

## Review Article

# Therapeutic targeting of the crosstalk between cancer-associated fibroblasts and cancer stem cells

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**Abstract:** Cancer-associated fibroblasts (CAFs) play critical roles in cancer progression and treatment failure. CAFs display extreme phenotypic heterogeneity and functional diversity. Some subpopulations of CAFs have the ability to reconstitute cancer stemness by promoting the expansion of cancer stem cells (CSCs) or by inducing the generation of CSCs from differentiated cancer cells. CAFs regulate cancer stemness in different types of solid tumors by activating a wide array of CSC-related signaling by secreting proteins and exosomes. As feedback, the CSCs can also induce the proliferation and further activation of CAFs to promote their CSC-supporting activities, thus completing the loop of CAF-CSC crosstalk. Current research on targeting CAF-CSC crosstalk could be classified into (i) specific depletion of CAF subpopulations that have CSC-supporting activities and (ii) targeting molecular signaling in CAF-CSC crosstalk, such as the IL6/STAT3, TGF- $\beta$ /SDF-1/PI3K, WNT/ $\beta$ -catenin, HGF/cMET and SHH/Hh pathways. Strategies targeting CAF-CSC crosstalk may open new avenues for overcoming cancer progression and therapeutic resistance.

**Keywords:** CAFs, CSCs, cancer stemness, therapeutic resistance, cancer therapy

## Introduction

The tumor microenvironment (TME) is one of the key unsolved issues that hampers effective cancer management in clinical practice [1] and is composed of extracellular matrix (ECM), cytokines interspersed in the ECM, and several different types of stromal cells, including fibroblasts, endothelial cells and immune cells [2]. Among the components of the TME, cancer-associated fibroblasts (CAFs) are key members of stromal cells and have been suggested to play essential roles in the complex process of tumor-stroma coevolution and tumorigenesis [3, 4]. CAFs have a strong ability to produce various factors that are related to ECM synthesis or remodeling, such as collagens, fibronectins and ECM-degrading proteases. CAFs can also secrete growth factors and proinflammatory cytokines and chemokines, such as transforming growth factor- $\beta$  (TGF- $\beta$ ), interleukin-6 (IL-6), CC-chemokine ligand 2 (CCL2), and stromal cell-derived factor 1 (SDF-1; also named CXCL-12) [3, 4]. Specific cell-surface proteins

on CAFs have been considered ideal markers for CAFs, and these markers include alpha smooth muscle actin ( $\alpha$ -SMA), fibroblast activation protein (FAP), S100 calcium binding protein A4 (S100A4), platelet-derived growth factor receptor- $\beta$  (PDGFR $\beta$ ) and fibroblast growth factor receptor 2 (FGFR2); however, none of these markers are exclusively expressed by CAFs [1, 5]. Increasing evidence suggests that CAFs are a complex and heterogeneous population of cells [4] with different cellular sources in cancers [1, 4]. Once activated by a broad range of stimuli from cancer cells or the TME, CAF progenitors acquire the phenotypes of CAFs, such as the expression of  $\alpha$ -SMA, FAP or S100A4 and the secretion of TGF- $\beta$ , IL-6 or CCL2 [1]. The diversity of CAF origins and stimuli may contribute to the phenotypic heterogeneity and functional diversity of CAFs. Most studies have suggested that CAFs have tumor-promoting effects, while some reports have indicated that CAFs have antitumor functions [1, 4]. The protumorigenic type of CAFs can enhance tumorigenesis, metastasis, angiogene-

sis and immune evasion of cancer cells by either the secretion of proteins (including chemokines, cytokines and growth factors) and exosomes or the direct cell-cell contact with tumor cells [4]. In addition to regulating cancer progression, CAFs also play critical roles in hampering the effect of cancer therapies, especially immunotherapy [6] and chemotherapy [1, 4]. Although most CAFs have potent protumorigenic roles, some CAF subsets actually have tumor-suppressive functions in specific cancers or at specific cancer stages. The anti-tumor functions of CAFs are mediated by their regulation of antitumor immunity [7]. The more recent studies showed that CAFs may induce the generation and expansion of cancer stem cells (CSCs) [8], which are involved in multiple steps of cancer progression and drug resistance development [8, 9].

CSCs are a small, self-renewing cell population within the bulk tumor mass and are capable of generating new tumors [9]. Compared with differentiated cancer cells, which have limited regenerative and tumorigenic capacities, CSCs have extensive proliferative potential to generate primary tumors and form disseminated metastatic tumors [10]. During tumor pathogenesis, genetic and epigenetic changes cause the transformation of normal stem cells, or even differentiated cells, into CSCs [11]. These mutated CSCs have extensive abilities to promote tumor growth, immune evasion, metastasis, chemoresistance and radioresistance, thereby leading to cancer progression and therapeutic failure [10]. Moreover, TME factors, including CAFs and their derived stimuli, can induce CSC clonal expansion and tumorigenic activities [8]. The amount and activity of CSCs within the tumor determine cancer stemness and correlate with disease progression and therapy efficacy. Notably, CSCs might undergo further mutations and thus increase their heterogeneity and generate diverse phenotypes of cancer cells [11]. The identification of CSCs is important to CSC studies and the development of CSC-targeting intervention strategies. CSC identification is generally based on cell surface markers. CD24, CD26, CD29 (also called  $\beta$ 1-integrin), CD34, CD44, CD133, CD166, aldehyde dehydrogenase (ALDH) and Ep-CAM (also called CD326) have been identified as CSC-specific surface markers in different cancers [8]. In addition, activated pathways, including

the WNT/ $\beta$ -catenin, MEK, Notch, Hedgehog (Hh), phosphoinositide 3 kinase (PI3K)/AKT and TGF- $\beta$  pathways, are utilized by CSCs for their survival, proliferation and stemness maintenance [8].

