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REVIEW ARTICLE

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The heterogeneity of cancer-associated fibroblast subpopulations: Their origins, biomarkers, and roles in the tumor microenvironment

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Abstract

The prognosis for patients with cancers known for a highly activated stromal reaction, including diffuse-type (scirrhous) gastric cancer, consensus molecular subtype 4 (CMS4) colorectal cancer, and pancreatic ductal adenocarcinoma, is extremely poor. To explore the resistance of conventional therapy for those refractory cancers, detailed classification and investigation of the different subsets of cancer-associated fibroblasts (CAFs) involved are needed. Recent studies with a single-cell transcriptomics strategy (single-cell RNA-seq) have demonstrated that CAF subpopulations contain different origins and marker proteins with the capacity to either promote or suppress cancer progression. Through multiple signaling pathways, CAFs can promote tumor growth, metastasis, and angiogenesis with extracellular matrix (ECM) remodeling; they can also interact with tumor-infiltrating immune cells and modulate the antitumor immunological state in the tumor microenvironment (TME). Here, we review the recent literature on the various subpopulations of CAFs to improve our understanding of the cell-cell interactions in the TME and highlight future avenues for CAF-targeted therapy.

KEYWORDS
cancer-associated fibroblast, heterogeneity, immune therapy, subpopulation, tumor microenvironment

1 | INTRODUCTION

Cancer-associated fibroblasts (CAFs) are the major component in the tumor microenvironment (TME) and account for almost 70% of the cells in tumor tissues, where they perform several tumorigenic functions.[1,2] The importance of the TME has been examined by numerous previous studies that have clarified the relationship between cancer cells, microenvironmental cells, and the resulting prognoses in patients with gastric, prostate, and colorectal cancer,[3–7] among others. By their interactions with cancer cells, CAFs remodel the extracellular matrix (ECM) and lead to the collective invasion of tumor cells, creating a supportive niche for cancer stem

Abbreviations: BM-MSCs, bone marrow–derived mesenchymal stem cells; CAF, cancer-associated fibroblasts; CRC, colorectal cancer; ECM, extracellular matrix; EMT, epithelial-to-mesenchymal transition; FAP, fibroblast activation protein; FSP-1, fibroblasts specific protein-1; GC, gastric cancer; PDPN, podoplanin; TGF-β, transforming growth factor-β; TIME, tumor immune microenvironment; TME, tumor microenvironment; α-SMA, α-smooth muscle actin.

cells, weakening the tumor immune microenvironment (TIME), and reprogramming cancer cell metabolism, resulting in the promotion of tumor metastasis and immune escape[8–12] (Figure 1). Using a single-cell transcriptomics strategy (single-cell RNA-seq) to profile the compositions of solid tumors, several groups have discovered various fibroblast subpopulations having distinct phenotypes and functions among pancreas, breast, and colorectal cancers.[13–15] Elyada and colleagues[13] first identified a new subset of fibroblasts in pancreatic cancer, called antigen-presenting CAFs (apCAFs) in addition to the previously identified myofibroblastic CAFs (myCAFs) and inflammatory CAFs (iCAFs).[16] The heterogeneity and plasticity of CAFs serve multiple mechanisms of cancer development by interaction with other cells in the TME. CAF-derived factors direct survival signals to cancer cells; they also affect the TIME by inhibiting immune-promoting cells and stimulating the recruitment of immune-suppressive cells, which enable cancer cells to evade immune surveillance.[5,16,17] An increasing number of studies about cancer immunotherapy including the use of PD-1/PD-L1 antibodies have revealed the involvement of CAFs in the TIME through various mechanisms, contributing to the formation of a tumor-permissive microenvironment.[18–20]

The aim of this review is to shed light on the complex nature of CAFs, including their identities, functions, and significance in cancer biology.

