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Cancer-associated fibroblasts subtypes and role in invasion and metastasis of gastric cancer

Minireview

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Gastric cancer (GC) is the fifth most common malignancy and the fourth leading cause of cancer-related death worldwide. Cancer-associated fibroblasts (CAFs), an important cell type in the tumor microenvironment, play an important role in GC development. In this review, we describe the current knowledge of CAFs' heterogeneity and their role in GC invasion and metastasis. Currently, CAF-targeted cancer therapies are being rapidly explored and developed. However, the heterogeneity of CAFs limits the application of this therapy, so it is urgent to find specific markers and divide them into different subpopulations. With the development of single-cell RNA sequencing technology, researchers have used this technology to classify CAFs in many tumors, but whether it is applicable to GC and other tumors needs further study. And we believe that this technology will be in the near future utilized to sort CAFs on the basis of different cell markers and functions, so as to target tumor-promoting CAFs and inhibit tumor progression. Targeting CAFs by cell surface markers or normalizing the activated CAFs subsets may be an effective therapy, alone or in combination with other therapeutic approaches for GC treatment. Therefore, in the coming decades, the interaction between CAFs and GC cells will be still the focus of our research.

Key words: cancer-associated fibroblasts, gastric cancer, subtypes, invasion and metastasis

Gastric cancer (GC) is the fifth most common malignancy and the fourth leading cause of cancer-related death worldwide. In China, GC remains the third cancer type and the third cause of cancer death by 2020, the number of GC cases in China accounts for about half of that in East Asian countries, which indicates that the burden of GC in China is still serious [1, 2]. A range of investigations has demonstrated that the signaling interactions between cancer cells and the TME play a powerful role in tumor progression. Inside, CAFs are one of the most abundant stromal components in the TME, owing to their preponderance, functional diversity, and inherent plasticity, they are considered potential targets for anti-cancer therapy [3]. Recently, researchers found that CAFs are not a homogenous population but rather include heterogeneous subpopulations, such as tumor-promoting CAFs (pCAFs), tumor-retarding CAFs (rCAFs), and a neutral subpopulation, which neither promotes nor retards tumor progression (nCAFs) [4]. Therefore, identifying pCAFs and exploring their specific molecular mechanism is an important precondition for developing anti-cancer drugs targeting CAFs in GC.

In this review, we describe the current investigations of CAFs in GC and predict possible research directions in future studies.

Definition and origin of CAFs

The cells that are negative for epithelial, endothelial, and leukocyte markers, with spindle-shaped morphology and lacking the mutations found within cancer cells might be considered CAFs [5]. A compelling body of evidence suggests that CAFs are heterogeneous subpopulations of cells, this may be due to the diversity of CAFs' origins.

According to the histological types of tumors, CAFs can be divided into six categories (Figure 1) [3, 5]. i) Resident