To date, very few reviews focusing on CAF-regulated cancer stemness have been reported. It is essential to provide an overview of CAF-regulated cancer stemness for developing more efficient cancer therapeutic approaches. In this review, we will focus on the current knowledge in identifying CAF subsets that regulate cancer stemness and discuss the crosstalk between CAFs and tumor cells for regulating cancer stemness in different cancer models. This review intends to open a new window for devising novel strategies to overcome cancer progression and therapeutic resistance.

### CAF support cancer stemness in different cancers

It has been reported that cancer stemness can be enhanced by CAFs in several cancer types (**Table 1**). A cell culture model in which tumor cells are cultured with conditioned media (CM) from CAFs or a coculture system with tumor cells and CAFs is often used to study the influence of CAFs on different CSCs *in vitro*. CAFs are able to induce the expression of stemness markers (e.g., Sox2, Bmi-1 and CD44), enhance sphere formation, and promote the self-renewal and expansion of CSCs in breast [12-14], prostate [15, 16], lung [14], colorectal [17, 18], gastric [19], and liver cancers [20]. Although the conditioned media from CAFs can regulate CSC properties in most *in vivo* models [1-9], it is noteworthy that direct cell-cell interactions are required for the regulation of cancer stemness by CAFs in some cases [17]. Additionally, *in vivo* experiments have also indicated the roles of CAF-regulated cancer stemness in enhancing the *in vivo* tumorigenicity in breast [13, 14], prostate [16], colorectal [21], gastric [19] and liver cancers [20]. Cancer stemness is correlated with chemoresistance and cancer metastasis. Both *in vitro* and *in vivo* experiments have demonstrated that CAFs can promote drug resistance [14, 17, 18, 20] and cancer metastasis [14, 16, 21] through the regulation of cancer stemness in different cancers. CAF-regulated cancer stemness may also affect cancer relapse [22].

## Targeting the CAF-CSC crosstalk for cancer therapy

**Table 1.** CAFs regulate cancer stemness in different cancers

Cancer types	CAF-regulated signatures		References
	Stemness marker	Induced/Expanded CSCs	
Breast	Sox2, Bmi-1, Nanog and CD44	CD24-CD44+ cells; PKH67 <sup>high</sup> cells; ALDH+ cells	[12-14]
Prostate	Oct4, Sox2, and Nanog	CD24-CD44+ cells; CD133+ cells LinSca1 <sup>high</sup> CD49f <sup>high</sup> cells	[15, 16]
Lung	ABCG2	ALDH+ cells	[14]
Colon	Not reported	ALDH+ cells	[17, 18, 21]
Gastric	ABCG2, CD44, Nanog and ALDH1 etc.	★SP (side population) cells	[19]
Liver	Oct4, and Nanog	CD44+ cells; CD47+ cells; CD90 cells; CD24+ cells	[20]

★SP cells were defined as the subset of cells that exhibited a low Hoechst33342 staining pattern and disappeared with the use of verapamil. Abbreviations: Sox2, SRY-box transcription factor 2; Oct4, Octamer-binding transcription factor 4; ABCG2, ATP binding cassette subfamily G member 2.

### Key components and pathways involved in CAF-regulated cancer stemness

As summarized in **Table 2**, the CAF-derived stimuli that regulate CSC properties may be different among various types of cancer. These stimuli include CAF-secreted chemokines, cytokines, growth factors and exosomes, and direct CAF-tumor cell interactions.

#### Chemokines

SDF-1 secreted by CAFs has been found to promote the generation of CSCs and sustain their tumorigenesis and metastatic activity in breast cancer [12] and colorectal cancer [21]. CAF-secreted SDF-1 regulates the CSC phenotype of cancer cells by interacting with the cancer cell-expressed receptor CXCR4 [12] and then activating the Wnt/ $\beta$ -catenin [12, 21] and/or PI3K/AKT pathways [21] in cancer cells. The SDF-1/CXCR4 chemokine axis also activates other CSC-related signaling pathways, such as NOTCH signaling in breast cancer cells [12, 23]. In cancers with SDF-1 secretion, CXCR4+ cancer cells can obtain enhanced stemness compared with CXCR4- cancer cells since CAF-secreted SDF1 can stimulate the CSC phenotype and induce the expansion of them. In line with this finding, CXCR4 has been considered a biomarker for radioresistant CSCs [24]. CCL2 is another chemokine released by CAFs and has the ability to regulate CSC properties. Tsuyada et al. showed that breast CAF-secreted CCL2 can induce self-renewal and expansion of CSCs in breast cancer. Consistently, a tumor model

generated by cotransplantation of primary breast cancer cells and CAFs into the mammary fat pads of NOD/SCID/IL-2Rg-null mice suggested that CCL2 is critical for CAF regulation of tumorigenesis *in vivo*. CAF-secreted CCL2 has also been found to regulate the CSC phenotype by activating NOTCH1 signaling in breast cancer cells [13].