2 | ORIGIN OF CAFs

Generally, the term CAF is used to describe morphologically spindle-like cells and functionally activated fibroblastic cells in the TME of solid cancers that have a phenotype distinct from the quiescent fibroblasts found in normal tissue. There is evidence that CAFs arise from bone marrow–derived precursors and bone marrow–derived mesenchymal stem cells (BM-MSCs),[6] and derive from local resident fibroblasts, adipocytes, adipose-derived MSCs, and pericytes.[21–25] Among them, we have clarified that MSCs could influence the progression of gastric cancer (GC); the expression of the MSC marker nerve growth factor receptor (NGFR), also called CD271, in stromal

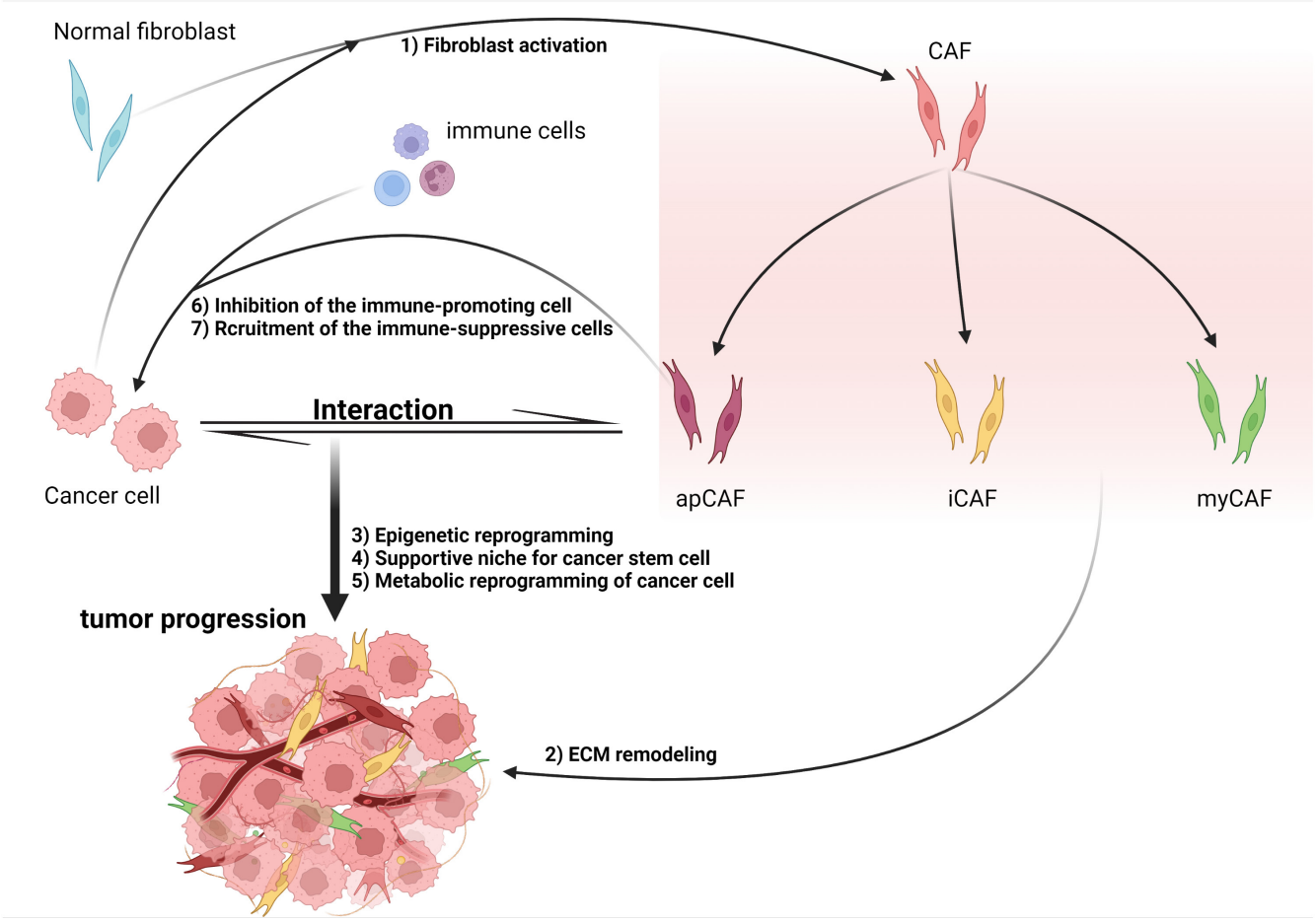

FIGURE 1 A schematic diagram of cancer-associated fibroblasts (CAFs) in the tumor microenvironment. Cancer cells stimulate normal fibroblasts to become CAFs. CAFs have been divided into three subpopulations, inflammatory CAFs (iCAFs), myofibroblastic CAFs (myCAFs), and antigen-presenting CAFs (apCAFs). CAFs, especially myCAFs, remodel the extracellular matrix (ECM). Interaction between CAFs (iCAFs and myCAFs) and cancer cells lead to metabolic reprogramming of cancer cells and epigenetic reprogramming of cancer stem cells, creating a supportive niche for the latter. Proliferation of apCAFs leads to the recruitment of immune-suppressive cells and inhibits immune-promoting cells

cells is related to a poor prognosis in GC patients.[6] We have also reported that CXCL1 from cancer cells stimulated the recruitment of BM-MCs into the tumor stroma via CXCR2 signaling in GC.[7,26] Additionally, Friedman et al recently reported that sCAF, a subpopulation expressing MHC class II antigen-presentation genes, might originate from a mesenchymal source, like BM-MSC, because the computational approaches for their trajectory show that the transcriptional makeup of sCAF is disconnected from tissue-resident fibroblasts, and the most differentially upregulated gene in sCAFs compared with other CAF subpopulations was *Clu*, which has been reported to play a tumor-promoting role in BM-MSC–derived CAFs.[27] Furthermore, Waghray et al identified a novel population of cancer-associated MSCs in pancreatic ductal adenocarcinoma (PDAC) that controls tumor progression via granulocyte-macrophage colony–stimulating factor.[28] In the pancreas, differentiation of tissue-resident pancreatic stellate cells (PSCs) to an activated, myofibroblast-like phenotype is thought to be a major source of PDAC CAFs.[29] Taken together, the heterogeneity of CAF subpopulations might derive from their origins and differentiations depending on autocrine and paracrine signaling in TME. These studies lay the foundation for our understanding of CAF heterogeneity in the TME and impel further investigation of the origins and functions of CAF subtypes.

