The recent advances of cancer associated fibroblasts in cancer progression and therapy

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As an abundant component of tumor microenvironment, cancer-associated fibroblasts (CAFs) are heterogeneous cell populations that play important roles in tumor development, progression and therapeutic resistance. Multiple sources of cells can be recruited and educated to become CAFs, such as fibroblasts, mesenchymal stem cells and adipocytes, which may explain the phenotypic and functional heterogeneity of CAFs. It is widely believed that CAFs regulate tumor progression by remodeling extracellular matrix, promoting angiogenesis, and releasing soluble cytokines, making them a promising cancer therapy target. In this review, we discussed about the origin, subpopulation, and functional heterogeneity of CAFs, with particular attention to recent research advances and clinical therapeutic potential of CAFs in cancer.

**KEYWORDS**
cancer-associated fibroblasts, tumor microenvironment, heterogeneity, tumor progression, tumor therapy

**Introduction**

As an important component of tumor microenvironment, CAFs are described as activated fibroblasts located in the vicinity of cancer cells without the phenotype of epithelial, cancerous, endothelial, and immune cells (1). They are elongated and spindle-shaped in morphology and have some positive markers, such as alpha-smooth muscle actin (α-SMA), fibroblast activation protein (FAP) and fibroblast specific protein 1 (FSP-1) (2). CAFs have merged as the hot-spot of cancer study; however, their phenotypic and functional heterogeneity hinders the clinical application (3). Studies have shown that CAFs could secrete a variety of chemokines, cytokines, and growth factors to facilitate tumor growth, chemotherapy resistance and immunosuppression (4). On the contrary, some studied have reported the tumor-suppressive function of CAFs in certain tumor
models (5). This review summarized the heterogeneity of biological origins, phenotypic markers, and biological functions of CAFs, as well as uncovered how their heterogeneity made identification, subtype classification and clinical therapy challenging. Our review provided a new perspective for CAF research and personalized therapy.

The origin and transition of CAFs

Increasing evidence suggest that CAFs have different cellular origins. Though precise lineage tracing study has shown the origin of fibroblasts in healthy or injured tissues, the origins and specific activation processes of CAFs are still lacking (6, 7). Several cells may be predecessors of CAFs, such as normal fibroblasts (8), mesenchymal stem cells (MSCs) (9), pancreatic stellate cells (PSCs) (10), epithelial cells (11), endothelial cells (12), adipocytes (13), pericytes (14), hematopoietic stem cells (15) and cancer stem cells (CSCs) (16). The changes in the microenvironment where these precursor cells exist in may be a primary inducer of CAF transition (3).

As the major source of CAFs, normal fibroblasts can transform to CAFs by cytokines secreted by stromal or tumor cells. Transforming growth factor-β (TGF-β) can induce the CAF phenotype through SMAD-dependent or independent pathway (17). For example, bladder cancer cells released exosomes contain TGF-β, leading to the activation of SMAD-dependent signaling and the stimulation of normal fibroblasts to CAFs (8). Platelet-derived growth factor-D (PDGF-D) secreted by cholangiocarcinoma cells could stimulate surrounding fibroblasts to produce VEGF-C and VEGF-A, resulting in the expansion of lymphatic vasculature and tumor cell intravasation (18). In addition to cytokines, non-coding RNAs from cancer cells can also induce the conversion of resident fibroblasts to CAFs. Exosomes derived from hepatocellular carcinoma cells were rich in miR-1247-3p, which activated SMAD-dependent signaling processes of CAFs (19). In lung adenocarcinoma, miR-200 deficiency in cancer cells promoted the expression of Jagged1/2 and the activation of Notch in adjacent CAFs, which reprogrammed CAFs from a quiescent state into an active pro-tumorigenic state (20). Additionally, the hypoxia microenvironment also contributes to the activation of resident fibroblasts. Hypoxia was related to the accumulation of ROS, the activation of the HIF-1α signaling pathway in hepatocellular carcinoma cells, and the enhanced expression of FAP in surrounding fibroblasts (21).

