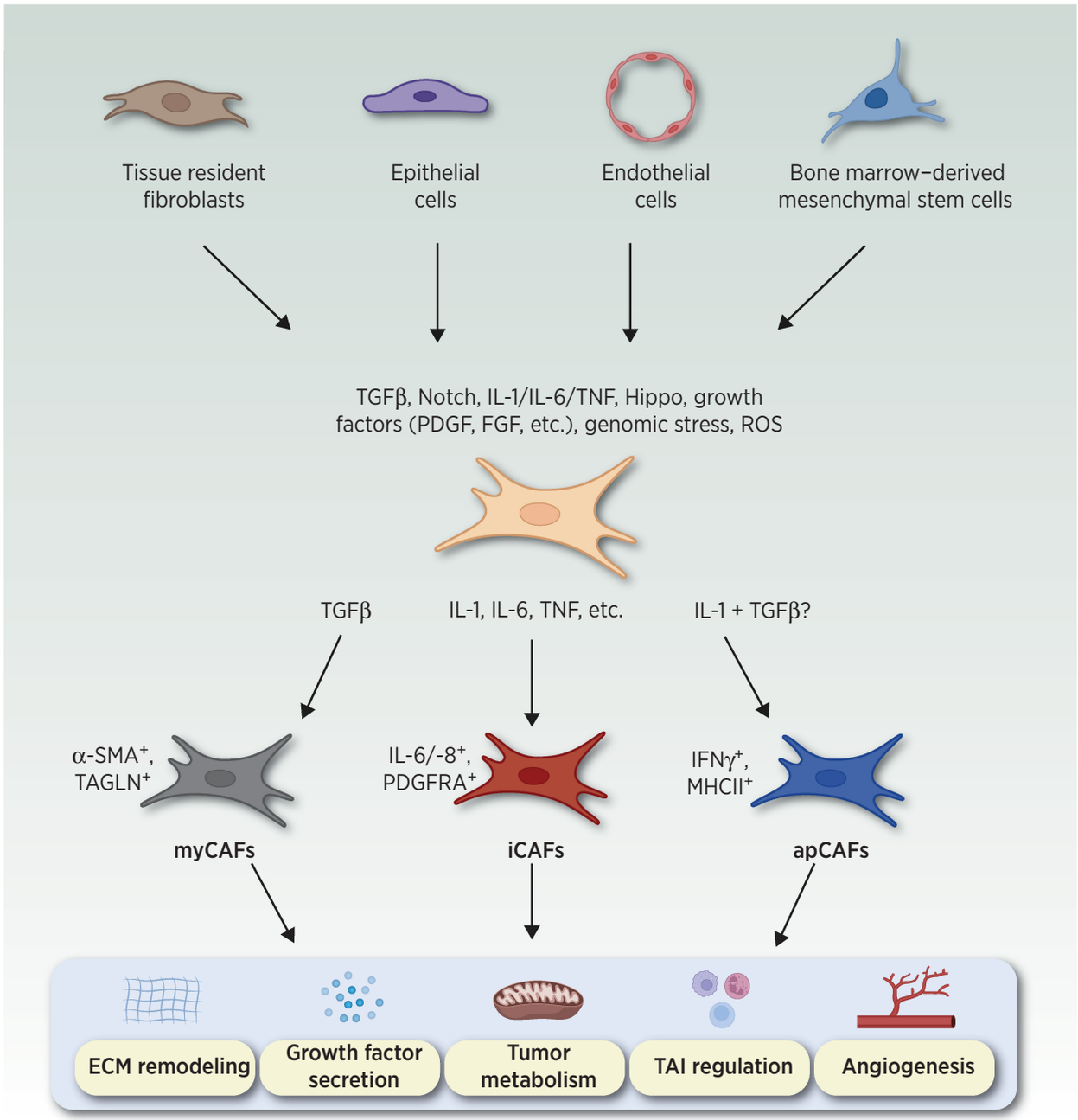

Figure 1.