3 | HETEROGENEITY OF CAF BIOMARKERS

Due to the complexity of CAFs, researchers are attempting to clarify specific CAF subtypes (Table 1). Currently, although α-SMA+ or FAP+ CAFs are the two most predominant CAF markers, deletion of each phenotypic CAF showed opposite results. Depletion of αSMA+ cells in PDAC development yielded poorly differentiated tumors and reduced survival.[30] Conversely, depletion of FAP+ cells resulted in the enhancement of antitumorigenic cytotoxic CD8+ T cells and slowed pancreatic tumor growth.[17] Recently, using a single-cell sequence, which is a breakthrough methodology to detect transcriptional levels of a single cell, Li et al defined different CAF subpopulations with α-SMA associating with other fibroblast markers such as transgelin (TAGLN) and platelet-derived growth factor subunit A (PDGFA), while FAP was associated with decorin (DCN) and COL1A2 expressions in CRC.[15] Öhlund et al defined α-SMAHighFAP+ pancreatic CAFs as a myofibroblastic subtype (myCAF) actively responsive to TGF-β, while the remaining α-SMALow CAFs were shown to secrete inflammatory mediators such as IL-6, which promotes the growth and proliferation of patient-derived PDAC organoids (iCAF).[16] Furthermore, Elyada et al reported a third subtype of CAFs that expressed MHC class II and CD74, named apCAFs.[13] Vimentin is a type III intermediate filament protein, which plays an important role in the formation of the cytoskeletal network. Vimentin is highly expressed in fibroblasts of all types; therefore, it widely used as a marker to visually identify fibroblast populations in immunohistochemical and immunofluorescent studies. However, as vimentin is also present in a number of different cell types of mesenchymal origin, such as adipocytes and myocytes, and even in epithelial cells undergoing epithelial-to-mesenchymal transition (EMT), its specificity as a marker of CAF is relatively low.[31] S100 calcium-binding protein A4 (S100A4), also known as fibroblast-specific protein 1 (FSP1) also marks tumor cells that have undergone EMT[32]; however, the genetic lineage–labeling approach has shown FSP1 expression in only a subset of CAFs and minimal overlap between FSP1 and αSMA expressions.[33] Another marker that is overexpressed in CAF populations, podoplanin (PDPN), is strictly membrane-bound.