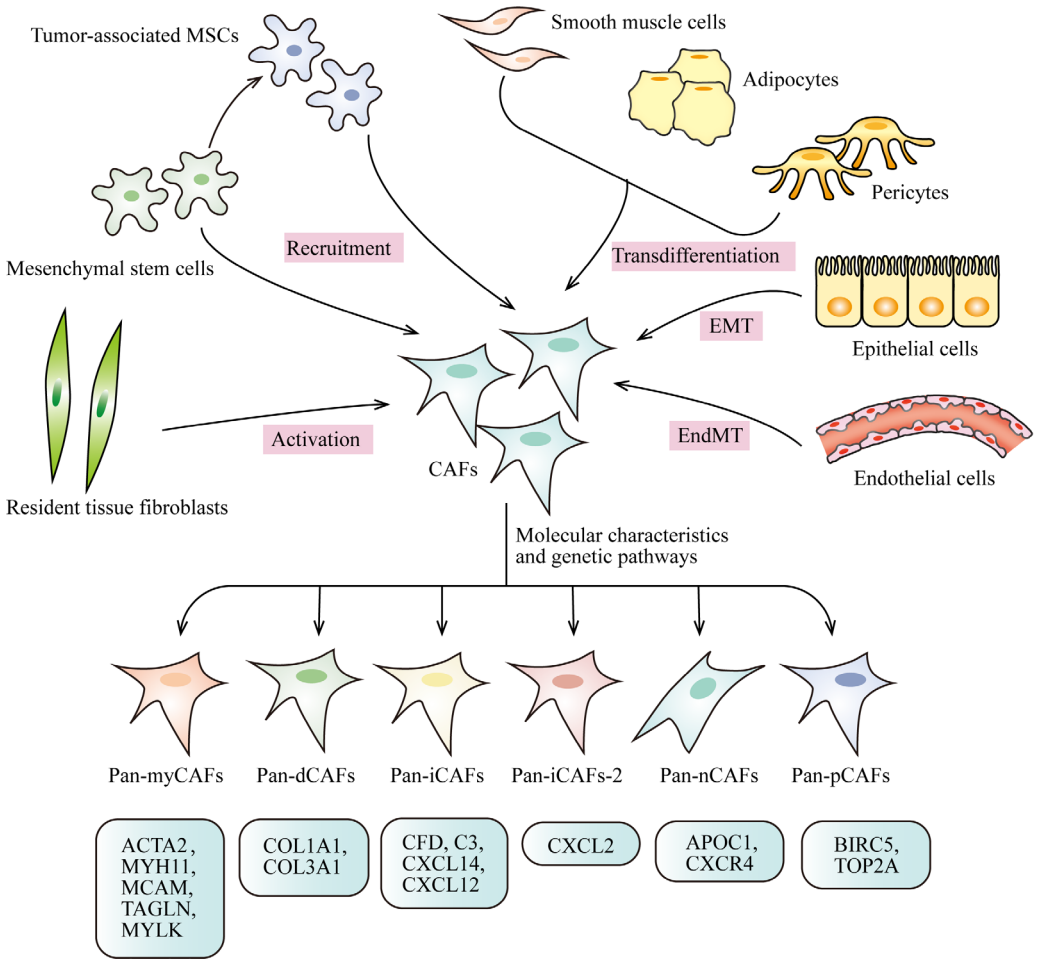

Figure 1. The origin and subtypes of CAFs on the basis of molecular characteristics and genetic pathways. Sources of CAFs include resident tissue fibroblasts, mesenchymal stem cells (MSCs), tumor-associated MSCs, epithelial cells, endothelial cells, as well as smooth muscle cells, adipocytes, and pericytes. Six CAF subtypes were identified: Pan-CAF 1 was classified as pan-myCAFs, expressing activated fibroblast markers (ACTA2) and smooth muscle cell markers (MYH11, MCAM, TAGLN, and MYLK), its markers are related to smooth muscle contraction and vascular wound healing. Pan-CAF 2 was classified as pan-dCAFs, expressing genes coding for collagen (COL1A1, COL3A1), its markers are associated with ECM remodeling. Pan-CAF 3 was classified as pan-iCAFs, expressing CFD, C3, CXCL14, and CXCL12, its markers are related to inflammation. Pan-CAF 4 was classified as pan-iCAFs-2, these cells had high expression of genes related to inflammation, including CXCL2. Furthermore, its marker genes found enrichment for NF-kB signaling pathway. Pan-CAF 5 was classified as normal fibroblast (pan-nCAFs) owing to the enrichment of its marker genes for homeostasis. Pan-CAF 6 was classified as pan-pCAFs, expressing genes related to cell cycle (BIRC5, TOP2A), its markers are associated with cellular proliferation. Abbreviations: ACTA2-actin α 2; APOC1-apolipoprotein C1; BIRC5-baculoviral IAP repeat containing 5; C3-complement C3; CFD-complement factor D; COL1A1-collagen type I α 1 chain; COL3A1-collagen type III α 1 chain; CXCL14-C-X-C motif chemokine ligand 14; CXCR4-C-X-C motif chemokine receptor 4; EMT-epithelial-to-mesenchymal transition; EndMT-endothelial-to-mesenchymal transition; MCAM-melanoma cell adhesion molecule; MYH11-myosin heavy chain 11; MYLK-myosin light chain kinase; TAGLN-transgelin; TOP2A-DNA topoisomerase II α

tissue fibroblasts are the main source of CAFs. In a specific microenvironment, resting fibroblasts become CAFs after being activated by various types of cytokines from the neighboring tumor cells [6]. ii) Mesenchymal stem cells (MSCs), there is sufficient evidence that quite a number of CAFs may be derived from MSCs. On the one hand, an *in vitro* study demonstrates that human BM-MSCs can differentiate and express markers of CAFs in human breast cancer, pancreatic cancer, and glioma cancer, such as α-smooth muscle actin (α-SMA), vimentin, fibroblast-associated protein (FAP), and fibroblast specific protein 1 (FSP1). On the other hand, murine tumor models and other studies also verify that BM-MSCs can develop into CAFs [3, 7, 8]. iii) Tumor-associated MSCs (TA-MSCs), are also derived from MSCs and can self-renew for many passages *in vitro*, which is different from CAFs. (Phenotypically, TA-MSCs exhibit lower expression of vimentin and fibroblast-specific protein 1 (FSP1) than CAFs, and *in vitro* experiments have shown that they can differentiate into CAFs. They are more closely related to the TME than other MSC type, and the correlations between TA-MSCs

and tumor immunity have been investigated, which can bring the prospect for tumor immunotherapy [7, 9]. iv) Epithelial cells, they can develop into CAFs by undergoing epithelial-to-mesenchymal transition (EMT). v) Endothelial cells, also by undergoing endothelial-to-mesenchymal transition (EndMT). vi) Other uncommon sources, such as myofibroblasts, vascular smooth muscle cells, pericytes, and adipocytes. Both these cells (include epithelial and endothelial cells) undergo transdifferentiation, with gene expression and biological changes to adopt a CAFs phenotype [4].