MSCs are another important source of CAFs. The transformational potential of MSCs into CAFs was first proved in breast cancer (9). TGF-β secreted by cancer cells recruited MSCs and maintained the differentiation of MSCs into CAFs (22). In colorectal cancer, the high level of stromal cell-derived factor-1 (SDF-1) upregulated the expression of chemokine receptor 4 (CXCR4) and TGF-β in MSCs, leading to the transformation of MSCs (23). In epithelial ovarian cancer, the elevated expression of STAT4 in epithelial cells induced MSCs derived from adipose and bone marrow to obtain CAF-like features, which in turn promoted EMT and peritoneal metastasis of ovarian cancer by secreting CXCL12, IL-6 and VEGF-A (24).

In addition to the stimulation of cancer cells, changes in tumor microenvironment like pH can also stimulate the transformation of MSCs. PH induced activation of MSCs to CAFs was decreased by upregulating the expression of proton-sensing G-protein-coupled receptor68 (GPCR68) and activating downstream effector Yes-associated protein (YAP) in MSCs (25).

The other cellular origins of CAFs have been reported. For example, PSCs could transform to CAFs in pancreatic cancer (10). In pancreatic ductal adenocarcinoma, the IL-1 signaling cascade led to JAK/STAT activation and induced an inflammatory CAF state (26). Epithelial or endothelial cells are found to be the probable origins of CAFs through epithelial-to-mesenchymal transition (EMT) or endothelial-to-mesenchymal transition (EndMT). The human nasal epithelial cells were activated and displayed CAF phenotypes such as FSP or FAP through EMT when they were exposed to matrix metalloproteinase (MMP)-9 (11). TGF-β could induce proliferating endothelial cells into fibroblast-like cells (12). In addition, a recent study reported that tumor cells induced adipocytes to CAFs by activating Wnt/β-catenin signaling in ovarian cancer (13). Cancer cells, especially cancer stem cells, have also been demonstrated to be a source of CAFs through the action of TGF-β (15). Besides these sources of CAFs mentioned above, there also exist some uncommon origins, such as pericytes, hematopoietic stem cells, which needs further exploration (14, 16).

In brief, the activation of CAFs is mainly regulated by different cytokines and signaling pathways of cancer niche (Figure 1). Although the origins of CAFs in solid tumors were not fully elucidated, using lineage tracing technologies to track CAF transition may provide a solution in the future.

Phenotypic identification and subtype classification of CAFs

The altered protein profiles can be used to identify or isolate CAFs. According to the distinct phenotypic markers, CAFs can be divided into several subpopulations and some of them partially overlap. In this part, we will present the phenotypic differences and subtype classification of CAFs, and provide some suggestions for identifying different CAF populations.

There are several typical CAF markers, such as FAP, α-SMA, FSP-1, PDGFR-α, PDGFR-β, and Thy-1 (27). Despite the diversity of biomarkers, the isolation of CAFs from cells remains a challenge due to low specificity. For example, α-
SMA and FAP were highly presented in pericytes, lymphatic endothelial cells and fibroblast reticular cells. Similarly, vimentin was present in endothelial cells, smooth cells and tumor cells (28). Additionally, with the continuous optimization of detection technology, the researchers identified uncommon PSC-derived CAF subsets in pancreatic ductal adenocarcinoma tissues. These CAFs located away from cancer cells, lacked elevated α-SMA expression, and secreted IL-6 and other inflammatory mediators (10). The results highlighted the importance of considering multiple indicators in CAF identification. In addition to classical phenotypic markers, some new ones are studied in recent years. In pancreatic cancer, the high expression of caveolin-1 (Cav-1) in CAFs was associated with the invasiveness of cancer cells and poor prognosis of patients (29). The same results were further proved in lung adenocarcinoma (30). Similarly, a recent study reported that the melanoma cell adhesion molecule+ (MCAM+) CAFs induced by TGF-β in colorectal cancer patients were associated with poor prognosis (31). Another study concluded that focal adhesion kinase (FAK) activity in CAFs was increased in PDAC tissues compared with healthy ones and the FAK+ CAFs could be an independent prognostic marker (32).