Friedman et al reported identifying eight CAF subtypes in two main CAF populations, based on selective expressions of the markers FSP1 or PDPN in breast cancer, noting that the ratio between PDPN+ and S100A4+ CAFs strongly correlates with clinical outcomes.[27] Platelet-derived growth factor receptors (PDGFR) α and β are tyrosine kinase receptors located on the surface of stromal cells, and both are commonly used as general markers for CAFs. In contrast to FAP and αSMA, the strength of PDGFRs lies not in their specificity for CAFs but rather in their global expression in the overall fibroblast population in the tumor.[34] Periostin (POSTN) is also highly expressed in fibroblast and CAF populations. Recently, the presence of CAFs acting as a cancer inhibitor in the tumor has also been reported, and in these cells, Meflin is expressed simultaneously with low expression of αSMA in the early stages of pancreatic cancer, which is essential for weakening the aggressiveness of the tumor.[35] Our laboratory previously reported that numerous bone-marrow–derived stromal cells (BM-SCs) infiltrated the gastric TME and that NGFR (CD271) expression in stromal cells could be used as a prognostic marker of BM-SCs for GC patients.[6]

Finally, negativity for several markers is used to help identify fibroblasts and CAFs. As there is no single definitive marker of CAFs, it is often critical to rule out other cell types contained in tumor tissue. Epithelial cell adhesion molecule can be used to rule out epithelial cells,[3637] and negativity for other markers such as CD45 and CD31 has been used to exclude leukocytes and endothelial cells.[13]

As mentioned above, even through the application of transcriptome analyses, no single marker has been found to completely define CAF subpopulations so far, and none of these subtypes was given a specific definition. With further advances, new markers for CAF subpopulations might be identified, which relate to the origin of CAF. Identifying the definitive markers of CAF subpopulations could help the development of novel targeted stromal therapies with CAF-targeted profiling.

4 | CANCER-PROMOTING OR CANCER-RESTRAINING CAF

Researchers have reported that CAFs have diverse and complex effects on cancer cells (Figure 2). A previous study reported that various solid tumors contain many stroma cells and a large quantity of ECM produced by these cells, the volume of which may exceed that of cancer cells in tumors.[38] In particular, CAF proliferation is prominent

TABLE 1 Cancer-associated fibroblasts (CAF) markers used for identification in human tissue

	Description	Surface marker
CAF markers		
ACTA2 (α-SMA)	Actin protein, a marker of myofibroblast	No
FAP	A 170 kDa membrane-bound gelatinase (transmembrane grycoprotein)	Yes
Vimentin	Type III intermediate filament protein, widely expressed in various fibroblast subpopulations	No
FSP-1 (S100A4)	A member of the S100 calcium-binding protein family; considered to be a marker of quiescent fibroblasts	No
PDGFR-α	A transmembrane protein consisting of an extracellular ligand-binding domain, a transmembrane domain, and an intracellular tyrosine kinase domain	Yes
PDGFR-β	An approximately 180-kDa receptor tyrosine kinase, belonging to the type III tyrosine kinase receptor (RTK) family	Yes
Podoplanin (PDPN)	A mucin-type, integral membrane, heavily O-glycosylated glycoprotein	Yes
COL1	The most abundant collagen of the human body, not exclusive to fibroblasts	No
POSTN	A secreted extracellular matrix protein, associated with the epithelial-mesenchymal transition in cancer cells	No
Tenascin-C	Extracellular matrix glycoproteins; a myofibroblast-associated marker	No
Negative markers		
EPCAM	A marker for epithelial cells	Yes
PECAM-1 (CD31)	A marker for endothelial cells	Yes
PTPRC (CD45)	Negative marker used for identification of leukocytes	Yes
SMTN	A marker for smooth muscle cells	Yes

in refractory cancers such as scirrhous gastric and pancreatic cancer, as well as in poorly differentiated cancers arising in a variety of organs.[1,39–44] Many studies have clarified that the CAFs that promote cancer progression (pCAFs) do so through numerous mechanisms, including ECM remodeling and production of cytokines, chemokines, and growth factors. These directly or indirectly promote cancer progression and metabolism as well as angiogenesis.[38] We previously reported that CXCL1 secreted from GC cell recruits BM-MCs into the TME, where the BM-MCs are differentiated into myofibroblasts. These myofibroblasts and cancer cells could mutually increase each other's proliferation, thus resulting in highly malignant scirrhous GC.[7,26]