Function and heterogeneity of CAFs

As mentioned above, CAFs originate from a variety of cells, depending on their origin, the function of such activated fibroblasts could be diverse and unique. On the one hand, CAFs induce tumorigenesis, angiogenesis, energy metabolism, immunosuppressive, invasion and metastasis, and eventually lead to tumor progression; on the other hand, tumor-suppressive functions of some CAFs subsets have been reported, which further supports the heterogeneity of CAFs in the TME. Indeed, it has been reported that the existence of anti-tumor CAFs in breast cancer and pancreatic ductal adenocarcinoma (PDAC) patients [10, 11]. Kalluri et al. [6] divide the CAFs into five subtypes on the basis of function: i) F1 subtype: tumor-restraining CAFs; ii) F2 subtype: tumor-promoting CAFs, and F1 and F2 can be converted into each other under certain conditions; iii) F3 subtype may have high growth factor secretion activity, thus affecting tumor immunity, angiogenesis, and cancer cell proliferation; iv) F4 subtype can produce and remodel the extracellular matrix (ECM); v) F5 subtype are CAFs with other functions.

CAFs are a heterogeneous population based on origins, functions, and markers, and there is a great quantity of different CAFs existing in TME. Many cell markers have been identified to better characterize activated CAFs, such as α-smooth muscle actin (α-SMA), fibroblast activation protein (FAP), fibroblast-specific protein 1 (FSP1), periostin (POSTN), integrin β1 (CD29), and platelet-derived growth factor receptor (PDGFR), both PDGFRα and PDGFRβ can be used to identify CAFs [12]. In addition to the well-known markers mentioned above, several recently discovered markers have also caught our attention. A gene expression analysis showed that yes-associated protein (YAP) is highly expressed in CAFs. YAP remodels ECM and promotes cancer cell invasion by regulating the contractile actomyosin cytoskeleton, so it can also be used as a CAFs marker [13]. Podoplanin (PDPN) is a membrane-binding marker that has been observed to be overexpressed in the CAFs population. Although it is also expressed in epithelial tumor cells and inflammatory macrophages, recent studies do show that this marker can be used to identify oncogenic fibroblast subsets, and it is also associated with poor prognosis [14, 15]. Besides, a proteomic analysis of human breast cancer tissues revealed that galectin-1 is upregulated in cancer-associated stroma tissue. And natriuretic peptide B (NPPB), increasing in ovarian cancer stroma compared with normal ovarian stroma, also has been identified as a potential candidate marker for CAFs [16, 17]. But none of them are specific to CAFs, they can also be expressed in other cell types, which highlight a certain degree of heterogeneity of CAFs in the TME [12].

Identifying reliable and specific cell surface markers is key to differentiate CAFs subsets. Once separated, functional studies can be conducted to clarify specific activities. Future research is likely to address these exciting questions. At present, the heterogeneity of CAFs and their various roles have not been fully elucidated. Further studies of single-cell RNA sequencing, translatable *in vivo* cancer models, discrete transgenic targeting and new matrix reagents, will provide new insights into the heterogeneity of these different types of CAFs [4]. Ela et al. have used single-cell RNA sequencing to divide CAFs into myofibroblastic CAFs (myCAFs), inflammatory CAFs (iCAFs), and antigen-presenting CAFs (apCAFs) in human and mouse PDAC tumors. And they found that the apCAFs that express MHC class II and CD74 but do not express classic costimulatory molecules have a potential immune-modulatory capacity because they can activate CD4 T cells in their model [18, 19]. In addition, Galbo [20] and his colleagues put forward six CAF subtypes (pan-CAFs) shared among three cancer types (melanoma, head and neck squamous cell carcinoma (HNSC), and lung cancer), and uncovered their molecular characteristics and genetic pathways (Figure 1).

i) Pan-CAF 1, was classified as pan-myCAFs, expressing activated fibroblast markers (ACTA2) and smooth muscle cell markers (MYH11, MCAM, TAGLN, and MYLK), its markers are related to smooth muscle contraction and vascular wound healing. ii) Pan-CAF 2, was classified as pan-dCAFs, expressing genes coding for collagen (COL1A1, COL3A1), its markers are associated with ECM remodeling. iii) Pan-CAF 3, was classified as pan-iCAFs, expressing CFD, C3, CXCL14, and CXCL12, its markers are related to inflammation. iv) Pan-CAF 4, was classified as pan-iCAFs-2, these cells had high expression of genes related to inflammation, including CXCL2. Furthermore, its marker genes found enrichment for the NF-κB signaling pathway. v) Pan-CAF 5, was classified as normal fibroblast (pan-nCAFs) owing to the enrichment of its marker genes for homeostasis. vi) Pan-CAF 6, was classified as pan-pCAFs, expressing genes related to cell cycle (BIRC5, TOP2A), its markers are associated with cellular proliferation.

Among them, pan-dCAFs overexpressed TWIST1, suggesting that EMT may be required for the transdifferentiation of this highly invasive CAF phenotype. Previous studies have shown that depletion of TWIST1 in CAFs derived from GC can reduce cancer cell migration and invasion. Their data showed that pan-dCAFs were correlated with poor prognosis in GC likewise, further highlighting the clinical relevance of pan-dCAFs in GC.