Based on surface markers, CAFs are classified into different subtypes that display distinctive secretory phenotypes and perform specific biological functions in dynamic tumor environment, as summarized in Table 1 (33). In a mouse model of pancreatic ductal carcinoma, the researchers demonstrated the existence of myofibroblastic CAFs (myCAFs), inflammatory CAFs (iCAFs) and antigen-presenting CAFs (apCAFs) by single-cell RNA sequencing. MyCAFs were characterized by the expression of α-SMA, TAGLN, MYL9, TPM1, TPM2, MMP11, POSTN and HOPX, which could promote the proliferation, invasion and metastasis of tumor cells. ICAFs could promote metastasis and angiogenesis by producing inflammatory cytokines and chemokines such as IL-6, IL-8, CXCL1, CXCL2, CCL2, CXCL12 and Ly6c. ApCAFs had immunomodulatory capacity in pancreatic ductal adenocarcinoma. They expressed MHC II, Saa3, Slp and could activate CD4+ T cells in an antigen-specific manner in the model system (34). Another study reported four CAF subtypes in pancreatic ductal adenocarcinoma based on transcriptomic analysis. These four subgroups, named A-D, could be distinguished by differential expression of three markers, periostin (POSTN), myosin-11 (MYH11) and podoplanin (PDPN). Patients with the dominant subtype-C had prolonged survival, whereas those with the dominant subtype D had the worst prognosis, suggesting that specific tumor-stromal interactions are associated with adverse outcomes (35). Furthermore, a novel subtype of CAFs with a highly activated metabolic state (meCAFs) was identified in PDAC. MeCAFs had highly activated glycolysis, and patients with abundant meCAFs had a higher risk of metastasis and poor prognosis, but showed a dramatically better response to immunotherapy (36).

In human breast cancer, four CAF subgroups, known as S1-S4, have been identified by flow cytometry, immunohistochemistry, and RNA sequencing. They can be distinguished according to the expression of FAP, CD29, αSMA, PDPN and PDGFRβ. CAF-S1 stimulated cancer cell migration and mediated EMT transition through the activation of CXCL12 and TGF-β. CAF-S4 induced...
| CAF subtypes | Phenotypic markers | Functions | Detecting techniques | Cancer types              | Refs |
|-------------|-------------------|-----------|----------------------|---------------------------|------|
| myCAF (myofibroblastic CAF) | α-SMA, TAGLN, MYL9, TPM1, TPM2, MMP11, POSTN, HOPX | Promoting proliferation, invasion and metastasis | Single-cell RNA sequence | Pancreatic ductal carcinoma (mouse) | (34) |
| iCAF (inflammatory CAF) | IL6, IL8, CXCL1, CXCL2, CCL2, CXCL12, Ly6c | Promoting metastasis and angiogenesis | | | |
| apCAF (antigen-presenting CAF) | MHC II, Saa3, Splt | Activating CD4+ T cells | | | |
| CAF-A | POSTN | Associated with intermediate prognosis | Single-cell RNA sequence | Pancreatic ductal carcinoma (human) | (35) |