CAF origins, important subtypes, and functions. Top, CAFs are derived from various cell types, the most important one being tissue-resident fibroblasts or fibroblast-like cells, including hepatic and pancreatic stellate cells. Other sources of CAFs are epithelial or endothelial cells that have undergone epithelial/endothelial-to-mesenchymal transition (EM- or EndMT) and circulating bone marrow–derived mesenchymal stem cells. Several external stimuli induce CAF activation, including TGFβ, pro-inflammatory cytokines, Notch signaling, and many more. Middle, Specific stimuli give rise to different CAF subsets. TGFβ can be considered as the key cytokine provoking a myofibroblastic (my)CAF phenotype, whereas pro-inflammatory cytokines such as IL1, IL6, and TNFα generate inflammatory (i)CAFs. The specific stimulus(i) governing formation of antigen-presenting (ap)CAFs have not been described yet, although a combination of IL1 and TGFβ has been proposed (45). Bottom, Importantly, myCAF, iCAF, and apCAFs all possess tumor-promoting and -suppressive functions by influencing the TME via different routes. PDGF, platelet-derived growth factor; FGF, fibroblast growth factor; α-SMA, alpha smooth muscle actin; TAGLN, transgelin; PDGFRA, platelet-derived growth factor receptor alpha; MHCII, major histocompatibility complex II. (Adapted from an image created with BioRender.com.)

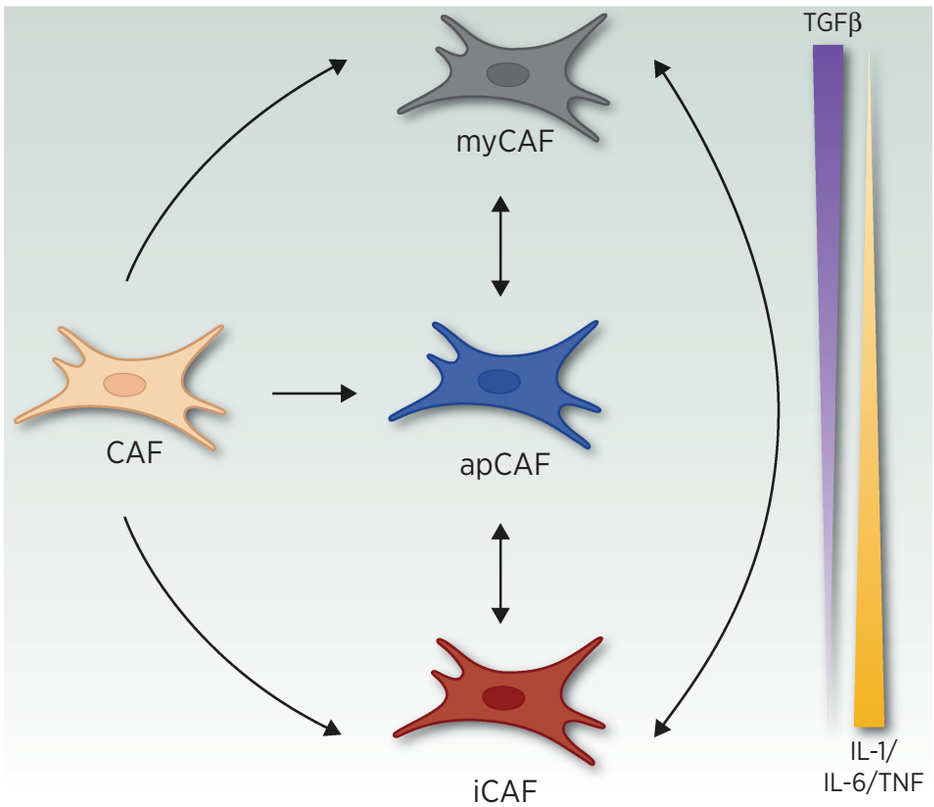

Figure 2.
CAF plasticity. CAF phenotypes are induced by inflammatory (IL1/IL6/TNF) or fibrotic (TGFβ) signaling. However, these phenotypes are not mutually exclusive and shift in response to environmental changes induced by tumor progression or therapeutic measures. (Adapted from an image created with BioRender.com.)

refer to in this review. For a comprehensive overview of the hitherto defined CAF subtypes and their marker genes, see reference (17). Intriguingly, CAF polarization is not defined by the activation of specific transcription factors as it is the case in T-cell polarization (e.g., FOXP3$^+$ T cells, also known as regulatory T cells) but phenotypic changes rather occur in response to exogenous stimuli and changes in the local cytokine milieu in the TME, thus, rendering these cells amenable for therapeutic exploitation (**Fig. 2**; refs. 15, 16). Here, we focus on the evidence for tumor-promoting and -suppressive functions of CAFs specifically in the context of TAI and immunosuppression and summarize findings related to CAFs positively modulating antitumor immunity. Finally, we briefly discuss avenues to enable targeting CAFs in an immunotherapeutic setting.