Furthermore, they found novel transcriptional driver genes (MEF2C, TWIST1, NR1H3, RELB, and FOXM1) which are the key to CAF heterogeneity.

Roles of CAFs in GC invasion and metastasis

The molecular mechanisms of tumor invasion and metastasis are complex and are closely related to cell adhesion molecules, ECM, EMT, highly aggressive tumor cell subclones, and tumor angiogenesis.

The invasion and metastasis of GC can be roughly summarized as the following steps: i) Separation of cancer cells from each other. ii) Cancer cells express more laminin (LN) receptors, increasing the attachment between cancer cells and the basement membrane. iii) Degradation of ECM, causing local defects in basement membrane to facilitate the passing of cancer cells. iv) Cancer cells migrate through the defect of the basement membrane by ameboid movement, further dissolve interstitial connective tissue and reach the vessel wall, it passes through the basement membrane of the vessel in the same manner and enters the vessel. v) Not all cancer cells that enter blood vessels can migrate to other organs and form metastases. Among them, cancer cells that accumulate with platelets are not easy to be destroyed by immune cells, they are easy to adhere to endothelial cells, and then cross the vascular endothelial cells and basement membrane to form new metastases.

The invasion and metastasis of cancer cells still pose great challenges to the treatment of GC even though immunotherapy and neoadjuvant chemotherapy have been well applied in GC, and almost 60% of GC deaths are due to peritoneal recurrence [21]. CAFs are the most important components in the microenvironment of GC. Unlike other normal fibroblasts, they can secrete many factors to promote invasion and metastasis, these factors include chemokines and cytokines such as CXCL12 (SDF1), CXCL14, IL-6, IL-33, and growth factors such as TGF-β, VEGF, as well as multiple microRNAs (Table 1) [21, 22].

Cytokines and chemokines. During tumor progression, different kinds of cytokines released by CAFs mediate the activation of different signaling pathways of tumor cells leading to the invasion and eventually metastasis. Zhou et al. have found that IL-33 released by CAFs promotes the migration and invasion of GC cells via ST2L, which is dependent on the activation of the ERK1/2-SP1-ZEB2 pathway. Conversely, TNF-α is released by GC cells and induces IL-33 overexpression in CAFs via the TNFR2-NF-κB-IRF-1 pathway. Thus, the cross-talk between CAFs and GC cells mediated by TNF-α/IL-33/ST2L signaling contributes to GC progression. And the overexpression of IL-33 and its receptor ST2L in GC can predict poor prognosis [23–25]. Other cytokines, such as IL-6, an important mediator in tumor-promoting effects of gastric CAFs, promote EMT and peritoneal metastasis via activating the JAK2/STAT3 signaling pathway in GC [26–28]. Additionally, they also participate in the formation of immunosuppressive TME, restricting the maturation of dendritic cells and remodeling monocytes toward macrophage differentiation [29]. Connective tissue growth factor (CTGF), discovered in human umbilical vein endothelial cells (HUVECs) by Bradham in 1991, is a multifunctional signaling modulator that can promote cancer progression and metastasis by regulating cancer cell proliferation, migration, invasion, drug resistance, and EMT. And its targeting drugs have been shown to inhibit tumor cell migration, peritoneal dissemination, and the EMT process in GC [30–32].

Pathological angiogenesis is a hallmark of cancer. Past studies have shown a positive correlation between the expression of factors related to tumor angiogenesis and poor clinical outcomes of GC patients [33, 34]. Neovascularization in cancer is regulated not only by tumor cells but also by stromal cells. Indeed, CAFs promote tumor angiogenesis directly by secreting pro-angiogenic factors, such as vascular endothelial growth factor A (VEGFA), VEGF receptor 2 (VEGFR2), CXCL12 (SDF1), fibroblast growth factor 2 (FGF2), and PDGFC. In GC, galectin-1 and hepatocyte growth factor (HGF) have already been confirmed to contribute to GC angiogenesis. Galectin [35–37] expression in CAFs was positively related to increased expression of endothelial cell marker, CD31. Ding et al. discovered that the phosphorylation of Akt and ERK1/2 was increased in GC cells treated with HGF and co-cultured with CAFs. Both Akt inhibitors and ERK1/2 inhibitors reduced the angiogenic and vasculogenic abilities of HGF [38–41].

Besides, chemokines are also involved in the regulation of CAFs in GC invasion and metastasis, such as CXCL12/CXCR4, activated by CAFs to mediate integrin β1 clustering at the cell surface and promote the invasive ability of GC cells. And the high level of CXCL12 correlates with tumor poor prognosis, the inhibition of CXCR4, Plerixafor (AMD3100), can reduce the invasion of GC cells [42–45]. It has been confirmed that the expression level of CXCL14 is elevated in pancreatic cancer and colorectal carcinoma but reduced in GC [46–51]. Recently, a study has shown that CXCL14 and NF-κB expression was positively related to miRNA-150 expression but negatively to iroquois homeobox (IRX1) expression. And then, they revealed that miR-150 downregulation constrains migration and invasion and facilitates apoptosis of GC cells by enhancing IRX1 expression. The inhibiting role of the miR-150/IRX1/CXCL14/NF-κB axis in GC needs further exploration [52]. According to this, CAFs can also inhibit the progression of GC.