#### Cytokines and growth factors

It has been shown that IL-6 secreted by CAFs promotes the stemness of CD24+ liver cells [25]. Similarly, IL-8 from CAFs can regulate the cancer stemness and chemoresistance of cancer cells [26]. In most cases, CAFs regulate the epithelial-mesenchymal transition (EMT) and CSC properties of cancer cells via the combined production of IL-6 and IL-8. Su et al. showed that IL-6 and IL8 can be secreted by CD10+GPR77+ CAFs and can promote tumorigenicity and chemoresistance by supporting cancer stemness in breast and lung cancers [14]. In line with this finding, simultaneous blocking of IL-6 and IL-8 completely inhibits CAF-induced human melanoma cell invasiveness [27]. CAF-derived IL-6 supports the CSC phenotype by activating the STAT3 pathway and/or TGF- $\beta$ /Smad signaling [28, 29]. IL-8 released by CAFs may sustain the stemness of cancer cells via interactions with its receptors, CXCR1 and CXCR2, which are expressed by cancer cells [30, 31].

CAF-secreted growth factors, such as TGF- $\beta$  and hepatocyte growth factor (HGF), are in-

## Targeting the CAF-CSC crosstalk for cancer therapy

**Table 2.** The signatures of CSC-supporting CAFs

Cancer types	CAF markers	Activated signaling in CAFs	CAF-derived mediators for CSC regulation	References
Breast	$\alpha$ -SMA, CD10, GPR77	NF- $\kappa$ B pathway; STAT3 pathway Hedgehog signaling; Autophagic pathway	IL-6, IL-8, SDF-1, CCL2 HMGB1	[12-14, 22]
Prostate	$\alpha$ -SMA-FAP+	IL-6/IL-6R signaling	Metalloproteases	[16, 36]
Lung	$\alpha$ -SMA, CD10, GPR77	NF- $\kappa$ B pathway	IL-6, IL-8	[14]
Colon	$\alpha$ -SMA, CD44, FAP and S100A4 etc.	Hypoxic inducible pathway	CD44, Exosomal lncRNA H19, Wnts	[17, 18, 42]
Gastric	$\alpha$ -SMA	Not reported	TGF $\beta$	[19]
Liver	$\alpha$ -SMA, FAP	Not reported	HGF, IL6	[20, 25]

Abbreviations:  $\alpha$ -SMA,  $\alpha$  smooth muscle actin; GPR77, G protein-coupled receptor 77; SDF-1, stromal cell-derived factor 1; HMGB1, high mobility group box 1; FAP, fibroblast activation protein alpha; S100A4, S100 calcium-binding protein A4; HGF, hepatocyte growth factor.

involved in the regulation of tumorigenicity, metastasis and drug resistance [1, 32]. Recent studies demonstrated that TGF- $\beta$  released by CAFs can sustain the stemness of scirrhous gastric cancer cells [19] and promote the EMT program by activating TGF- $\beta$ /Smad signaling in breast cancer [33]. Additionally, autocrine TGF- $\beta$  can promote SDF-1 secretion via the activation of HSF1 in breast CAFs and thus regulate the CSC properties of breast cancer cells through the SDF-1/CXCR4 axis [12, 34]. Therefore, TGF- $\beta$  released by CAFs may regulate cancer stemness either in a direct manner or in an indirect manner. Similarly, CAF-secreted HGF can also regulate CSC phenotypes through different pathways. CAF-secreted HGF is able to activate cMET-dependent signaling in liver cancer cells [20] and colorectal cancer cells [35]. The cMET/FRA1/HEY1 cascade induced by the CAF-secreted HGF plays critical roles in the regulation of liver cancer stemness [20]. It remains unclear whether the cMET-dependent signaling activated by CAF-secreted HGF can regulate the CSC properties of colorectal cancer cells. However, it has been shown that colorectal CAF-secreted HGF can support the stemness of colorectal cancer cells by activating the Wnt/ $\beta$ -catenin and PI3K signaling pathways [21].

### Other secreted proteins

Except for chemokines, cytokines and growth factors, other CAF-secreted proteins, such as metalloproteases and high-mobility group box 1 (HMGB1), may also regulate the CSC phenotype. Giannoni et al. showed that metalloproteases secreted by prostate CAFs elicit a Rac1b/COX-2-mediated release of reactive oxygen species (ROS) in cancer cells, which is mandatory for supporting the stemness of met-

astatic prostate cancer cells [16, 36]. In these studies, the EMT signaling and proinflammatory signature induced by the CAF-secreted metalloproteases can increase the expression of stem cell markers, sphere-forming capacity and self-renewal activity of prostate cancer cells. HMGB1, a nuclear protein, can also induce proinflammatory signaling when released by apoptotic cells or autophagic cells [37]. It has been demonstrated that HMGB1 released by autophagic CAFs can enhance the stemness and tumorigenicity of luminal breast cancer cells by activating its receptor, toll-like receptor 4 (TLR4), expressed by cancer cells [22].

### Exosomes

Exosomes, which are closely associated with cancer progression and treatment failure [38], are critical mediators of tumor-CAF crosstalk. Exosomes released by CAFs can contribute to cancer progression and drug resistance by regulating the proliferation, metastasis and CSC properties of cancer cells [39, 40]. Donnarumma et al. showed that exosomal miRNAs, including miRs-21, -378e, and -143, are essential for CAF-regulated stemness and the EMT phenotype of breast cancer cells [41]. Ren et al. demonstrated that CAFs promote the stemness and oxaliplatin resistance of colorectal cancer via exosomal lncRNA H19 [18]. To regulate the CSC properties of cancer cells, miRNAs [41] or lncRNAs [18] should be shuttled by CAF-derived exosomes into cancer cells. Additionally, after being transferred to tumor cells, lncRNA H19 can activate the  $\beta$ -catenin pathway, acting as a competing endogenous RNA sponge for miR-141 [18]. In addition to noncoding RNAs, exosomal proteins such as Wnt ligands can also promote cancer stemness. Hu et al. demonstrated that CAF-secreted exosomal Wnts

can support CSC characteristics and drug resistance by inducing colorectal cancer cell de-differentiation [42]. In pancreatic ductal adenocarcinoma (PDAC), CAFs can provide a stem cell niche for PDAC cells without the ability to produce Wnts and support their *in vivo* tumorigenesis by secreting Wnts [43]. Moreover, Wnts secreted by esophageal CAFs can induce the EMT and invasiveness of cancer cells, which are considered hallmarks of CSCs [5, 44]. Taken together, CAFs induce the activation of the WNT/ $\beta$ -catenin pathway in cancer cells and thus regulate their CSC phenotypes by secreting different factors, such as soluble SDF-1 and HGF, exosomal lncRNA H19 and exosomal Wnt ligands.