Our previous study reported that CXCL12 (also known as SDF1) from tumor stromal cells may stimulate the proliferation of GC cells through the CXCR4 axis in a hypoxic microenvironment.[45,46] In addition to these reports, it has been reported that pCAF functions are exerted via various cytokines such as CCL2,[47] CXCL9, and CXCL10[48] and factors such as IGF1,[49] PDGF[50,51] VEGF,[52] and TGF-β.[53] Furthermore, CAF has been reported to promote cancer angiogenesis via CXCL12[54] and VEGFA.[52]

These studies suggest the new strategy of targeting pCAFs to inhibit cancer progression, and several attempts to develop such therapeutic agents have advanced to clinical evaluation.[55,56] However, some clinical trials of agents targeting pCAF or stroma have been unsuccessful.[57,58] Furthermore, it has also become clear that there are CAFs that have inhibitory roles in cancer progression.[59–61] Chen et al[62] suggested that these cancer-restraining CAFs (rCAFs) perform functions such as acting as a barrier against cancer cell invasion and seeding, promoting anticancer immunity, proinflammatory secretome and signaling for tumor suppressors, and producing specific ECM components as barriers to tumor cell invasion and dissemination. Mizutani et al identified that Meflin-positive cells might be a candidate surface marker of rCAF in pancreatic and colon cancer.[35] Mizutani et al hypothesized that Meflin suppresses the activity of Lox family proteins, thereby reducing ECM cross-linking in TME, and that this mechanism may improve chemosensitivity if Meflin expression in CAFs softens the cancer stroma.[35] As mentioned previously, α-SMA+ or FAP+ CAFs are the two major CAF markers, but depletion of CAFs of each phenotype has been shown to have opposite results. Özdemir et al demonstrated that selective αSMA+ cell depletion resulted in poorly differentiated primary tumors, increased metastasis and decreased survival. These changes were accompanied by a decrease in F4/80+ monocytes and TIME due to increased regulatory T (Treg) cell infiltration into the tumor.[30] However, depletion of FAP+ cells resulted in an increase in antitumor cytotoxic CD8+