MicroRNAs. As mentioned above, the miRNAs are considered one of the most important regulators in GC invasion and metastasis, involved in the regulation of posttranscription of gene expression. Based on this particular feature, miRNAs have been shown to be involved in multiple signaling pathways within the TME [22]. A growing body of evidence has demonstrated that several miRNAs showed a differential function in GC. For instance, miRNA-17-5p, which was upregulated in GC, promoted the proliferation and invasion

Table 1. The role of factors secreted by CAFs in invasion and metastasis of gastric cancer.

Marker	Expression level	Involved critical molecule/pathway	Effect on cancer	References
Cytokines				
IL-33	Upregulated	TNF-α/IL-33/ST2L signaling	contributing to GC progression	[23–25, 104]
IL-6	Upregulated	JAK2/STAT3 signaling pathway	promoting EMT and peritoneal metastasis	[26–29, 105]
Gal-1	Upregulated	TGF-β1/Smad signaling pathway	promoting angiogenesis in GC; promoting the migration and invasion of GC cells	[35–37]
Growth factors				
CTGF	Upregulated	NF-κB signaling pathway	enhancing the migration and metastasis of GC cells	[30, 31, 106]
HGF	Upregulated	PI3K/AKT and ERK1/2 signaling	promoting vascularization in GC	[38–41, 109]
Chemokines				
CXCL12	Upregulated	CXCL12/CXCR4 signaling; upregulate integrin β1	promoting invasiveness and mobility of GC cells	[42, 43, 45]
CXCL14	Downregulated	ERK1/2 signaling	promoting tumor development and invasion	[46, 47, 49–51]
MicroRNAs				
miRNA-17-5p	Upregulated	targeting RUNX3	promoting proliferation and invasion of GC cells	[53, 56, 58, 59]
miRNA-214	Downregulated	targeting FGF9 and EMT	contributing to migration and invasion of GC cells	[60–62, 108]
miRNA-149	Downregulated	reducing IL-6 and EP2 expression; targeting FOXC1	suppressing the proliferation and metastasis of GC cells	[63–67]
Exosomes				
miRNA-139	Downregulated	repressing MMP11 expression	inhibiting GC progression	[72–74]
miRNA-522	Upregulated	targeting ALOX15; blocking lipid-ROS accumulation	suppressing ferroptosis in GC	[98–100, 109]
Extracellular Matrix Production and Remodeling				
MMPs	Upregulated	enhancing the stiffness of GC matrix	enhancing metastatic behavior of GC cells and migration to the blood vessels	[76, 110–113]
LOX	Upregulated	modifying collagens and elastin in the ECM	increasing stiffness; promoting liver metastasis of GC	[82–85, 114]
Resistance to Chemotherapy				
AnxA6	Upregulated	activating β1 integrin-focal adhesion kinase (FAK)-YAP	inducing drug resistance	[86–88, 94]
IL-11	Upregulated	JAK/STA T3/BCL2 signaling pathway	regulating the drug resistance of GC	[33, 93–97]

Abbreviation: IL-6-interleukin-6; IL-11-interleukin-11; IL-33-interleukin-33; Gal-1-galectin-1; CTGF-connective tissue growth factor; HGF-hepatocyte growth factor; CXCL12-chemokine ligand 12; CXCL14-chemokine ligand 14; CXCR4-CXC-chemokine receptor 4; MMP-matrix metalloproteinase; LOX-lysyl oxidase; AnxA6-Annexin A

of GC cells by targeting runt-related transcription factor 3 (RUNX3), and also as miR-146a [53–59]. Conversely, miRNA-214, as a novel tumor suppressor gene, downregulating in CAFs contributes to migration and invasion of GC cells through targeting FGF9 and EMT, but the proliferation ability of tumor cells is not changed. Moreover, FGF9's high expression in CAFs of lymph node metastatic sites was associated with poor prognosis in GC [60–62]. miRNA-149 also plays a role in inhibiting tumors [63–67].

Moreover, Zhang et al. put forward a survival predicting nomogram, which can be used to predict the prognosis of patients with GC, and identified one downregulated miRNA (miR-135b) and four upregulated miRNAs (miR-106b, miR-141, miR-145, and miR-20a), which were related to the clinical outcome of patients with GC. These five miRNAs separately regulate several signaling pathways, including Wnt, TGF-β, Hippo, AMPK, and MAPK signaling pathways. In a word, this nomogram can predict the prognosis of post-surgery patients with GC but whether it applies to other types of GC remains to be investigated [68].