CAFs in Tumor-Promoting Inflammation and Immunosuppression

The involvement of fibroblasts in inflammation was suggested already decades ago (18). Since then, substantial effort has gone into deciphering the specific mechanisms by which CAFs mediate inflammation-driven tumorigenesis. Of these, we will here discuss attraction of pro-tumorigenic and immunosuppressive myeloid cell populations, direct stimulation of tumor cell growth and malignancy via secretion of pro-inflammatory cytokines as well as modulation of antitumor T-cell responses.

CAFs attract pro-tumorigenic myeloid cells

Myeloid cells such as macrophages, granulocytes, dendritic cells (DC) and myeloid-derived suppressor cells (MDSC) infiltrate tumors and promote tumorigenesis by facilitating tumor cell invasion and metastasis, supporting angiogenesis, and suppressing adaptive immune responses (19). However, some myeloid cell subpopulations such as conventional (c)DCs can also boost antitumor immunity (20). Nevertheless, generally spoken, the presence of myeloid cells in tumors is seen as an indicator of negative prognosis (19).

Erez and colleagues provided the first functional and molecular evidence how CAFs support squamous cell carcinoma growth demonstrating that CAFs attract macrophages by secreting pro-inflammatory cytokines IL1β, IL6, and chemokines CXCL1 and CXCL2 (21). Importantly, this pro-inflammatory CAF phenotype was dependent on IL1β-triggered activation of NF-κB, a master transcriptional regulator of inflammation (21). In line with that, loss of IL1 receptor 1 in pancreatic stellate cells and colon fibroblasts greatly reduced their pro-inflammatory potential and reduced the infiltration of myeloid cell populations into colorectal tumors (15, 16). The outstanding role of IL1 signaling in shaping pro-inflammatory CAFs has been confirmed by several studies (15, 16, 22–24). Furthermore, loss of the pathogen recognition receptor Toll-like receptor 4 (TLR4) and its downstream effector MyD88 in fibroblasts reduces tumorigenesis in a colorectal cancer model and associates with decreased tumoral infiltration of macrophages and neutrophils (25). On the basis of these results, it is conceivable that bacterial lipopolysaccharide (a TLR4 ligand) might as well be responsible to generate an inflammatory fibroblast population that attracts myeloid cells in colorectal cancer. Of note, inflammatory fibroblasts are suggested to reside at the luminal surface of human colorectal cancers which would allow them to interact with luminal bacteria (26). Furthermore, HIF2α—a transcription factor stabilized under hypoxic conditions—in fibroblasts mediates macrophage migration into pancreatic tumors and polarizes

them towards an immunosuppressive phenotype (27). In addition, fibroblast-secreted Chitinase-3-ligand-1 stimulated breast cancer cells to secrete CCL2 and other chemokines which attracted macrophages and fostered tumor growth in a murine breast cancer model (28).

CAF-secreted pro-inflammatory cytokines nurture tumor growth

Another mechanism by which CAFs impact tumor growth is the secretion of pro-inflammatory cytokines that act directly on tumor cells and induce cell proliferation, resistance to cell death and epithelial-to-mesenchymal transition (EMT). IL6, IL11 and leukemia inhibitory factor (LIF), which belong to the IL6 family of pro-inflammatory cytokines, are crucial mediators of inflammation-driven tumorigenesis (29). CAFs secrete IL6, IL11, and LIF in response to various stimuli including IL1β and TGFβ (15, 30, 31). IL6 generated by CAFs has pro-proliferative effects on various types of cancer cells (32, 33). Also, CAF-derived IL6 confers chemoresistance in an autochthonous model of pancreatic and facilitates EMT in esophageal cancer (34, 35). IL11, secreted by colonic fibroblasts, activates STAT3 and ERK signaling in cancer cells and promotes tumor growth and metastasis (30, 36). Finally, pancreatic stellate cell-derived LIF acts on pancreatic cancer cells to induce de-differentiation and resistance to chemotherapy (37).

CAFs suppress adaptive immune responses

The third way by which CAFs support tumor progression is immunosuppression involving MDSCs and regulatory T cells (T_{reg}), which are the main drivers of a suppressive TME by inhibiting cytotoxic (i.e., $CD8^+$) T-cell function employing various mechanisms (38, 39).