### *Membrane proteins*

Except for secreted factors, direct cell-cell contact is also required for CAFs in regulating cancer stemness [17], suggesting the critical role of membrane proteins in CAF regulation of the CSC phenotype. Kinugasa et al. demonstrated that CD44 expressed on CAFs acts as a functional cell-surface molecule that is essential for supporting the stemness and drug resistance of colorectal cancer cells [17]. CD44 expressed on CAFs is implicated in the regulation of CAF-secreted SDF-1, which has been reported to stimulate the CSC properties of cancer cells. It remains unclear whether CD44 is involved in the direct cell-cell interaction of CAFs and cancer cells. More membrane molecules on CAFs have been reported to play indirect roles in the regulation of cancer stemness. As described above, CD10+GPR77+ CAFs act as a protumorigenic CAF subpopulation that can sustain the stemness and enhance the chemoresistance of breast and lung cancer cells [14]. GPR77 is a functional CAF membrane molecule that can be activated by cancer-derived stimuli and then induces CAF intracellular NF- $\kappa$ B signaling, which is involved in cancer stemness regulation via the production of IL-6 and IL-8 [14]. CD10 serves as a marker for the CAF subpopulation that supports the CSC phenotype and tumorigenesis of tumor cells in breast, lung and colorectal cancers [14, 45]; however, the function and mechanism of CD10 on stemness regulation remain unknown.

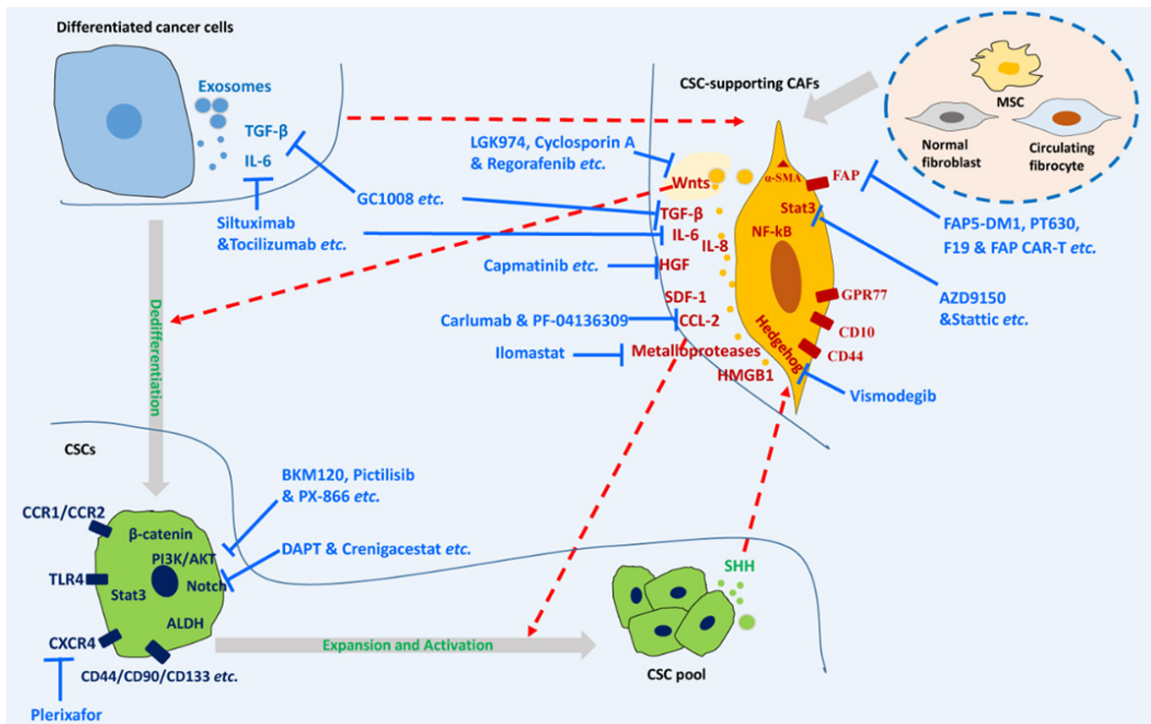
### **CAF-CSC interaction loop**

The bi-directional activation between cancer cells and stromal cells is critical to cancer cell