Exosomes. The main function of exosomes is to participate in intracellular material delivery, and they also participate in the transfer of information within the TME. The contents of exosomes can be proteins, miRNAs, or other substances. In GC cases, kinds of evidence showed that they can transfer information from neighbor or distant cells into target cells to produce biological effects, and then modulate GC metastasis and invasion [69, 70]. Recently, a study has shown that exosome-dependent molecular transfer or signaling pathway activation is regarded as a crucial process in the four stages of peritoneal dissemination of GC [71]. However, Xu found that exosomal miRNA-139 in CAFs inhibits GC progression by retarding matrix metalloproteinase 11 (MMP-11) expression [72–74]. So, they can be involved in both, the promotion and inhibition process. Human gastrokine 1 (GKN1), made of 185 amino acids, plays an important role in maintaining mucosal integrity and homeostasis, and in regulating cell proliferation and differentiation. Yoon et al. demonstrated that GKN1 was secreted in exosomes and could be internalized by the gastric epithelium, thereby preventing cell prolif-

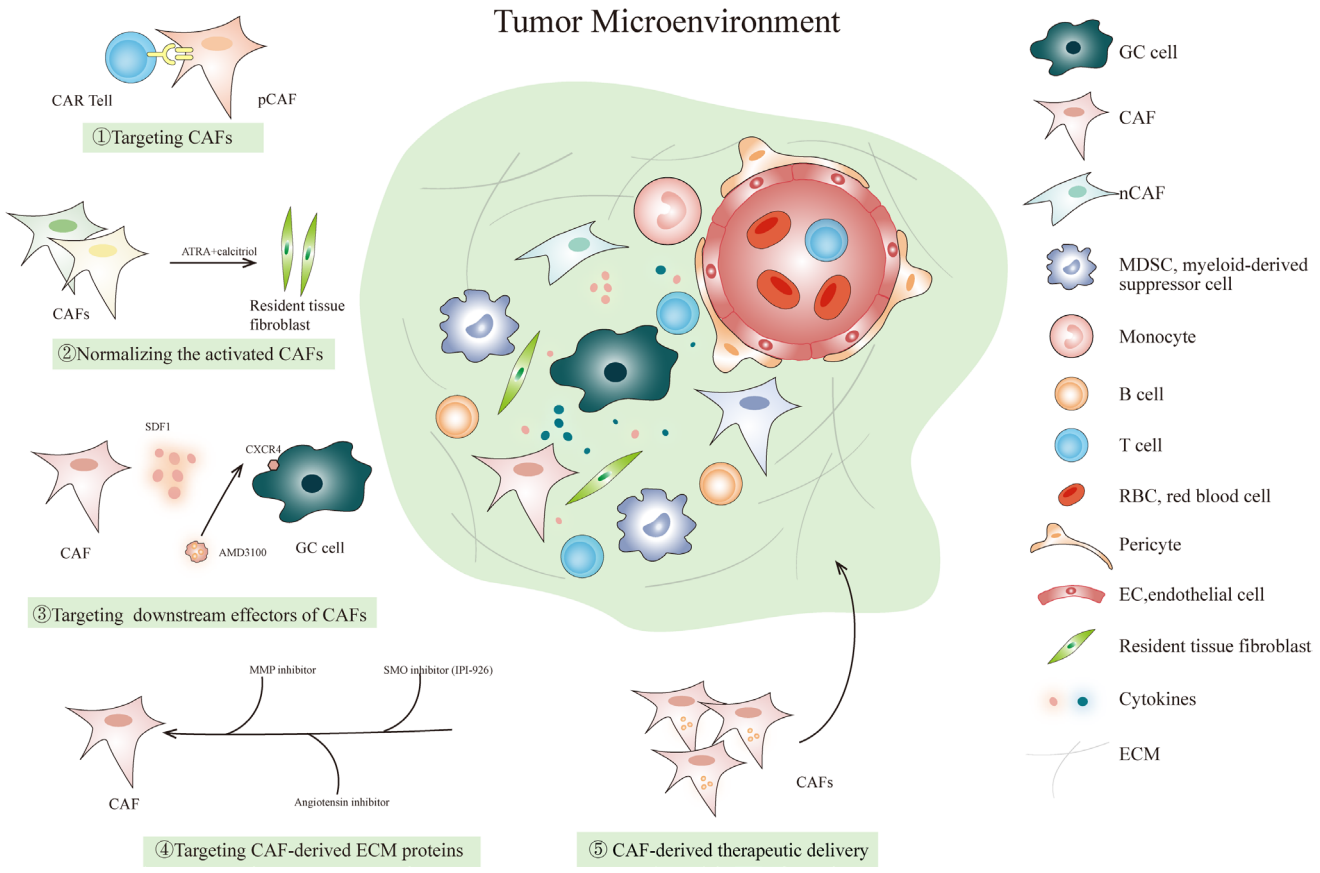

Figure 2. CAFs in tumor microenvironment and therapies of CAFs-targeting in GC. CAFs play an important role in the development of GC. They can secrete many factors to mediate GC invasion and metastasis, and there are several treatment methods for CAFs: 1) Targeting CAFs by cell surface markers, such as α-SMA, FAP, CD10+GPR77+. 2) Normalizing the activated CAFs, such as the application of ATRA and calcipotriol. 3) Targeting activation signaling and downstream effectors of CAFs, targeting IL-6, IL-6 receptor or JAKs, kinase inhibitor imatinib, and targeting the SDF1-CXCR4 axis with AMD3100 have been well verified. 4) Targeting CAF-derived proteins and associated signaling. Angiotensin inhibitor, MMP inhibitor, and SMO inhibitor belong to this category. 5) CAF-directed therapeutic delivery, utilizing *ex vivo* modified CAFs delivery anticancer drug has become an attractive cellular vehicle. Abbreviations: FAP-fibroblast-associated protein; ATRA-all-trans retinoic acid; CXCR4-C-X-C motif chemokine receptor 4; SDF1-stromal cell-derived factor 1; MMP-matrix metalloproteinase; CAR-T-chimeric antigen receptor T cell; MDSC-myeloid-derived suppressor cell; RBC-red blood cell; EC-endothelial cell

eration and inducing apoptosis of GC cells. In addition, the concentration of GKN1 in the sera of patients with GC was significantly lower than that of healthy people, patients with hepatocellular carcinoma, and colorectal cancer [69, 75].

Extracellular matrix production and remodeling. Extracellular matrix (ECM) provides mechanical and structural support for tissue functions, and its degradation is now considered one of the murderers of tumor progression. This process is mediated by the actions of metalloproteinases (MMPs), which are produced by various cells, including tumor cells and fibroblasts, as well as by CAFs [76].