By secreting IL1β, CAFs stimulate the recruitment of MDSCs into primary and metastatic breast cancers, which has a tumor-promoting effect (40). Furthermore, chemokines CXCL1, 2, 5, CCL2 and CCL3 produced by CAFs attract tumoral MDSCs (41, 42). In addition, pancreatic CAFs produce IL6 and other cytokines to differentiate monocytes into MDSCs, which in turn, suppress T-cell proliferation (43).

Apart from that, CAFs also induce T_{reg} formation. For example, while apCAFs can present antigens to $CD4^+$ T cells via MHCII, they lack the necessary co-stimulatory molecules needed to induce full T-cell activation and clonal proliferation (11). This combination of MHCII-T cell receptor binding and suboptimal co-stimulation can induce T_{reg} formation (44). Consequently, apCAFs stimulate the differentiation of $CD4^+$ T cells into T_{reg}s and apCAF-induced T_{reg}s reduce $CD8^+$ T-cell proliferation in vitro (45). In line with that, another T_{reg} subset, $CD73^+$ $\gamma\delta T_{reg}$s, is induced by CAF-derived IL6 and reduces proliferation of $CD4^+$ T cells in a model of breast cancer (46). Moreover, using single-cell RNA sequencing, Costa and colleagues described a CAF subpopulation termed "CAF-S1" found in breast and ovarian cancers which increased T_{reg} infiltration via production of CXCL12 and enhanced T_{reg} differentiation (12, 47). In a follow-up study, the authors further elaborated that a subpopulation of the "CAF-S1" subset, namely "ecm-myCAFs" raised the expression of immune checkpoints PD-1 and CTLA-4 on T_{reg}s and associated with primary resistance to ICB (48).

Apart from these indirect effects on $CD8^+$ T-cell function, direct interactions between CAFs and $CD8^+$ T cells contribute to CAF-mediated immunosuppression. For example, Lakins and colleagues demonstrated that apCAFs trigger antigen-specific, FASL and PD-L2–dependent apoptosis of $CD8^+$ T cells and thereby hamper antigen-specific tumor control *in vivo* (49). Besides, CAF-derived FGF2 increases exhaustion of $CD8^+$ T cells by upregulating SPRY1, an inhibitor of T-cell receptor signaling (50). Lastly, CAF-secreted βig-h3 (also known as TGFβi) diminished antigen-specific proliferation of $CD8^+$ T cells which resulted in a tumor-promoting effect in a model of pancreatic cancer (51).

Finally, density, composition, and stiffness of the tumoral ECM which is mainly determined by CAF activity influences infiltration of T cells into tumor cores, associates with specific T-cell subsets and, thus, has an impact on antitumor immunity (52–54). For example, a dense pattern of collagen in the TME inhibits T-cell infiltration (52) Furthermore, in ovarian cancer, a protein signature including fibroblast-produced matrix molecules such as collagens and fibronectin positively associated with expression of T_{reg} and T_{H2} differentiation markers which is indicative of an immunosuppressive and tumor-promoting TME (54). In contrast, a recent study demonstrated that loss of ECM density in the aged skin promotes exclusion of T cells from melanomas (53). In line with that, deletion of type I collagen in an α smooth muscle actin $(\alpha\text{-SMA})^+$ CAF subset associated with decreased T-cell presence in pancreatic cancers (55).

In summary, CAFs leverage a multitude of different mechanisms to establish and maintain pro-tumorigenic inflammation and immunosuppression.

CAFs in Antitumor Immunity

Compared with the extensively studied tumor-promoting effects, tumor-suppressive CAF functions in the context of antitumor immunity are less well described. First evidence was generated in 2014, which demonstrated that an $\alpha\text{-SMA}^+$ myofibroblast population was required to suppress regulatory T_{reg}s and to maintain $CD8^+$ T-cell function (56). This has recently been confirmed by another study from the same group reporting that indeed $\alpha\text{-SMA}^+$ CAFs possess tumor-limiting functions whereas fibroblast activation protein alpha $(FAP)^+$ CAFs induce tumor progression in pancreatic cancer by reinforcing immunosuppression (8, 35). Importantly, loss of type I collagen in $\alpha\text{-SMA}^+$ CAFs accelerated pancreatic tumor progression and correlated with increased infiltration of MDSCs and, likely as a consequence, a decreased infiltration of T cells that could in part explain the tumor-restrictive function of $\alpha\text{-SMA}^+$ CAFs (55). Notably, loss of IKKβ in $Col1a2^+$ fibroblasts associated with an increase of T_{reg}s and augmented tumor growth in an inflammation-driven model of colorectal cancer (57). Surprisingly, loss of IKKβ in $Col6^+$ fibroblasts led to a decreased tumoral immune cell content and hampered tumor growth in the very same model (24). The contrasting results of these studies support the notion that specific genes exert opposing functions in different CAF subsets, which in turn, calls for careful selection of marker genes when performing genetic manipulations in fibroblasts to study CAF-immune cell interactions.