| CAF-B | POSTN, MYH11, PDGPN | Associated with intermediate prognosis | | | |
| CAF-C | PDGPN | Associated with better prognosis | | | |
| CAF-D | Not determined | Associated with poorer prognosis | | | |
| mcCAF (Metabolic state CAF) | CD74 and HLA-DRA | Promoting metastasis | Single-cell RNA sequence | Pancreatic ductal carcinoma (human) | (36) |
| CAF-S1 | FAP<sup>High</sup>, CD29<sup>Med-High</sup>, α-SMA<sup>High</sup>, PDGFRα<sup>High</sup> | Mediating EMT | Flow cytometry, immunohistochemistry and RNA-sequencing | Breast cancer (human) | (37) |
| CAF-S2 | FAP<sup>Low</sup>, CD29<sup>Low</sup>, α-SMA<sup>Neg</sup>, PDGFRα<sup>Neg-Low</sup> | Making up of healthy tissues | | | |
| CAF-S3 | FAP<sup>Low-Med</sup>, CD29<sup>High</sup>, α-SMA<sup>Neg-Low</sup>, PDGFRα<sup>Neg-Low</sup> | Making up of healthy tissues | | | |
| CAF-S4 | FAP<sup>Neg</sup>, CD29<sup>Low</sup>, α-SMA<sup>Neg</sup>, PDGFRα<sup>Neg-Low</sup> | Inducing cancer invasion | | | |
| CD10+ GPR77+ CAF | CD10, GPR77 | Promoting tumor formation and chemoresistance | Single-cell RNA sequence | Breast and lung cancer (human) | (38) |
| vCAF (vascular CAF) | Desmin, Fibulin-1, PDGFRα | Invading tumor stroma | Single-cell RNA sequence | Breast cancer (human) | (39) |
| mCAF (matrix CAF) | Similar with vCAF | Regulating tumor immune response | Similar with vCAF | | |
| eCAF (cycling CAF) | Scrg1 | Promoting tumor formation | | | |
| CAF-C1 | BMP4 | Modulating cancer cells proliferation and stemness | Single-cell RNA sequence | Oral carcinoma (human) | (40) |
| CAF-C2 | α-SMA | Inhibiting cancer proliferation | | | |
| eCAF (extracellular matrix CAF) | POSTN | Promoting cancer invasion | Single-cell RNA sequence | Gastric cancer (human) | (41) |
| CAF-A | MMP2, DCN, COL1A2 | Remodeling extracellular matrix | Reference component analysis(RCA) | Colorectal cancer (human) | (42) |
| CAF-B | ACTA2, TAGLN, PDGFA | Expressing cytoskeletal genes | | | |
| Subtype I | HGF, FGF7 | Broad tumor promotion | Single-cell RNA sequence | Non-small lung cancer (human) | (43) |
| Subtype II | FGF7 | Modest tumor promotion | | | |
| Subtype III | Low HGF and FGF7 | Minimal tumor promotion | | | |
| Activated myofibroblast Phenotype | α-SMA, vimentin, FAP, collagen 1α, PDGFRα | Enhancing the stemness of cancer cells | Flow cytometry | Hepatocellular carcinoma (human) | (44) |
| Mesenchymal stromal cell phenotype | CD90, CD73, CD165, CD29, CD44, CD166 | Regulating immunosuppression | | | |
| FAP-high CAF | FAP, TGF-β, IL-6, COL1A1, SULF1, CXCL12 | Regulating cancer invasion and immune regulation | Quantitative RT-PCR | High-grade serous ovarian cancer (human) | (45) |
| FAP-low CAF | DLK1, COLEC11, TCF21 | Regulating glucose homeostasis and lipid metabolism | | | |
Functional heterogeneity of CAFs in cancer biology

