Heterogeneity of primary and metastatic CAFs: From differential treatment outcomes to treatment opportunities (Review)

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Abstract. Compared with primary tumor sites, metastatic sites appear more resistant to treatments and respond differently to the treatment regimen. It may be due to the heterogeneity in the microenvironment between metastatic sites and primary tumors. Cancer-associated fibroblasts (CAFs) are widely present in the tumor stroma as key components of the tumor microenvironment. Primary tumor CAFs (pCAFs) and metastatic CAFs (mCAFs) are heterogeneous in terms of source, activation mode, markers and functional phenotypes. They can shape the tumor microenvironment according to organ, showing heterogeneity between primary tumors and metastases, which may affect the sensitivity of these sites to treatment. It was hypothesized that understanding the heterogeneity between pCAFs and mCAFs can provide a glimpse into the difference in treatment outcomes, providing new ideas for improving the rate of metastasis control in various cancers.

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Abbreviations: CAFs, cancer-associated fibroblasts; mCAFs, metastatic CAFs; pCAFs, primary CAFs; RFs, resident fibroblasts; ECM, the extracellular matrix; MSCs, mesenchymal stem cells; BM-MSCs, bone marrow-derived MSCs; NFs, Normal fibroblasts; PSCs, pancreatic stellate cells; HSCs, hepatic stellate cells; LMs, liver metastases; myCAFs, myofibroblastic CAFs; iCAFs, inflammatory CAFs; PFs, portal fibroblasts; apCAFs, antigen-presenting CAFs; scRNA-seq, single-cell RNA sequencing; αSMA, smooth muscle actin-α; TGF-β, transforming growth factor-β; Met-LNs, lymph node metastasis; EMT, epithelial-mesenchymal transition; FSP1, fibroblast-specific protein 1; FAP, fibroblast activation protein; FAPi, FAP inhibitor; PDGF, platelet-derived growth factor; PDGFR, PDGF receptor; SDF-1, stromal cell-derived factor 1; OPN, osteopontin; COL, collagen; CXCR, C-X-C chemokine receptor; IL, interleukin; SUVs, standardized uptake values; LRG1, leucine-rich α-2-glycoprotein 1; SAM, S-adenosyl methionine; LOX, lysyl oxidases; HA, hyaluronan

Key words: metastatic cancer-associated fibroblast, primary cancer-associated fibroblast, heterogeneity, tumor microenvironment, treatment outcomes, treatment opportunities

Contents

1. Introduction

The simultaneous treatment of both primary and metastatic tumors is the main treatment option following tumor metastasis. However, the treatment for the primary tumor often has limited efficacy on the metastasis (1), resulting in different responses. Metastatic and primary tumors show varying degrees of resistance after several treatments (2,3). Compared with the metastatic sites of the primary tumors, they often exhibit a more malignant progression state (4). Accompanying this is the rapid loss of patient symptom management and the failure of antitumor treatment. The differential response of primary tumors and metastases to the treatment may be related to the heterogeneity in their tumor microenvironments (TMEs) (5,6).

The TMEs are the internal and external micro-landscape of tumor cells formed by the response of normal organs to evolving cancer cells, mainly composed of tumor cells, infiltrating immune cells, cancer-associated stromal cells, such as cancer-associated fibroblasts (CAFs), endothelial cells and lipocytes, along with the extracellular matrix (ECM) and multiple signaling molecules (7). These environmental factors play a key role in both the development of tumors and their response to therapy (5). CAFs, as key components of the tumor microenvironment, have been found to be closely associated with the heterogeneity between primary tumors and metastases. This heterogeneity may affect the drug resistance of primary and metastatic sites (8).

CAFs promote tumor proliferation, therapeutic resistance and immune rejection by secreting growth factors, inflammatory ligands and ECM proteins (9-11). Previously, CAFs were considered cell populations that were not single but complex subclusters with different functions (9). Significant heterogeneity in the subsets of CAFs associated with primary tumors and metastases (pCAFs and mCAFs, respectively) have been shown to exhibit different sensitivities to treatment (8). The heterogeneity of pCAFs and mCAFs may be the key to the different treatment responses of primary tumors and metastases.