In addition, a CD105-negative CAF population was shown to impede tumor growth in pancreatic cancer (9). This phenotype was dependent on an intact adaptive immune system, suggesting that CD105-negative CAFs foster antitumor immunity (9).

Intriguingly, and in contrast to previous studies, Kerdidani and colleagues demonstrated that apCAFs also have tumor-suppressive properties, which was due to C1q secreted by apCAFs that acted on $CD4^+$ T cells to protect them from apoptosis (58).

Tertiary lymphoid structures [TLS, also known as "Ectopic lymphoid-like structures" (ELS) or "Tertiary lymphoid organs" (TLO)] are accumulations of B and T cells formed in inflamed and cancerous tissues and display some of the key features of secondary lymphoid organs (59). Importantly, the presence of TLS

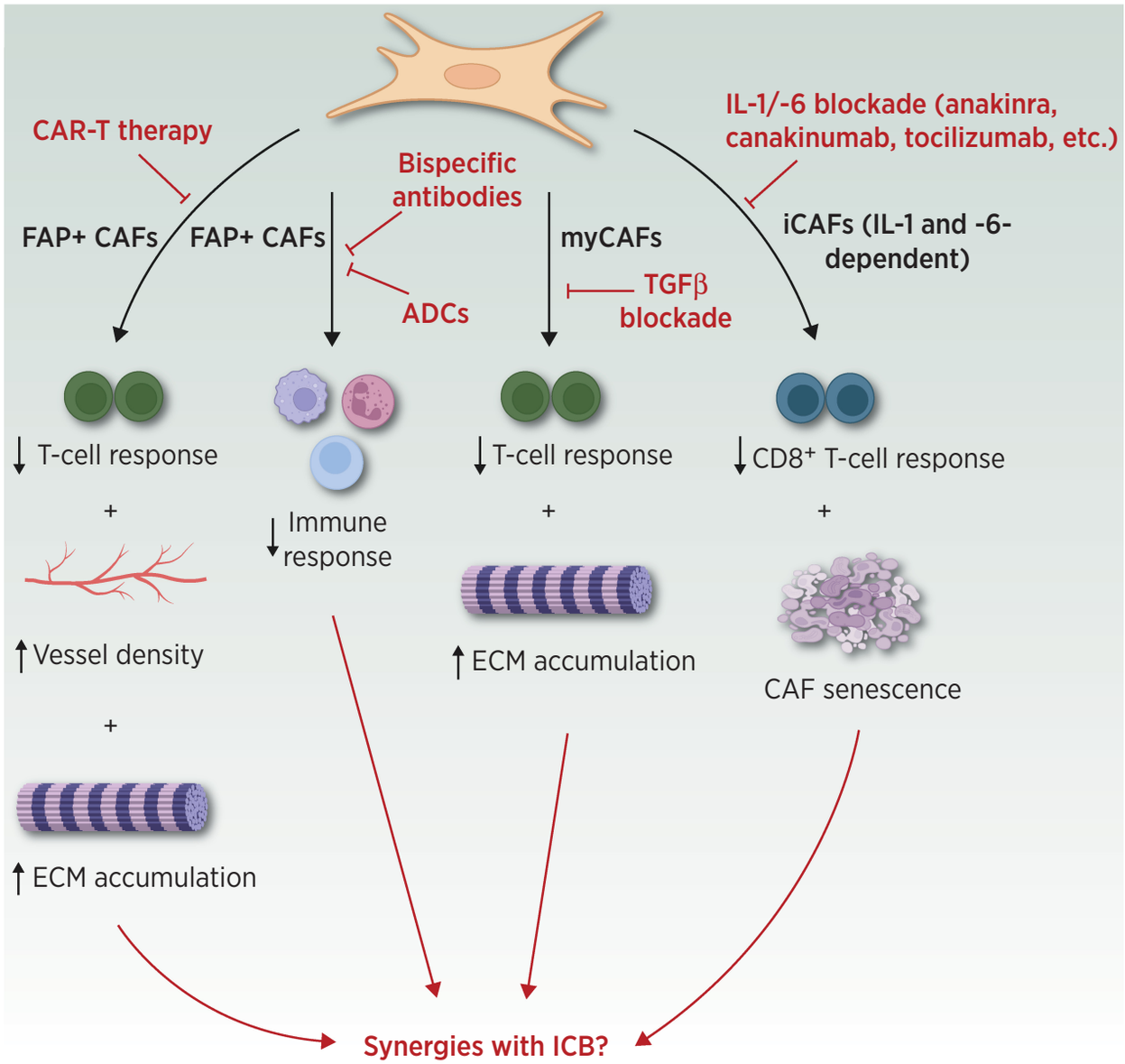

Figure 3.
CAFs as targets in cancer immunotherapy. Immunosuppressive and otherwise pro-tumorigenic CAF subsets such as FAP$^+$, my-, and iCAFs can be targeted via various routes. CAF-directed therapy might be synergistic with ICB. (Adapted from an image created with BioRender.com.)

correlates with better prognosis and response to ICB in multiple cancer entities (59). FAP-negative CAFs orchestrate the formation of TLS in part by accumulation of B cells via the CXCL13-CXCR5 axis in mouse models of melanoma and colorectal carcinoma (60). Conversely, in a mouse model of Sjögren's syndrome, FAP$^+$ fibroblasts were mainly involved in the assembly of TLS, again hinting toward different functions of fibroblasts subsets depending on localization and type of pathology (61). In essence, there is an increasing amount of evidence implying antitumor immune functions of CAFs, which include interactions with T_{reg}s, MDSCs and the B-cell compartment.

CAFs as Targets in Cancer Immunotherapy

The translation of immunotherapies and, specifically, ICB into the clinical setting represented a breakthrough in oncology. However, while some tumors like melanomas show excellent response to and, thus, can be sustainably controlled or even cured by ICB treatment in metastasized stages, others such as pancreatic and most colorectal cancers do not (62, 63). Importantly, among other factors, a high tumoral fibroblast content is associated with a diminished response to anti–CTLA-4, anti–PD-1 and anti–PD-L1 therapy in an array of