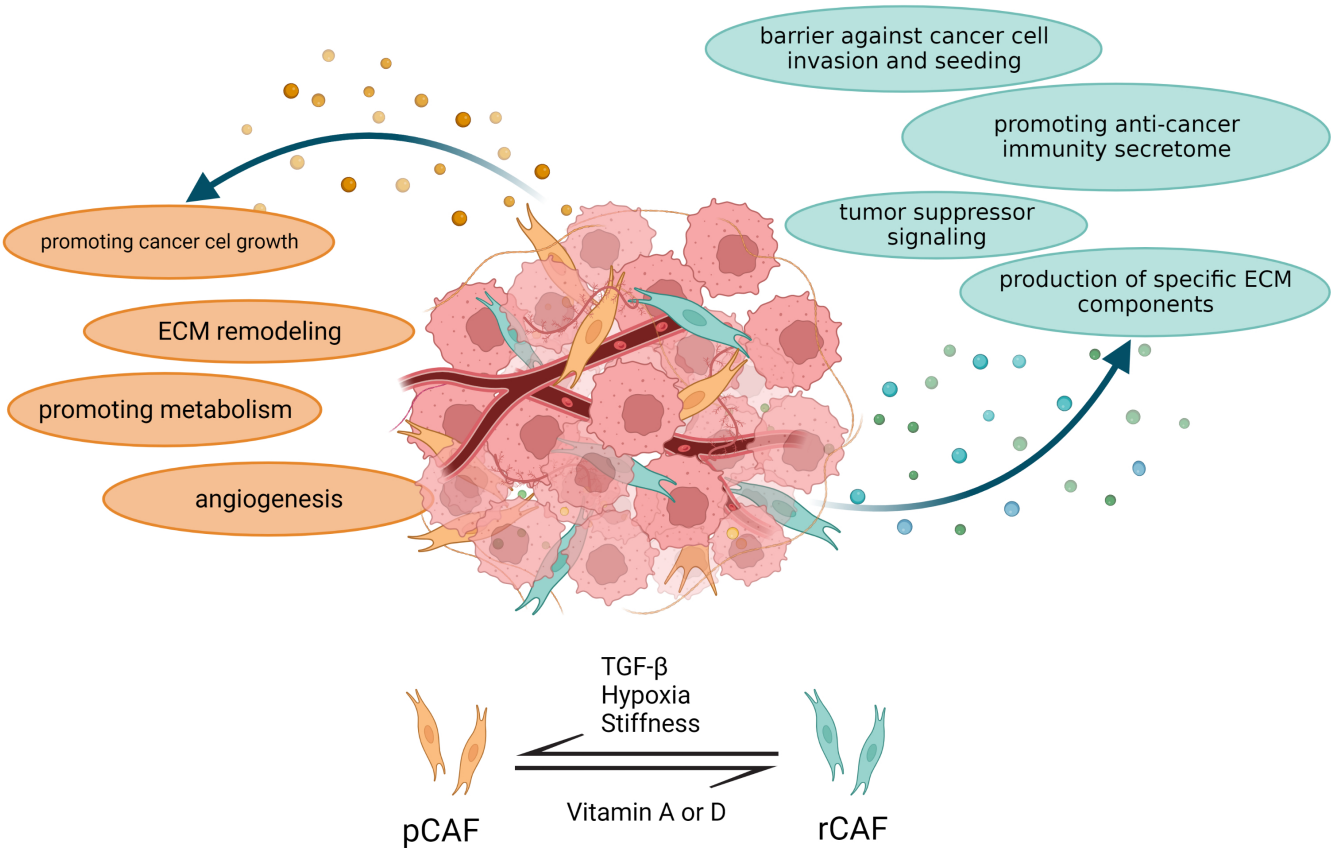

FIGURE 2 Heterogeneity of cancer-associated fibroblasts (CAFs) in cancer-promoting and cancer-restraining functions. Schematic diagram of subpopulations of potential cancer-promoting CAFs (pCAFs) and cancer-restraining CAFs (rCAFs). pCAFs act on the cancer-promoting system through various factors, while rCAFs act on the cancer-restraining system, for example, by softening the extracellular matrix (ECM)

T cells, showing a tendency to inhibit pancreatic tumor growth.[17] Furthermore, genetic deletion or pharmacological targeting of FAP+ cells reduced tumor growth in mouse models of colorectal, lung, and breast cancer.[63–66] These suggest that, even in these two major CAF markers, αSMA-expressing cells act as rCAF and FAP-expressing cells act as pCAF involving their TIME. In addition, our colleagues found that, in biliary tract cancers, αSMA+ CAFs play a suppressive role (rCAF) via interleukin-8, but not in pancreatic cancer.[67] Future efforts are needed to identify the definitive tumorigenic mechanisms of αSMA+ and FAP+ CAFs in the TME.

Hedgehog (Hh) signaling in CAF is also known for its role that potentially mediates the tumor-suppression as rCAF. Rhim et al reported that Hh-driven tumor stroma suppressed tumor growth in part by restraining tumor angiogenesis.[61] Furthermore, Gerling et al suggested that activation of stromal Hh signaling resulted in loss of stromal bone morphogenetic protein (BMP) inhibitors and might have the potential to restrain colon cancer initiation and progression.[59]

However, there are possibly opposing reports that Hh signaling in CAFs plays not only a tumor-suppressive but also a tumor-progressive role. A previous study suggested that sonic hedgehog (SHH) protein expressed on PDAC cells contributed to tumor progression via differentiation and motility of resident fibroblasts already present in PSCs and pancreatic tissue.[68] Moreover, in the study of Steele et al, Hh signaling inhibition alters fibroblast composition and immune infiltration in the pancreatic cancer microenvironment.[69]

Importantly, owing to their heterogeneity, it is also hypothesized that stromal switch involves the conversion of rCAFs to pCAFs, contributing to cancer progression.[38]

Keeping all of this in mind, although these issues are presumably due to the diversity of CAFs resulting from their origin and heterogeneity, it has now been widely accepted that CAFs can have a dual role in tumorigenesis. It is incredibly vital that understanding the function and behavior of CAFs in TME improves the capacity to identify the therapeutic target in the future.