Compared with pCAFs, mCAFs generally have a stronger ability to shape the ECM, and in immunosuppression and angiogenesis (12-16). The results of preclinical studies suggest that targeting mCAFs can alleviate the progression of metastatic cancer and mitigate therapeutic resistance, indicating that mCAFs are a promising target for metastatic cancer (17). Therefore, comparing the heterogeneity between mCAFs and pCAFs from the biological characteristics is necessary to find opportunities to target mCAFs. Among them, the identification of the cells of origin in CAF subtypes is a central question, as it may partially determine the functions of distinct CAF populations (18) (Fig. 1A). Furthermore, fibroblasts heterogeneity can be partly explained by variable activation levels of the resident fibroblasts (RFs) with organ-specific features (19). The phenotypic heterogeneity of activated CAFs can be manifested by a wide range of biological markers with selective expression patterns in the context of specific TMEs (10) (Fig. 1B). Moreover, the biological heterogeneity of CAFs is also reflected in secreted molecules, exosomes, transcriptional features and matreotypes (20,21) (Fig. 1C). The present study attempted to make sense of the different treatment outcomes between primary tumors and metastases from the perspective of heterogeneity and treatment resistance of pCAFs and mCAFs, identifying treatment opportunities for metastases.

2. Heterogeneity of mCAFs and pCAFs

Lineage-dependent heterogeneity of CAFs. Any cell with properties associated with 'activated' fibroblasts, myofibroblasts and mesenchymal stem cells (MSCs) can be defined as fibroblasts (21). In several experimental models of human tumors, validation at the transcriptional and protein levels revealed that differences in the spatial separation of CAF subclasses could be attributed to their respective sources (22,23), which is called lineage-dependent heterogeneity (18). In tumors, various cells, such as RFs, MSCs, including bone marrow-derived MSCs (BM-MSCs), pancreatic stellate cells (PSCs), hepatic stellate cells (HSCs), smooth muscle cells, pericytes, adipocytes, monocytes, mesothelial cells, epithelial cells and endothelial cells, are activated into CAFs through different mechanisms (24-31).

The present study summarized the functional convergence of CAFs from the perspective of their sources and CAFs from different sources may also have functional synergy (Table I). HSCs and PSCs share homology and have similar morphologies and functions (32), CAFs derived from HSCs and PSCs are prone to stromal deposition (33-35), similar to their function in mediating tissue fibrosis in non-malignant diseases (36,37). Tumor-resident MSCs also function in ECM production and remodeling after activation as CAFs and could partially promote angiogenesis (38-40). CAFs derived from BM-MSCs may be involved in angiogenesis and the maintenance of an inflammatory environment in tumors (41,42). Pericytes are cells embedded between the capillary basement membrane and endothelial cells that physiologically regulate vasoconstriction and source of neovascular walls (43), CAFs from this source may be predominantly involved in tumor angiogenesis (44). CAFs undergoing mesenchymal transformation change their adhesion properties, which is closely related to the enhancement of tumor aggressiveness (45); due to differences in organ anatomy and physiology, RFs activate CAFs in different ways via different pathways, differentiating into elusive functional phenotypes (46-49).

Pancreatic cancer (PDAC), colorectal cancer (CRC), breast cancer (BC) and lung cancer are malignant tumors characterized by high enrichment of CAFs and stromal hyperplasia and they have received a great deal of attention in the field of CAF research (50). Here, we take these several types of cancer as examples to compare the heterogeneity of pCAFs (Fig. 2A) and mCAFs (Fig. 2B) sources. It is of great significance in describing the overall picture of CAF composition and understanding the microenvironment heterogeneity between primary and metastatic tumors. The screening and ablation of CAF precursor cells of specific origins may be an effective means of improving the sensitivity of secondary tumors to drugs (17).

Main sources of pCAFs and liver mCAFs in PDAC and CRC. CRC: The main sources of pCAFs in CRC include RFs and intestinal MSCs (51). CRC cells secrete transforming growth factor-β (TGF-β) to activate RFs, which are then converted into smooth muscle actin-α (α-SMA)+ CAFs (52). Different mouse lineage traces show that most proliferating α-SMA+ CAFs originate from leptin receptor+ resident cells during the development of CRC (53). Shinagawa *et al* (54) injected PKH-labeled MSCs into the tail vein of CRC-bearing mice and detected MSCs in the stroma of both primary tumor and liver metastases (LMs). By contrast, MSCs were not detected in non-cancerous tissues, such as normal colon mucosa and liver, which proves that MSCs are specifically recruited into the CRC stroma and play an important role in tumor growth and metastasis (54). In addition, some studies have shown that epithelial cells, smooth muscle cells and pericytes are also part of the pCAFs pool in CRC (44,51,55).