phenotypes and functions and influences cancer progression and treatment resistance [1, 4]. Only when specific signaling pathways are activated by corresponding stimuli from cancer cells or the TME can CAFs acquire their phenotypes for sustaining cancer stemness. For example, CM from the breast cancer cell lines BT474 and MDA361 but not from the noncancerous mammary epithelial cell line MCF10A can activate STAT3 signaling in CAFs and then induce CCL2 production, which is essential to regulating the stemness of cancer cells [13]. The NF- $\kappa$ B pathway in CAFs is also implicated in the regulation of CAF phenotype-stimulating cancer stemness. Persistent activation of the NF- $\kappa$ B pathway in CD10+GPR77+ CAFs from breast or lung cancer is required for the production and secretion of IL-6 and IL-8, exerting the ability to promote cancer stemness [5]. NF- $\kappa$ B signaling in CD10+GPR77+ CAFs can be activated by autocrine or tumor-derived C5a, one of the complement mediators [14]. Additionally, TGF- $\beta$  and IL-6 are two well-known factors that regulate the crosstalk between cancer cells and CAFs. In lung cancer, tumor-derived TGF- $\beta$  can drive  $\alpha$ -SMA+ CAFs to produce IL-6, which supports the stemness of cancer cells and in turn increases TGF- $\beta$  secretion by cancer cells [29]. Furthermore, tumor-derived IL-6 can promote the differentiation and activation of  $\alpha$ -SMA-FAP+ CAFs and regulate their CSC-stimulating abilities by inducing metalloprotease production [16, 36]. After being induced by CAFs or other stimuli, CSCs will in turn release specific factors to maintain the CAF phenotype with CSC-stimulating functions. Valenti et al. showed that CSCs from mammary gland tumors secrete the Hedgehog ligand SHH, which activates the CAF Hedgehog signaling pathway and thus induces CAFs to secrete factors promoting the expansion and self-renewal of CSCs [46].

The origin of CAFs and the stimuli they receive influences CAF phenotype with specific secretomes, whereas the cancer cells featured with specific membrane receptors and intracellular signaling pathways reciprocally have impacts on the response of cancer cells to CAF-derived stimuli. In breast cancer, CAF-secreted CCL-2 failed to induce CSCs in MCF-7 cells because of their lack of CXCR-2 receptor; however, CAF-secreted CCL-2 strongly induces CSCs in BT-474 and MDA361 cells that have high expres-

## Targeting the CAF-CSC crosstalk for cancer therapy



**Figure 1.** Targeting CAF-CSC crosstalk for cancer therapy. CSC-supporting CAFs can be activated by surrounding stimuli, including cancer cell-derived factors, and then regulate cancer stemness by inducing the dedifferentiation of differentiated cancer cells or inducing the expansion and activation of CSCs via their secreted proteins and exosomes. CAF-CSC crosstalk can be blocked by direct depletion of CSC-supporting CAFs or various drug candidates targeting molecular signaling pathways between CAFs and CSCs. CSCs: cancer stem cells; CAFs: cancer-associated fibroblasts; MSC: mesenchymal stem cell; FAP: fibroblast activation protein.

sion of CXCR-2 [13]. Therefore, the regulation of cancer stemness by CAFs is an effect of complicated cancer-stroma crosstalk.

### Targeted strategies for disrupting CAF-CSC crosstalk

CAFs have emerged as one of the key factors that regulate the CSC phenotype of cancer cells, so targeting cancer-CAF crosstalk for cancer-stemness regulation should be a feasible strategy to suppress cancer progression or reduce treatment failure. Strategies targeting CAF-CSC crosstalk can be mainly classified into two categories: direct depletion of CAF subpopulations that have CSC-stimulating abilities and interference of the activation of signaling or downstream effectors (**Figure 1**).

#### Direct depletion of CSC-stimulating CAFs

Recently, researchers have put much effort into identifying specific CAF cell-surface markers for *in vitro* sorting of CAFs and *in vivo* depletion of CAFs.  $\alpha$ -SMA or FAP is often expressed

on the CSC-stimulating CAFs (**Table 2**). Since selective depletion of the  $\alpha$ -SMA<sup>+</sup> CAFs in a mouse model of spontaneous PDAC not only enhances tumor hypoxia, the EMT, and the CSC phenotype but also increases the tumor infiltration of Treg cells that suppress antitumor immunity and promote tumor progression, targeting  $\alpha$ -SMA<sup>+</sup> myofibroblasts may not be a proper strategy for cancer treatment [7]. However, as shown in **Table 3**, depletion of FAP<sup>+</sup> CAFs via different strategies demonstrated promising antitumor efficacy in various preclinical studies [47-50]. More importantly, inhibition of FAP<sup>+</sup> CAFs with the small-molecule dipeptidyl peptidase inhibitor PT100 enhanced the efficacy of cancer therapy with oxaliplatin in a mouse colon cancer model [51], suggesting that direct depletion of FAP<sup>+</sup> CAFs could lead to a reduction in the CSCs that are associated with chemoresistance. However, clinical investigations for such treatment paradigms did not show significant antitumor efficacy [52, 53]. In addition, FAP can also serve as a target for chimeric antigen receptor T (CAR-T) cell strategies in can-

## Targeting the CAF-CSC crosstalk for cancer therapy

**Table 3.** Strategies for direct depletion of FAP+ CAFs

Strategy types	Method or drug candidate	Cancer types	Clinical trial	References
Genetic depletion	LacZ knock-in	Lung, Colon	Not reported	[47]
Conditional depletion	Diphtheria toxin	Pancreatic, Lung	Not reported	[48]
FAP-targeting killing	FAP5-DM1: maytansinoid-conjugated antibody	Pancreatic, Lung, Colon	Not reported	[49]
	$\alpha$ FAP-PE38: toxin-conjugated antibody	Breast, Melanoma	Not reported	[50]
Pharmacological inhibition: Inhibitors	PT630, PT100	Lung, Colon	Not reported	[47, 51]
Pharmacological inhibition: Antibodies	F19 (sibrotuzumab)	Lung, Colon	(1) Clinically safe; (2) Showed tumor inhibiting potential in a phase I trial of advanced cancers with FAP-expressing stroma; (3) Failed in another early phase II trial in patients with metastatic colorectal cancer	[52, 53]
CAR-T strategy	FAP-specific CAR-T	Lung, Pancreatic	No	[54, 55]