CAFs promote tumorigenesis and metastasis

CAFs play a dynamic role in proliferation, invasion and metastasis of tumors, and its mechanism is gradually elucidated. In lung adenocarcinoma, CAFs secreted SDF-1 to promote the expression of CXCR4, β-catenin and peroxisome proliferator activated receptor δ (PPARδ) in tumor cells, and enhance cancer invasiveness and EMT (51). In breast cancer, CAFs secreted IL-32 to induce an interaction between integrin β3 and the RGD motif, activate p38 MAPK in tumor cells, leading to increased expression of EMT markers (52). TGF-β and inflammatory cytokines secreted by breast cancer cells induced CAFs to express gremlin 1 (GREM1), abrogating BMP/SMAD signaling.
and promoting stemness and invasion of cancer cells (53). In gastric cancer, downregulation of miR-214 in CAFs resulted in a high expression of Fibroblast Growth Factor 9 (FGF9), promoting EMT and tumor metastasis (54). In human colorectal cancer, CAFs promoted cancer proliferation, EMT and metastasis by secreting pro-inflammatory factors, such as IL-6, IL-8 and exosomal miRNA-92a-3p to activate Wnt/β-catenin pathway as well as inhibit mitochondrial apoptosis (55, 56).

**CAFs induce chemoresistance**

Tumor matrix is not only the material support but also an important regulator of cancer cells. They create a complex signaling network to promote drug resistance in tumor cells after drug treatment (57). In patients with breast and lung cancer, phosphorylation and acetylation of p65 activated NF-κB to produce CD10+GPR77+ CAFs. They provided a survival niche for cancer stem cells to achieve tumor formation and chemoresistance (37). Similarly, IL-11 secreted by CAFs induced STAT3 phosphorylation and increased the expression of anti-apoptotic proteins Bcl-2 and Survivin in lung adenocarcinoma. These protected cancer cells from cisplatin-induced apoptosis, thereby promoting chemoresistance (58). Exosomes derived from CD63+ CAFs contained miR-22 and mediated tamoxifen resistance in breast cancer by targeting ERα and PTEN (59). In gastric cancer, the USP7/hnRNPA1 axis was activated and miR-522 was expressed in CAFs after cisplatin and paclitaxel treatment, leading to ALOX15 inhibition and reduced survival niche for cancer stem cells to achieve tumor formation and chemoresistance (57). CAFs could also secrete IL-8 and activate the NF-κB signaling pathway in gastric cancer to mediate chemoresistance (61). In pancreatic ductal carcinoma, CAFs secreted SDF-1 to upregulate the expression of SATB-1 in cancer cells and mediate gemcitabine resistance (62). Similarly, CAFs promoted pancreatic cell proliferation and drug resistance by releasing exosomes containing the chemoresistance inducing factor, Snail (63).

**CAFs mediate immunosuppression**

CAFs can promote the immunosuppression of cancer cells by secreting TGF-β, IL-6, CXCL12 and CCL2, thereby preventing cytotoxic T cell activity and recruiting immunosuppressive populations (64). There was a significant increase in regulatory T cells (Tregs) in paracancerous tissues, which secreted TGF-β and IL-10 to inhibit the activation of tumor-site effector T cells. In breast cancer, CAF-S1 enhanced the ability of Tregs to suppress T effector proliferation, and then promoted immunosuppressive (65). A new subset of CAFs that expressed CD68 was found in esophageal squamous cell carcinoma. The recurrence rate of patients with low-CD68 CAFs was higher. Knockdown of CD68 in CAFs upregulated the secretion of CCL17 and CCL22 by tumor cells to enhance Treg recruitment (66). MiR-92-containing exosomes from CAFs induced the expression of programmed cell death receptor ligand 1 (PD-L1) in breast cancer and raised the apoptosis of T cells (67). Similarly, in melanoma and colorectal cancer cells, CAFs led to the high expression of PD-L1 and the activation of PI3K/AKT signaling, resulting in the disappearance of T cells in the anti-tumor immune response (68). Furthermore, CAFs could inhibit an anti-tumor immune response by inhibiting dendritic cells which are necessary for T lymphocytes activation. In a recent study, CAFs secreted WNT2 in esophageal squamous cell carcinoma and colorectal cancer. WNT2 suppressed the dendritic cells to act on the anti-tumor T cell response via SOCS3/p-JAK2/p-STAT3 signaling (69).

Additionally, CAFs could also reduce immune efficiency by recruiting granulocytes and monocytes, and suppressing dendritic cell functions (70, 71). For example, increased expression of IL-33 in metastases-associated fibroblasts stimulated type 2 immunity and mediated the recruitment of eosinophils, neutrophils and inflammatory monocytes, influencing the function of these immune cells in tumor tissues (72).

**CAFs exert tumor suppression effect**

Although the studies mentioned above have revealed the cancer-promoting function of CAFs, some studies have also reported the tumor suppression effects of CAFs. In a mouse model of pancreatic ductal carcinoma, ablation of CAFs was first proven to be associated with worse tumor progression, further supporting the concept of CAFs heterogeneity in the tumor microenvironment (73). In mice with pancreatic cancer, the absence of α-SMA+ myofibroblasts led to hypoxia enhanced and EMT turnover. In patients with pancreatic ductal carcinoma, fewer myofibroblasts were related to increased drug resistance and reduced survival. Another study reported that deletion of sonic hedgehog (SHH) decreased the formation of fibroblast-rich desmoplastic stroma, increased vascularity and enhanced tumor proliferation (74). In estrogen receptor-positive (ER+) breast cancer, CD146+ CAFs could maintain ER expression, estrogen-dependent proliferation and tamoxifen sensitivity (75). Furthermore, a recent study reported the presence of two populations of CAFs with different functions, namely, cancer-promoting and cancer-restraining. Meflin, a marker of mesenchymal stromal cells to maintain their undifferentiated state, was expressed on pancreatic stellate cells in pancreatic ductal carcinoma. The results of situhybridization analysis of 71 human pancreatic ductal carcinoma tissues showed that the infiltration of Meflin-positive CAFs was related to good prognosis. In a mouse model of pancreatic ductal carcinoma,
Meflin deficiency led to significant tumor progression in poorly differentiated histology (76). The functional heterogeneity of CAFs in certain cancer types was highlighted in Figure 2.