PDAC: PSCs produce most of the ECM in PDAC (56) and were previously considered the main source of myofibroblasts in PDAC (57). Öhlund *et al* (58) identified myofibroblastic CAFs

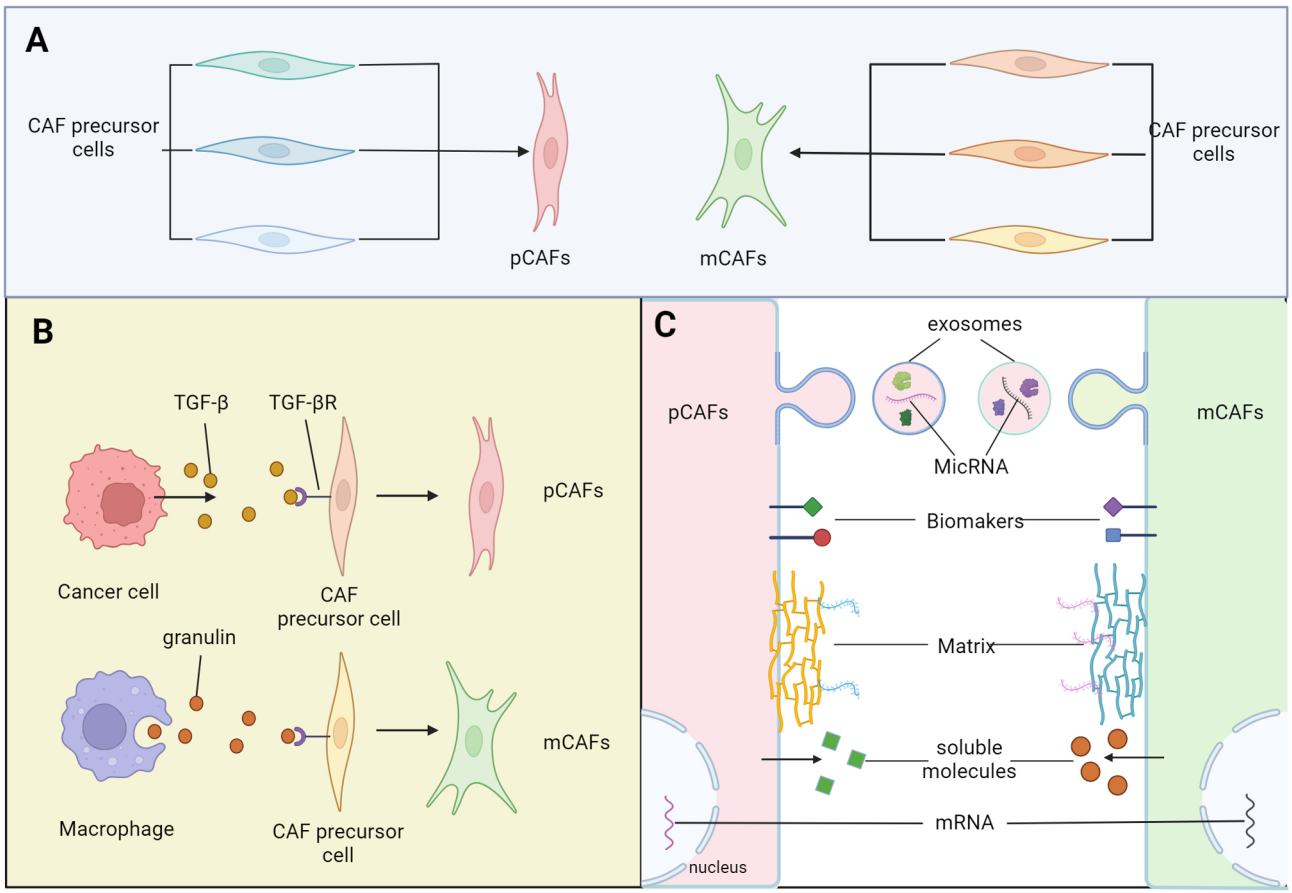

Figure 1. Heterogeneity of mCAFs and pCAFs. (A) mCAFs and pCAFs come from different sources. (B) mCAFs and pCAFs are activated in different ways. For example, in PDAC, the precursor of pCAFs are activated by the tumor-secreted TGF-β and in LMs, mCAFs are activated by macrophage-secreted granulin. (C) mCAFs and pCAFs differ in terms of exosomes, transcriptomes, biomarkers, matreotype and soluble molecules. CAFs, cancer-associated fibroblasts; mCAFs, metastatic CAFs; pCAFs, primary CAFs; TGF-β, transforming growth factor-β; TGF-βR, TGF-β receptor; MicRNA, microRNA.

(myCAFs) as responsible for ECM deposition close to tumors and inflammatory CAFs (iCAFs) responsible for secreting inflammatory and chemokines factors far from tumors in KPC mouse models and human PDAC tissue. Subsequently, through a three-dimensional co-culture platform of mouse-derived PSCs and PDAC cells, they found that PSCs can differentiate into myCAFs and iCAFs *in vitro*. Moreover, Elyada *et al* (59) then corroborated the gene signatures of these two cell types via single-cell RNA sequencing (scRNA-seq). They identified antigen-presenting tumor-associated fibroblasts (apCAFs) in addition to these two cell subtypes, which were specifically labeled with serum amyloid A3 (Saa3) and expressed MHC-II class molecules but not co-stimulatory molecules (59). It has been proposed that apCAFs act as a direct positive regulator of the adaptive immune system to activate T cells (60). Dominguez *et al* (61) analyzed apCAFs derived from mesothelial cells by mouse PDAC RNA-seq data, which Huang *et al* confirmed via lineage tracing (62).