5 | INTERACTION BETWEEN CAFs AND THE TIME

Cancer-associated fibroblasts in the TME have been shown to play an important role in regulating the antitumor activity of tumor-infiltrating

immune cells, including innate and adaptive immune cells in the TIME[70] (Figure 3). CAFs also indirectly affect immune cell recruitment and activity by promoting the expression of immune checkpoint molecules and ECM remodeling.[70] Previous studies have shown that CAFs promote immune cells through the production of various factors such as TGF-β, CXCL2, collagen, MMPs, and laminin, as well as performing ECM degradation and remodeling, but have also been reported to interact with immune elements to promote cancer development and progression.[71–73] Many studies have been conducted on the TIME, suggesting that the interaction of CAF with immune cells and other immune components may modulate the TIME and thus inhibit antitumor immune responses.[72,74,75] In detail, by secreting various chemokines, CAFs can limit the mobilization of immune effector cells such as CD8+ T cells into tumor tissue.[76] Furthermore, it has been shown that the proportion of immunosuppressive cells such as M2-type macrophages, Treg cells, and MDSCs, which are modified by CAFs, is markedly increased in the TIME, thereby facilitating tumor immunosuppression.[77–79] In addition, several cytokines secreted by activated immune cells, such as IL-1β, have been reported to induce the conversion of normal fibroblasts into iCAF, which may further suppress immune function in the TIME.[80]

Recently, Elyada et al identified a new subset of fibroblasts, called apCAF, in PDAC, and it has high activity against STAT1, which is known to mediate MHC class II expression in response to IFNγ, suggesting that apCAF is regulated by IFNγ signaling in vivo.[13] They then postulated that MHC class II expression by apCAFs may act as a decoy receptor to induce withdrawal of CD4+ T cells, preventing their clonal proliferation, thus leading to T cell anergy or differentiation into Tregs, contributing to an immunosuppressive TIME. Interestingly, recent reports have indicated that apCAFs are derived from mesothelial cells.[81] Then, during pancreatic cancer progression, mesothelial cells reduce the mesothelial features induced by IL-1 and TGF-β and acquire fibroblast features to form apCAFs. apCAFs are induced by direct antigen-specific ligation of naive CD4+ T cells to Tregs. Furthermore, the authors showed that treatment with antibodies targeting mesothelin, a mesothelial cell marker, can effectively inhibit the mesothelial cell-to-apCAF transition and thus the Treg formation induced by apCAFs.