**Treatment strategies for CAFs**

CAFs play a vital role in cancer occurrence and development by regulating the proliferation, invasion and chemoresistance of tumor cells. The abundance in tumor microenvironment and the diverse tumor-supportive roles of CAFs make them an ideal therapeutic target (77). The recent advances in cancer therapy by targeting CAFs were summarized in Table 2 and Figure 3.

**CAF-targeted ablation**

Targeting CAFs by inhibiting surface markers such as FAP and α-SMA has been extensively explored in pre-clinical studies. Sibrotuzumab, an antibody against FAP, has been tested in phase I clinical trials of colorectal cancer and non-small cell lung carcinoma. In patients with advanced FAP-positive cancer, repeat infusions of sibrotuzumab were safe, but the efficiency in Phase II trials was limited (78). The first clinical inhibitor against FAP activity, Val-boroPro, was used in phase II trials in patients with metastatic colorectal cancer. However, the results were not satisfactory and Val-boroPro had minimal clinical activity (79). In a mouse model, SynCon, a novel FAP DNA vaccine, was able to break tolerance and induce CD8+ and CD4+ immune responses (80). Similarly, the FAP-targeting immunotoxin αFAP-PE38 was used to deplete FAP+ CAFs in a metastatic breast cancer model, thereby decreasing the recruitment of tumor-infiltrating immune cells in the tumor microenvironment and suppressing tumor growth (81). Similar to the depletion of FAP+ CAFs, reduction of α-SMA+ content of stroma through Cellax therapy was confirmed to have effects in inhibiting tumor progression (82). Furthermore, CD10+GPR77+ CAFs were a novel subset that was identified in breast cancer. A neutralizing anti-GPR77 antibody could restore the chemosensitivity of cancer cells (37). Although CAF ablation is effective in some tumor models, the reduction of FAP+ stromal cells are proved to have a relationship with the loss of muscle mass and anemia (83). In addition, CAFs lack specific markers and alter phenotypes at different stage, making targeted therapy
difficult. In conclusion, ablation of CAFs in cancer therapy needs cautionous consideration, as non-selective removal may have the opposite effect, and the combined application of markers may contribute to more accurate subtype localization.

### Restoring CAFs to a quiescent state

Sustained stimulation of tumor cells will activate some signaling pathways in progenitors, and promote their acquisition of CAF phenotypes and tumor-promoting functions. Strategies to inhibit the expression of some genes in activated CAFs may restore them to a quiescent state, which fails to promote tumor growth and even has tumor-suppressive effects (98). TGF-β and PDGF play crucial roles in the activation of CAFs. Dasatinib, the inhibitor of PDGFR, could reverse the phenotype of CAFs into normal fibroblasts. The proliferation of lung cancer cells was reduced if they were incubated with conditioned medium from CAFs pre-incubated with Dasatinib (84). Similarly, artemisunate and dihydroartemisin from Artemisinin (ART) were shown to suppress TGF-β signaling in CAFs and inhibit tumor growth and metastasis (85). The combination of JAK inhibitor (ruxolitinib) and DNMT inhibitor (5-azacytidine) could restore the fibroblast phenotype and reverse the pro-invasive activity of CAFs in lung cancer and head and neck carcinomas (86). The ROS-producing enzyme NOX4 was upregulated by CAFs in many human cancers, and gene inhibitors convert fibroblasts to CAFs, preventing CAF accumulation and slowing tumor growth (98). Pharmacologic inhibition of NOX4 by GKT137831 [Setanaxib] reversed CAFs to a quiescent state, overcame cancer immune resistance, and improved the

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### Table 2: Treatment strategies based on CAFs.