**Table 4.** Drug candidates targeting molecular signaling for CAF-CSC crosstalk

Molecular Pathways or key effectors	Cancers types	Targets	Drug candidates★	References
IL-6/IL-6R/STAT3 signaling and their effectors	Breast, Prostate, Lung, Liver, Glioblastoma	IL-6/IL-6R	<b>Siltuximab, Tocilizumab, Olamkicept</b>	[2, 13, 14, 25, 28, 36, 58-63]
		STAT3	<b>Stattic, C188-9, OPB-31121, OPB-51602, AZD9150, STAT3 decoy oligonucleotide</b>	
		CCL2/CCR2	<b>Carlumab, PF-04136309</b>	
		NOTCH	<b>DAPT, INCB3619, GSI II, Crenigacestat</b>	
TGF- $\beta$ /SDF-1/CXCR4/PI3K	Gastric, Breast, Colon, Prostate, Renal	TGF- $\beta$ /TGF- $\beta$ R	<b>GC1008, T<math>\beta</math>M, Ki26894, LY2109761, LY3022859, PF-03446962</b>	[12, 19, 34, 64-76]
		SDF-1/CXCR4	<b>Plerixafor</b>	
		PI3K/AKT	<b>BKM120, Ly294002, Pictilisib, Buparlisib, Ipatasertib, Alpelisib, PQR309, PX-866</b>	
HGF/cMET	Colon, Liver	HGF/cMET	CpdA, Volitinib, <b>Capmatinib</b> , Crizotinib, SU11274, PHA665752, TC-N19, LY2875358, LY2801653, JNJ-61186372	[20, 35, 77-87]
WNT/ $\beta$ -catenin	Colon, Pancreatic, Esophageal	WNT $\beta$ -catenin	<b>LGK974, Cyclosporin A, Wnt-C59</b>	[42-44, 88-92]
SHH/Hedgehog	Breast, Colon, Prostate	SHH/Hedgehog	<b>Vismodegib, Sonidegib</b>	[46, 93-96]

★: (1) All the drug candidates listed here have shown antitumor activities in preclinical studies; (2) The underlined drug candidates listed here have been tested for their CAF-CSC blocking abilities; (3) The drug candidates listed here in bold have been used in clinical investigations. Abbreviations: SDF-1, stromal cell-derived factor 1; HGF, hepatocyte growth factor; cMET, tyrosine-protein kinase Met; SHH, sonic hedgehog signaling molecule.

cer treatment. In a preclinical study, FAP-specific CAR-T cells arrested the growth of desmoplastic human lung cancer xenografts and syngeneic murine pancreatic cancers [54]. In contrast, another study demonstrated that CAR-T cells had limited antitumor efficacy and even induced lethal toxicity since they also recognized and killed multipotent bone marrow stromal cells (BMSCs) that expressed FAP [55].

Collectively, the direct targeting of CAFs still faces numerous obstacles and challenges. Neither FAP nor  $\alpha$ -SMA is exclusively expressed by CAFs. Moreover, some subsets of CAFs may suppress the CSC phenotype, and thus their depletion may enhance the EMT and stemness of cancer cells [56]. Further studies are needed to develop novel strategies targeting more specific cell-surface markers of CSC-stimulating CAF subpopulations. Previous studies showed that the cell-surface molecule CD44 is associated with the CSC-stimulating activity of  $\alpha$ -SMA+ CAFs in colorectal cancer [17], and CD10 or GPR77 can serve as a cell-surface marker for CSC-stimulating CAFs in breast, lung or colorectal cancer [14, 45]. Thus, the recognition of combined markers, including membrane molecules associated with CSC-stimulating properties and canonical CAF cell-surface markers, such as FAP, should be able to enhance the specificity of targeting CSC-stimulating CAFs. Roybal et al. designed a novel CAR-T cell, the dual-receptor AND-gate T cell, which is only armed and activated in the presence of dual antigen tumor cells [57]. Novel dual-receptor AND-gate T cells can also be developed to only recognize CAFs with dual surface markers and thus deplete CAFs more specifically.

### *Targeting CAF-CSC crosstalk molecular signaling*

**Targeting the IL-6/STAT3 pathway and its downstream effectors:** Tumor-derived IL-6 has the ability to activate the STAT3 pathway in CAFs [58], thus sustaining the CSC-stimulating phenotype of CAFs by inducing their metalloprotease secretion [36]. Additionally, the activation of STAT3 signaling by autocrine or CAF-derived IL-6 in cancer cells is also implicated in the mediation of cancer stemness [14, 25, 28]. Blocking IL-6/IL-6R/STAT3 signaling with the STAT3 inhibitor Stattic has been shown to reduce the stemness of breast cancer cells in preclinical studies [2]. Other agents for inhibi-