For example, increased deposition and cross-linking of collagen I and III enhance the stiffness of the GC matrix, which is the beginning of the enhanced metastatic behavior of GC cells. Increased stiffness also correlates with the infiltration of macrophages, which promotes malignant progression [44]. Furthermore, stiffness increases N-cadherin expression on endothelial cells, which promotes GC cells binding to the endothelium and migration to the blood vessels [44, 77]. Therefore, in addition to paracrine signaling, CAFs also contribute to angiogenesis indirectly via remodeling ECM proteins such as periostin, tenascins, fibronectin, osteopontin, frizzled-related protein 2 (SFRP2), and collagens [78]. Besides, ME is rich in the lactic acid environment and also promotes the formation of neovascularization [79]. In a recent study, CAFs-derived lysyl oxidase (LOX), which modifies collagens and elastin in the ECM, thereby catalyzing the covalent cross-linking of collagen fibers and thus increasing stiffness, promoting liver metastasis of GC thus predicting poor prognosis. Upon stimulation by TGF-β secreted by tumor cells, these CAFs were found to secrete more LOX, which facilitates tumor growth and progression [80–85]. Extracellular vesicles (EV) from CAFs are composed of diverse payloads, but the impact of CAF-EV on GC progres-

sion has not been illustrated. Recently, an investigation has shown that the abundance of CAFs in GC tissues is associated with poor prognosis in GC patients who received chemotherapy. Moreover, CAF-EV induced tubular network formation and drug resistance of GC cells in ECM. Comprehensive proteomic analysis of CAF-EV identified that Annexin A6 plays an important role via activation of β1 integrin-focal adhesion kinase (FAK)-YAP in this process, and inhibition of FAK or YAP could effectively reduce drug resistance of GC *in vitro* and *in vivo* [47]. And in the pancreatic tumor, Peran et al. found that Cadherin 11 promotes immunosuppression and ECM deposition, and that might be developed as a therapeutic target for pancreatic cancer [86–89].

Resistance to chemotherapy. Although the mortality rate of GC has decreased with the application of neoadjuvant chemotherapy and targeted therapy, drug resistance has also gradually emerged, Emerging evidence has demonstrated that CAFs confer substantial resistance to cancer therapeutics via impaired drug delivery and biochemical signaling. CAFs have been confirmed to regulate chemoresistance by secreting cytokines, such as IL-6, IL-17A, insulin-like growth factor (IGF)-1, insulin-like growth factor (IGF)-2, nitric oxide (NO) and platinum-induced polyunsaturated fatty acids [81, 90]. Some subsets of CAFs, such as the CD10+GPR77+ subset, can induce therapeutic resistance in cancer, this conclusion was confirmed by Su and his colleagues in an experiment in which tumor cells were dramatically enhanced upon co-culture with CD10+GPR77+ CAFs under chemotherapy. Besides, they also suggested that CD10+GPR77+ CAFs are not only resistant to chemotherapy themselves, but also can induce chemoresistance of the tumor cells in TME [91]. Recent evidence has shown that the chemokines, cytokines, and growth factors secreted by CAFs are key factors in promoting tumor progression and promoting drug resistance [92]. Ma et al. have concluded that IL-11 secreted by CAFs regulates the drug resistance of GC in a paracrine manner through the JAK/STAT3/BCL2 signaling pathway, so the application of IL-11R inhibitor might be a potential strategy to GC therapy [93–97]. Arachidonate lipoxygenase 15 (ALOX15) is closely related to lipid-ROS production in GC, and exosome-miR-522 serves as a potential inhibitor of ALOX15. The present study [98] demonstrates that CAFs secrete exosome-miRNA-522 to inhibit ferroptosis in cancer cells by targeting ALOX15 and blocking lipid-ROS accumulation, leading to ALOX15 suppression and decreased lipid-ROS accumulation in cancer cells, and ultimately resulting in decreased chemosensitivity [99, 100].