| Drugs | Mechanism | Cancer models | Biological effects | State | Refs |
|-------|-----------|---------------|--------------------|-------|------|
| Sibrotuzumab | Deplete FAP+ CAFs | Colorectal cancer and non-small cell lung cancer | Inhibit tumor growth | Phase I | (78) |
| Val-boroPro | Deplete FAP+ CAFs | Colorectal cancer | Inhibit tumor growth | Phase II | (79) |
| SynCon FAP DNA vaccine | Deplete FAP+ CAFs | Lung, prostate, breast cancer | Enhance immune response | Preclinical | (80) |
| αFAP-PE38 | Deplete FAP+ CAFs | Breast cancer | Inhibit tumor growth | Preclinical | (81) |
| Cella | Deplete αSMA+ CAFs | Breast cancer | Deplete tumor stroma | Preclinical | (82) |
| Neutalizing anti-GPR77 antibody | Deplete CD10+ GPR77+ CAFs | Breast and lung cancer | Inhibit tumor growth | Preclinical | (37) |

### Restoring CAFs to a quiescent state

- **Dasatinib**: Inhibit PDGFR
- **Artemisinin**: Suppress TGF-β signaling
- **Ruxolitinib and 5-azacytidine**: Restore the fibroblast phenotype of CAFs
- **GKT137831 [Setanaxib]**: Inhibit NOX4
- **Minnelide**: Decrease viability of CAFs
- **Losartan and FOLFIRINOX**: Restore the fibroblast phenotype of CAFs
- **GKT137831 [Setanaxib]**: Inhibit NOX4
- **Minnelide**: Decrease viability of CAFs
- **Losartan and FOLFIRINOX**: Restore the fibroblast phenotype of CAFs

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**TABLE 2** Treatment strategies based on CAFs.
prognosis of multiple cancers in a CAF-rich mouse tumor model (87). Minnelide is a water-soluble triptolide prodrug in phase I clinical trials. It is effective in multiple animal models of pancreatic cancer. Minnelide was observed to decrease the viability of CAFs and reduce ECM components such as hyaluronan and collagen, resulting in the suppression of cancer cells (88). Additionally, the use of angiotensin receptor blockers (ARBs) like losartan, converted myofibroblast CAFs to a quiescent state by decreasing the activation of TGF-β, and then alleviated immunosuppression and improved T lymphocyte activity (99). In a phase II clinical trial, the researchers combined losartan with FOLFIRINOX to assess the efficacy of locally advanced pancreatic cancer, and the results showed that the treatment prolonged the prognosis of patients (89).

Blocking the interaction between CAFs and cancer cells

Compared with depletion of CAFs or reversion of their state, other treatments, such as blocking the interaction between CAFs and cancer cells may be more practical. TGF-β signaling pathway has been proven to be vital in CAF activation and tumor promotion. LY2109761, the TGF-β receptor inhibitor, could suppress tumor growth and metastasis by inhibiting the release of connective tissue growth factor (CTGF) and interrupting the cross-talk between cancer cells and CAFs (90). In preclinical models of pancreatic tumor, neuregulin 1 (NRG1), the ligand of HER3 and HER4 receptors, was secreted by both cancer cells and CAFs. 7E3, as an antibody to NRG1, was demonstrated to prevent tumor growth and metastasis by inhibiting NRG1-mediated HER3 and AKT/MAPK signaling pathways, providing a novel therapeutic option for pancreatic cancer (91). In gastric cancer, IL-17a secreted by CAFs promoted the migration and invasion of cancer cells by activating JAK2/STAT3 signaling pathway. As a neutralizing antibody against IL-17a or JAK2 inhibitors, AG490, could significantly inhibit the effect of CAFs on cancer progression and improve prognosis (92). Furthermore, CAFs in pancreatic cancer were found to interact with tumor cells and hyperactive SHH signaling. A commercial SHH inhibitor, GDC-0449 was reported to reverse fibroblast-induced resistance to doxorubicin in smoothened-positive pancreatic cancer cells. Importantly, the synergistic combination of GDC-0449 with PEG-PCL-
Dox exhibited robust antitumor efficiency in a BxPC-3 tumor xenograft model, suggesting a potential strategy for the treatment of fibroblast-enriched pancreatic cancer (93). In hepatocellular carcinoma, the utilize of Resolvin D1 (RvD1) inhibited the paracrine of CAFs-derived cartilage oligomeric matrix protein (COMP) by targeting FPR2/ROS/FOXM1 signaling pathway, and repressed EMT and cancer stemness feature, which might be a potential agent contributing to treatment outcomes (94). The expression of CXCL12 in fibroblasts was considered to be associated with the presence of axillary metastases in HER2 breast cancer, and the suppression of its receptor provided some therapeutic potential. Researchers inhibited CXCR4, the receptor of CXCL12, through the administration of AMD3100 and treating outcomes (94). The expression of CXCL12 in primary esophageal squamous cell carcinoma and colorectal cancer, WNT2+ CAFs were negatively correlated with active CD8+ T cells. Direct interference with CAF-derived WNT2 could restore DC differentiation and DC-mediated antitumor T-cell response (69). In a phase II clinical trial of pancreatic cancer, ruxolitinib combined with capcitabine was used in patients with metastatic pancreatic cancer who had failed to respond to gemcitabine. The results showed that patients treated with ruxolitinib had longer overall survival and better prognosis, supporting the potential clinical benefit of JAK1/JAK2 inhibitor ruxolitinib (96). Additionally, the stromal-disrupting effect of Nab-paclitaxel was reported in pancreatic cancer therapy (100). In a phase III clinical trial, nab-paclitaxel combined with atezolizumab was tested in patients with unacceptable, locally advanced or metastatic triple-negative breast cancer and showed longer overall survival (97).