tion of IL-6/IL-6R/STAT3 signaling have also exhibited antitumor efficacy in preclinical studies, and the early phase clinical trials for some of these inhibitors in cancer patients are ongoing [59] (**Table 4**). Among these agents used in current clinical trials, AZD9150, a second-generation STAT3 antisense oligonucleotide, has shown antitumor activity with a maximum-tolerated dose of 3 mg/kg in a clinical trial of treatment-refractory lymphoma and NSCLC [60] (**Table 5**). Most of the clinical trials for blocking IL-6/IL-6R/STAT3 did not show effective antitumor activity in patients with solid tumors [59]. The failure of these clinical trials may be partially caused by the absence of reliable markers that are predictive of treatment response. In the crosstalk between cancer cells and CAFs, the CCL2/CXCR2/NOTCH pathway can be activated in response to IL-6/IL-6R/STAT3 signaling. Treatment with CCL2 neutralizing antibodies or inhibitors of the NOTCH activating  $\alpha$ - and  $\gamma$ -secretases effectively attenuated the stemness of breast cancer cells or glioblastoma cells and suppressed their metastasis in preclinical studies [13, 61]. However, clinical investigations on CCL2/CXCR2/NOTCH blocking show limited clinical activity in patients with solid tumors [62, 63]. Matrix metalloprotease (MMP) secretion serves as a downstream effector of IL-6/IL-6R/STAT3 signaling in prostate cancer, and the MMP inhibitor Ilomastat is sufficient to suppress the CAF-regulated CSC phenotype of prostate cancer cells [36]. However, to the best of our knowledge, no clinical trials investigating the safety, tolerance, and bioactivity of MMP inhibitors in cancer patients have been reported.

**Targeting the TGF- $\beta$ /SDF-1/PI3K pathway:** Autocrine signaling loops mediated by TGF- $\beta$  and SDF1 in CAFs have been found to promote cancer-stemness-supporting CAF phenotypes in breast and gastric cancers [12, 19, 34]. CAF-derived SDF1 plays roles in cancer progression through its interaction with its receptor CXCR4 [12]. As reported in preclinical studies, the gastric CSC population that was upregulated by CM from gastric CAFs was significantly decreased by anti-TGF $\beta$ 1 neutralizing antibodies or by a TGF- $\beta$ R inhibitor (Ki26894) [19], and blocking SDF-1/CXCR4 signaling with the CXCR4 antagonist AMD3100 (plerixafor) significantly inhibited CSC populations in breast [12], colon [64] or renal cancer cells [65]. Unfortunately, none of the clinical trials in patients with solid tumors



have shown effective antitumor activity for these strategies targeting TGF $\beta$ /TGF- $\beta$ R or SDF-1/CXCR4 [66-69]. PI3K/AKT activation in cancer cells induced by CAF-derived factors, such as SDF-1, TGF- $\beta$  and HGF, can mediate CSC phenotypes [21]. Blocking PI3K/AKT signaling with the PI3K inhibitor BKM120 or Ly294002 is capable of killing CSCs in different cancers, including colon [21, 70], prostate [71] and breast cancers [72], by single-drug or combination therapy in preclinical studies. Interestingly, PI3K inhibitors, including PX-866 [73], alpelisib [74], PQR309 [75] and pictilisib [76], have shown antitumor activity in clinical trials for patients with solid tumors (**Table 5**).

*Targeting the HGF/cMET or WNT/ $\beta$ -catenin pathway:* HGF regulates CSC phenotypes in liver or colorectal cancer cells by acting on their cMET signaling [20, 35] or  $\beta$ -catenin signaling [21]; CAF-secreted WNT ligands induce CSCs or metastasis in colorectal, pancreatic and esophageal cancers through the WNT/ $\beta$ -catenin pathway [42-44]. Numerous preclinical studies have demonstrated that specific cMET inhibitors [77-84] or multitargeted inhibitors [79, 85, 86], which inhibit HGF/cMET signaling and other signaling simultaneously, have the ability to suppress cancer cell growth, enhance cancer cell sensitivity to radiotherapy and medical therapy, and reduce cancer recurrence in different cancer models (**Table 4**). Recently, a phase I study in Japanese patients with advanced solid tumors showed that capmatinib (INC280), a multitargeted cMET inhibitor, exhibited antitumor activity in 8/44 patients [87], thereby supporting further drug development of capmatinib. WNT/ $\beta$ -catenin inhibitors, including LGK974 [88], Wnt-C59 [89] and cyclosporin A [90], have been found to suppress the survival or proliferation of CSCs in different cancers in preclinical studies. Phase I clinical trials using LGK974 or cyclosporin A in patients with advanced solid malignancies that are dependent on WNT ligands are ongoing [91, 92].

*Targeting the SHH/Hedgehog pathway:* It has been demonstrated that the Hedgehog inhibitor vismodegib is effective in reducing CAF and CSC expansion and thus inhibiting proliferation and triggering apoptosis of cancer cells in different cancers, including breast [46], colon [93] and prostate cancers [94]. Moreover, vismodegib (GDC-0449/Erivedge) has been approved by the FDA for the treatment of basal cell

carcinoma [95]. Another Hedgehog inhibitor, sonidegib, also exhibited the ability to suppress CAF activation and reduce CSCs in triple negative breast cancer (TNBC) [96]. More importantly, a phase I clinical trial has shown that 3 of 12 patients with metastatic TNBC derived a meaningful clinical benefit from combination therapy with sonidegib and docetaxel [96]. Therefore, vismodegib and sonidegib treatment could be considered new strategies for overcoming cancer stemness and increasing the sensitivity of cancer cells to chemotherapy. However, smoothened (Smo) mutations that confer constitutive activity and drug resistance might emerge during vismodegib treatment [95]. For this reason, new effective Hh inhibitors are being developed, and their CSC-inhibiting bioactivities are being studied [95].