CAFs-targeting therapy

As early as 1889, Paget and his colleagues put forward the theory of "seed and soil", which shows that TME plays an important role in tumor development [101]. In the past several years, CAFs-targeting therapy has aroused people's interest, many related clinical trials are in progress. Currently, there are several treatment methods for CAFs (Figure 2) [3, 102]: i) Targeting CAFs by cell surface markers, such as α-SMA, FAP, CD10+GPR77+. Because FAP represents a major cell surface marker for immunosuppressive CAFs and eliminating FAP+ CAFs has also been proved to be associated with increased CD8+ T cells infiltration. It has been explored as the main target for eliminating CAFs. Depletion of FAP+ CAFs via genetic deletion or chimeric antigen receptor (CAR) T cells (enhancing anti-tumor immunity and reducing desmoplasia and vascular density) has shown promising anti-tumor activities in preclinical animal models [12]. The combination of CD10 and GPR77 markers helps to define a new group of tumor-promoting CAFs, targeting GPR77 with a neutralizing mAb leads to reduced tumorigenesis and enhanced chemosensitivity in breast cancer patients. It remains to be seen whether dual inhibition of CD10 and GPR77 has better therapeutic potential than blocking GPR77 alone [91]. ii) Normalizing the activated CAFs, such as the application of all-trans retinoic acid (ATRA) and vitamin D receptor ligand (calcipotriol). And in the pancreatic tumors, a new potential target has been found, Netrin G1 [103], by targeting NetG1 in CAFs, tumor-promoting CAFs can be reverted back into tumor-restrictive CAFs, ultimately limiting tumorigenesis. iii) Targeting activation signaling and downstream effectors of CAFs, targeting IL-6, IL-6 receptor or JAKs, kinase inhibitor Imatinib, and targeting the SDF1-CXCR4 axis with AMD3100 have been well verified. Hedgehog signaling is essential for CAF development. A preclinical study of IPI-926, a specific inhibitor of the Hedgehog signaling pathway, can reduce tumor-associated stromal tissue and stabilize the tumor temporarily [12]. iv) Targeting CAF-derived proteins and associated signaling, losartan (an angiotensin inhibitor that reduces collagen and hyaluronan production by CAFs), MMP inhibitors, and a SMO inhibitor (IPI-926) (which can suppress pro-stromal SHH pathway and then increase gemcitabine uptake) belong to this category. v) CAF-directed therapeutic delivery, utilizing *ex vivo* modified MSCs or CAFs delivery anticancer drug have become attractive cellular vehicles.

In a word, owing to CAFs' pro-tumor functions makes them become a potential therapeutic target. However, targeting CAFs has faced numerous obstacles and challenges. In particular, the lack of specific CAFs cell surface markers restrains their direct application, and it is difficult to precisely target CAFs without damaging normal tissue. Therefore, it is urgent to find specific markers and divide them into different subpopulations.

Conclusions and perspectives

CAFs, activated fibroblasts surrounding cancer, are one of the most abundant stromal components in the TME. And they have a variety of cellular origins, which makes them a heterogeneous population. Currently, representative CAFs markers include but are not limited to α-SMA, FAP, FSP1,

POSTN, PDPN, CD29, PDGFRα, PDGFRβ, nevertheless, none of them is specific to CAFs, they can also be expressed in other cell types. So combined application of these markers might be a better strategy for distinguishing the heterogeneous populations of CAFs in future investigations.

In truth, the development of GC is a complex process, in which CAFs play an important role. Unlike other normal fibroblasts, they can secrete many factors to mediate GC invasion and metastasis, including chemokines and cytokines as well as multiple microRNAs, exosomes, and remodeling ECM. Currently, CAF-targeted cancer therapies are rapidly being explored and developed. However, due to lacking specific markers, targeted therapy of CAFs has been hindered to some extent. Along with advances in techniques such as single cell RNA sequencing, translatable *in vivo* cancer models, discrete transgenic targeting and new matrix reagents, such as specific CAR-T cell methods, will provide new insights into the heterogeneity of these different types of CAFs. And researchers have used single-cell RNA sequencing technology to classify CAFs in many tumors, but whether it is applicable to GC and other tumors needs further study. The six-classification proposed by Galbo has been verified in gastric cancer and proposed that pan-dCAFs were correlated with poor prognosis in GC. So, the pan-dCAFs have caught our attention. So, we can focus on targeting pan-dCAFs and inhibit tumor progression. Targeting CAFs by cell surface markers or normalizing the activated CAFs subsets may be an effective therapy that can alone or in combination with other therapeutic approaches for GC treatment. Therefore, in the coming decades, the interaction between CAFs and tumors is still the focus of our research.

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