Conclusions

Since the concept of CAFs was proposed in the early 1990s, CAFs have attracted extensive attention in cancer biology. Previous studies have led to a better understanding of the heterogeneity of CAF origins, phenotypes and functions. CAFs are the main cell types in tumor microenvironment which affect the occurrence, and development of cancer cells. They have rich cellular sources and precursor cells such as normal fibroblasts and mesenchymal stem cells have been shown to be the major sources. CAFs are not a cell type but heterogeneous functional subpopulations. Based on the surface markers, CAFs are divided into several subtypes, which have different biological functions. CAF subtypes identified in different cancer types may play opposite roles in cancer progression, such as tumor-promoting and tumor-suppressive functions. CAFs have great potential in clinical applications. Several preclinical studies and ongoing clinical trials have shown that strategies targeting CAFs are possible in cancer therapy. However, there are still some challenges in translating CAF research into clinical benefit. First, the concrete origins of CAFs in specific cancer types remains elusive. In addition, most studies on the origin of CAFs have been performed in vitro and lack appropriate clinical validation. The use of lineage tracing methods will greatly solve these problems in future studies. Second, the lack of uniform nomenclature for CAF subpopulations in different cancer types makes it difficult to compare CAF subgroups in distinct tumors. It would be useful to name them by combining analysis of cell lineage, surface markers, functions and clinical relevance. Additionally, there is still a lack of curate classification of CAF subtypes. Advanced strategies, such as single-cell RNA sequencing, mass spectrometry-based time-of-flight flow cytometry (CyTOF), multiple flow cytometry and multiple immunostaining, may be helpful to accurately classify CAF subtypes. Finally, although many experiments targeting CAFs to improve cancer therapy have been conducted in preclinical models and clinical trials, most of them have failed to pass phase II clinical trials. It has not yet reached practical application. To overcome this limitation, more detailed experimental designs and more clinical samples are needed, and the combination of these CAF-targeting approaches with existing therapies may be beneficial. Overall, it is critical to accurately understand the underlying mechanisms of action between CAFs and tumor cells. It is also important to understand CAF-targeting therapies at the molecular, cellular, and systemic levels based on the interactions between CAFs and tumor cells, to find the most appropriate strategies and avoid adverse effects. In addition, tracing the origins of CAFs may be a key factor in achieving the clinical application of CAF-targeting strategies and avoiding side effects. With the resolution of these problems, CAF-derived therapies are expected to provide new support for clinical cancer therapy in the near future.

Author contributions

CW wrote the manuscript and designed the figures. JG, HG, and XXZ assisted in the manuscript writing and figures drawing. XZ and RJ revised the manuscript. All authors contributed to the article and approved the submitted version.
The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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