cancers (6). Therefore, targeting of CAFs represents a promising strategy to overcome resistance to immunotherapies. However, as stated above, CAF depletion also had adverse effects on the outcome in preclinical models, which is why utmost caution needs to be exercised when deleting CAFs from the TME. Current immunotherapeutic approaches directed at or mediated by CAFs include, among others, the depletion of the immunosuppressive FAP^+ CAF population, utilization of FAP^+ TME cells as a vehicle to deploy drugs and antibodies in a tumor-specific manner, as well as blocking of TGFβ and IL1 signaling (**Fig. 3**).

In line with the immunosuppressive role of FAP^+ CAFs in lung and pancreatic cancer (8, 35), chimeric antigen receptor T (CAR-T) cells engineered to specifically target FAP^+ cells significantly reduced tumor burden in a lung cancer model (64). Similarly, Wang and colleagues demonstrated that FAP-specific CAR-T therapy increased the endogenous $CD8^+$ T-cell response against tumors in various subcutaneous models (65). Moreover, removal of FAP^+ cells via CAR-T cells stunted tumor growth also by immune-independent effects which included a reduction of tumor vessel density and diminished ECM deposition (66). However, FAP-specific CAR-T therapy imposed significant bone toxicity and cachexia in several subcutaneous tumor models, thus warranting caution when translating these results into the clinic (67). Furthermore, a bispecific antibody that both targets FAP and functions as an CD40 agonist elicited a potent antitumor immune response with limited systemic side effects in two subcutaneous tumor models (68). In line with that, a novel FAP-targeting antibody–drug conjugate (ADC) termed "OMTX705" induced complete regressions by augmenting $CD8^+$ T-cell infiltration even in PD-1–resistant tumor models (69).