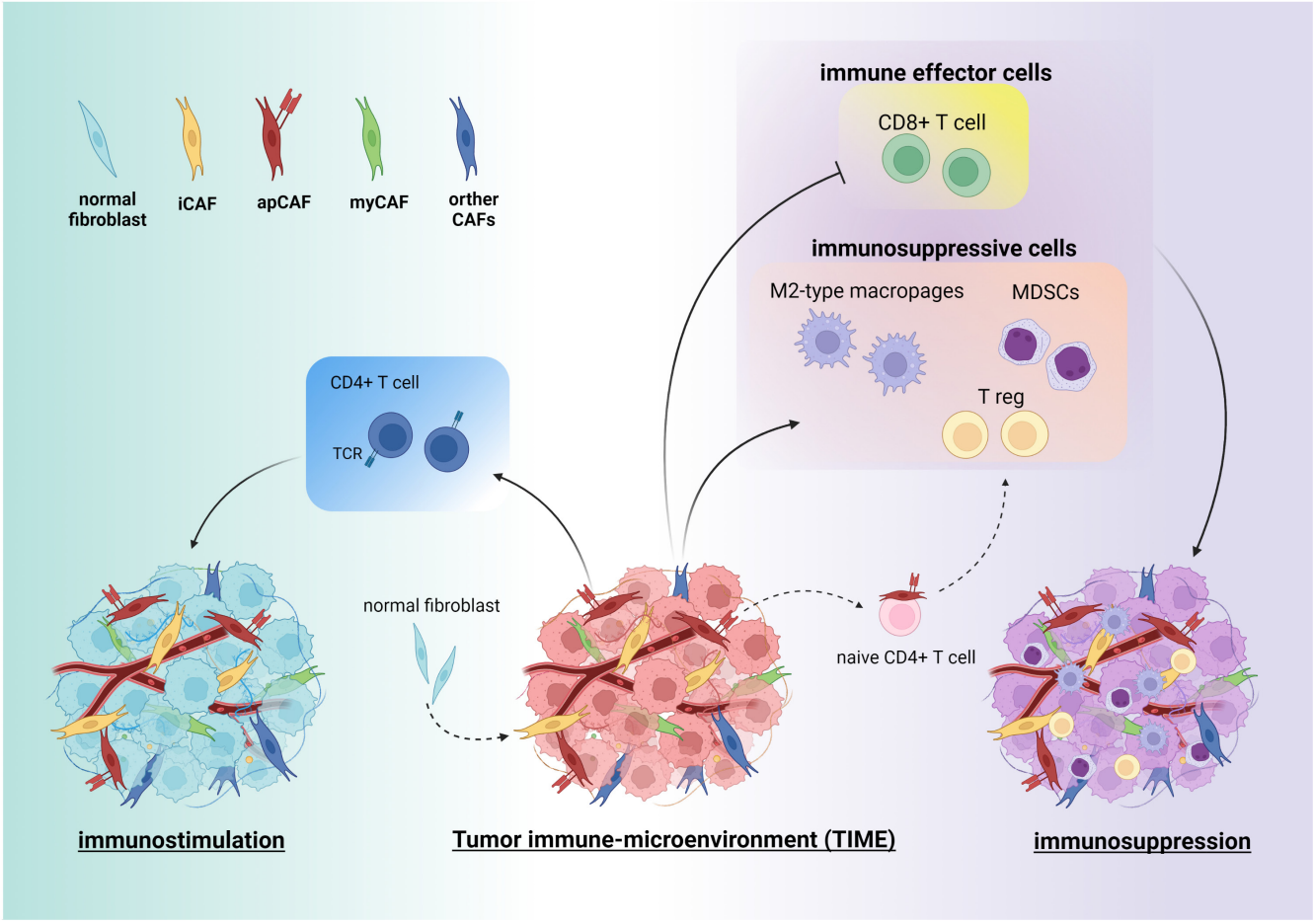

FIGURE 3 Cancer-associated fibroblasts (CAFs) in the tumor immune microenvironment (TIME). Schematic diagram of functions of CAFs in the TIME. CAFs limit the mobilization of immune effector cells (CD8+ cells) and markedly increase immunosuppressive cells, resulting in the immunosuppression of the TIME. On the other hand, MHC class II immunity via tumor antigen presentation by CD4+ cells helps the immunostimulation of the TIME. The interaction of CAFs with immune cells and other immune components may modulate the TIME and thus inhibit/stimulate antitumor immune responses. MDSCs, myeloid-derived suppressor cells; Treg, regulatory T cells

Importantly, on the other hand, apCAFs in lung cancer play, conversely, not only an immune-suppressive but also a tumor-suppressive role with MHC class II immunity via tumor antigen presentation.[82] As mentioned above, if the function of apCAF is highly dependent on the tissue/cell type from which it is derived, several other questions arise. In view of the advent of tumor stroma as a new immunotherapy target, these are clearly questions of therapeutic relevance and not simply theoretical ones.

All in all, it is crucial to obtain a precise understanding of the roles of CAFs within the TIME, and the multidimensional interactions of infiltrating immune cells will help researchers determine the immune modulation mechanisms induced by CAFs; further exploration of these interactions will likely identify the potential for CAF-targeted immunotherapy.

6 | CONCLUSIONS

In this review, we have briefly summarized and described the origin, heterogeneity, and tumor-promoting and -suppressive roles of CAFs with cancer within the TME, as well as with immune cells within the TIME. The precise molecular profile, classification of CAF subpopulations, and a better understanding of interaction in the TME are warranted that will provide us with better prognostic biomarkers and CAF-targeting drugs to ultimately improve patient survival.

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CONFLICT OF INTEREST

The authors have no conflict of interest.

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