### Conclusions and perspectives

Here, we want to emphasize the importance of CAFs in supporting the CSC properties of human cancers. CAFs have heterogeneous phenotypes and functions and can be classified as protumorigenic and antitumorigenic CAFs [1, 4]. The CSC-promoting CAF subpopulations have different phenotypes compared with those without CSC-supporting activity. Additionally, the phenotypes of CSC-promoting CAF subpopulations may vary among different cancer types and cancer niches. As shown in **Table 2**, the CSC-promoting abilities of CAFs in different cancers are related to their specific membrane markers, signaling activation and secretomes. These molecular characteristics of CAFs facilitate the phenotype identification of CAF subpopulations with CSC-supporting activity in a specific cancer type; however, further studies are required to improve these identifications. Additionally, specific membrane markers, such as CD10, GPR77 and CD44, could be used for the *in vivo* depletion of CSC-supporting CAFs. Since CAFs also contain antitumorigenic subpopulations, strategies targeting CSC-supporting CAFs may be more appropriate for cancer intervention than depleting all CAF populations [1, 4]. However, more studies are required to identify specific membrane markers for differentiating CSC-supporting CAF subpopulations in human cancers.

CSCs can transform between states in response to signals from CAFs, exhibiting distinct

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**Table 5.** Clinical trials showing bioactivity of CAF-CSC blocking strategies

Drug	Description	Indication	Study phase	Trial results	References
AZD9150	STAT3 antisense oligonucleotide	Advanced-stage pancreatic cancer, NSCLC, and CRC; Metastatic HNSCC; Advanced-stage and/or metastatic hepatocellular carcinoma	I, II	Showed antitumor activity with a maximum-tolerated dose of 3 mg/kg in patients with treatment-refractory lymphoma and NSCLC	[60]
PX-866	Pan-isoform inhibitor of Class I PI3K	Recurrent or metastatic castration-resistant prostate cancer	II	At 12 weeks, 33% patients were progression-free with 5% of partial objective responses when treated with single-agent PX-866	[73]
Alpelisib	Alpha-specific PI3K inhibitor	Japanese patients with advanced solid tumors; Estrogen receptor-positive advanced breast cancer; HER2-positive metastatic breast cancer	I, Ib	Single treatment with alpelisib, or combined therapies with alpelisib and fulvestrant, or T-DM1 all exhibited clinical activity	[74]
PQR309	Dual PI3K and mTORC1/2 inhibitor	Advanced solid tumors	I	Partial response in a patient with metastatic thymic cancer, 24% disease volume reduction in a patient with sino-nasal cancer, and stable disease for more than 16 weeks in a patient with clear cell Bartholin's gland cancer	[75]
Pictilisib	Pan-PI3K inhibitor	Advanced breast cancer	Ib	Two (3.4%) patients experienced complete responses, and 17 (29.3%) patients had partial responses when treated with pictilisib and paclitaxel together	[76]
Capmatinib	Multitarget cMET inhibitor	Japanese patients with advanced solid tumors	I	Exhibited antitumor activity in 8/44 patients	[87]
Vismodegib	Hedgehog inhibitor	Basal cell carcinoma	FDA approved	N/A	[95]
Sonidegib	Hedgehog inhibitor	Triple negative breast cancer (TNBC)	I	In total, 25% of patients with metastatic TNBC derived clinical benefit from combination therapy with sonidegib and docetaxel	[96]

Abbreviations: NSCLC, non-small cell lung cancer; CRC, colorectal cancer; HNSCC, head and neck squamous cell carcinoma; PI3K, phosphatidylinositol 3-kinase.

features and abilities to disseminate and give rise to metastatic lesions, which further influence cancer progression, cancer relapse and treatment response [10, 11]. Thus, beneficial therapies could be derived from blocking the CAF-CSC crosstalk in human cancers (**Figure 1**). However, it seems that most of the drug candidates targeting a single pathway in CAF-CSC crosstalk fail to significantly improve the antitumor efficacy in cancer patients [59, 62, 63, 66-69]. Only a few candidates, such as the PI3K inhibitor, have shown gratifying antitumor effects when used singly in broad clinical studies [73-76, 87, 95] (**Table 5**). One possible explanation for the single drug treatment failure is that the CAF subsets with CSC-supporting activities in one cancer can sustain the CSC phenotype of cancer cells through different pathways. Thus, combination therapy targeting multiple pathways of CAF-CSC crosstalk is required to enhance the antitumor activities. Moreover, identification of reliable biomarkers that are predictive of treatment response would also be indispensable for the clinical application of CAF-CSC crosstalk blockade. The failure in some clinical trials of CAF-CSC targeting strategies was due to the lack of reliable biomarkers for predicting treatment response [59, 62, 63, 66-69]. As CSCs have the ability to drive cancer chemoresistance or radioresistance [1, 4], proper strategies blocking CAF-CSC crosstalk could also be designed to enhance the sensitivity to these therapies, eventually leading to the suppression of cancer progression and relapse. Moreover, some signal transductions involved in the CAF-CSC crosstalk can regulate the suppression of antitumor immunity. For example, TGF- $\beta$ /SDF-1 signaling [97], STAT3/CCL2 signaling [98], and WNT/ $\beta$ -catenin signaling [99] have been reported to regulate both CAF-CSC crosstalk and antitumor immunity. Thus, strategies blocking these pathways could be used to enhance the antitumor efficacy of immunotherapy, including PD-1/PDL-1 blockade. In addition, CAF-CSC crosstalk-targeted strategies could also enhance the efficacy of antiangiogenesis therapeutics, as it has been reported that cancer intervention with VEGF antibodies can induce CAF-regulated CSCs and reduce their antitumor effect [17]. Targeting CAF-CSC crosstalk is still a crucial challenge in cancer interventions. Further studies on CAF-regulated cancer stemness will open a new window for designing effective

strategies to overcome cancer progression and therapeutic resistance.

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### Disclosure of conflict of interest

None.

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