As stated above, TGFβ and IL1 signaling pathways are pivotal for CAF activation. Hence, blocking of these two pathways could be beneficial for antitumor immunity. Indeed, two landmark studies from 2018 describe how TGFβ receptor 1 inhibitor galunisertib or a TGFβ antibody synergize with ICB to unleash a powerful and sustained T-cell response (70, 71). Surprisingly, Jiao and colleagues demonstrated that TGFβ blockade cooperated with ICB in models of bone metastasis but not primary prostate cancer, suggesting that stage- and host organ-specific factors determine response to combinatorial treatment (72). In another study, TGFβ blockade gave rise to a population of $CD73^+$ IFN-licensed (il)CAFs which exhibited a transcriptomic signature compatible with a response to IFN signaling (73). Furthermore, ilCAFs produced T-cell attractants CXCL9, -10, and -11, thereby likely increasing T-cell infiltration, retarding tumor growth and synergizing with ICB (73). This suggests that ilCAFs might contribute to increased antitumor immunity in TGFβ inhibitor-treated tumors. Because of these promising preclinical results, several clinical studies are currently investigating a putative beneficial effect of combinatorial TGFβ blockade and ICB for various cancer entities (74).

Finally, tumor-promoting iCAFs in pancreatic cancer were suggested to be susceptible to blockade of IL1 signaling using the recombinant IL1-receptor 1 antagonist (anakinra; ref. 15). Consequently, co-administration of anakinra with radiotherapy prevented iCAF polarization, blocked radiation-induced CAF senescence and decelerated tumor progression in a model of rectal cancer (16). Importantly, in that study, treatment with anakinra was associated with a substantial increase of $CD8^+$ T cells within irradiated tumors suggesting that ICB might have additional synergistic effects in that setting (16). The potentially beneficial combination of chemoradiotherapy with anakinra is currently being investigated in rectal cancer patients in a phase I study (75). Taken together, CAF-directed immunotherapeutic approaches including combinatorial treatment with immune checkpoint inhibitors generated promising results in preclinical models that are currently being translated into the clinic (**Table 1**).

Concluding Remarks

Within the last years, it has become evident that CAFs cannot be viewed as purely tumor-promoting agents in the TAI context. In fact, CAFs represent a highly diverse and extraordinarily plastic cell population within the TME. Their versatile functions can be divided into "physical" and "chemical" aspects: CAFs "physically" block or facilitate immune cell infiltration through regulation of the ECM and "chemically" interact with immune cells via cytokine and chemokine secretion.

Table 1. Ongoing or completed clinical trials evaluating combinatorial targeting of CAFs (and other TME cells) and immune checkpoints.

Target	Drug	Targeted CAF populations	Mechanism	Reference (NCT identifier)
IL1 signaling	Canakinumab (anti-IL1β antibody) + ICB	iCAFs	Inhibition of iCAF activation	e.g., NCT04028245
TGFβ signaling	Galunisertib (TGFβ1 receptor inhibitor) and various others + ICB	myCAFs	Inhibition of myCAF activation	e.g., NCT02423343 (For a comprehensive overview, see Ref. 75)
Angiotensin receptor 1 (AT1R)	AT1R antagonist Losartan + ICB	myCAFs	Reduction of ECM synthesis	NCT03563248
IL6 receptor (IL6R)	IL6R antagonist Tocilizumab	iCAFs	Inhibition of iCAF activation	NCT03999749
FAP	CAR-T preparations, ADCs, bispecific antibodies, FAP-activated prodrugs with or without ICB	FAP^+ CAFs	Deletion of FAP^+ CAFs or exploitation of FAP^+ CAFs for drug delivery	NCT03932565 NCT04826003 NCT05098405 NCT04857138 NCT04969835
FAK	FAK inhibitor Defacitinib + Pembrolizumab + Gemcitabine	CAFs	Inhibition of CAF activity	NCT02546531
Vitamin D receptor (VDR)	VDR agonist Paracalcitol + Pembrolizumab	CAFs	Reversion of CAF activation	NCT03331562
Retinoic acid receptor (RAR)	RAR agonist ATRA + Ipilimumab	CAFs	Reversion of CAF activation	NCT02403778

Abbreviations: FAK, focal adhesion kinase; PSC, pancreatic stellate cells; ATRA, all-trans retinoic acid.

Importantly, these soluble factors are likely to be systemically active and might therefore influence metastatic spread, e.g., by creating pre-metastatic niches. Thus, these putative pleiotropic CAF effects as well as other factors such as tumor entity, stage, TME composition and previous therapeutic interventions need to be carefully considered when tailoring therapies aimed at modulating CAF function to boost the antitumor immune response. Yet, their versatility and plasticity provide a great opportunity to modulate their respective functions for therapeutic benefit.

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