LMs: Mouse experiments have shown that 95% of liver myCAFs originate from HSCs and portal fibroblasts (PFs) (63). In some benign liver diseases, HSCs and PFs are the main sources of myofibroblasts (64). HSCs play a critical role in hepatic fibrosis (65), while PFs are the first responders in biliary fibrosis (66). Through gene tracking, scRNA-seq and Cre-lox mediated gene deletion methods, Bhattacharjee *et al* (35) showed that the CAFs of PDAC LMs mainly originate from HSCs. Xie *et al* (67) found the exosomal CD44v6/C1QBP complex is delivered to the plasma membrane of HSCs, resulting in the phosphorylation of insulin-like growth factor 1 (IGF-1), leading to HSC activation and liver fibrosis. In another RNA-Seq analysis of CRC LMs mCAFs, comparing ECM-CAFs subtypes functioning in ECM remodeling and collagen (COL) production with fibroblast gene features in the normal or cirrhotic liver, found that ECM-CAFs significantly overlap with scar-associated MSCs (SAMes), which express PFs markers. The Ctr-CAF-I subtype (with contractile function), which expresses PLN and variants of actin gamma 2, may originate from vascular smooth muscle cells (VSMCs); while the Ctr-CAF-II subtype (the average CAF phenotype) may originate from HSCs (68).

The most common metastatic site for PDAC and CRC is the liver and some studies have found that the fate of this directed metastasis appears to be mediated by fibroblasts before the metastatic event occurs (34,69-71). When genome-wide transcription data on the heterogeneity of human fibroblasts between organs were acquired through cDNA microarray analysis of human skin fibroblast cultures from different ages and anatomical locations, the fibroblasts were clustered next to other fibroblasts from the same site rather than cells from the same individual, which indicates that fibroblasts

Table I. Different sources of CAFs.

First author/s, year	Source cells	Cancer species	Model/Method	Activation/ recruitment factors	Markers	Function	(Refs.)
Bachem *et al*, 2005	PSC	PDAC (primary)	CDX/IHC	PDGF, FGF-2, TGF-β1	FN, α-SMA, Vimentin, Desmin	Produces COL-1 and COL-3	(33)
Helms *et al*, 2022		PDAC (primary)	CDX/GFP	/	α-SMA, PDPN, FAP	Cell adhesion, ECM-receptor interaction and axonal guidance	(75)
Bhattacharjee *et al*, 2021	HSC	CRC and PDAC (LM)	CDX/GFP	/	α-SMA, Desmin	Produces COL-1 to restrict tumor growth; produces HA and HGF to promote tumor growth	(35)
Erez *et al*, 2016		PDAC (LM)	CDX/scRNA-seq Adoptive bone marrow trans-plantation/GFP	/ metastasis-associated macrophage derived granulin	Lrat, Lum, PDGFRb, α-SMA	COL deposition, produces periosteum proteins to promote liver metastasis	(34)
Tan *et al*, 2020		CRC (LM)	Co-cultivation/ quantitative PCR, western blotting	TGF-β1/SDF-1/ CXCR4	α-SMA, Vimentin, FSP1, FAP	Promotes liver metastasis	(69)
Hosaka *et al*, 2016	Pericytes	BC (primary)	Co-cultivation/ IFS, CDX/RFP	PDGF-BB/ PDGFRβ	α-SMA, FSP-1	Promotes angiogenesis, tumor growth and metastasis	(44)
Shinagawa *et al*, 2010	MSC	CRC (primary and LM)	CDX/PKH26 maker	SDF-1/CXCR4, CCL2 /CCR2, PDGF	α-SMA, PDGFRb, Desmin, FSP, FAP	Promotes tumor proliferation	(54)
Suetsugu *et al*, 2011		CRC (LM)	CDX/GFP	/	Desmin	/	(209)
Klopp *et al*, 2007		BC (primary)	CDX/GFP	Irradiation (TGF-β1, CCR2, VEGF, PDGFBB)	GFP	Promotes fibrosis, participate in tissue repair and vascular reconstruction	(38)
Mi *et al*, 2011		BC (primary)	CDX/quantitative PCR, western blotting	OPN	a-SMA, Tenascin-c, SDF-1, FAP-1	Secretes MMP-2 and MMP-9, reshapes the stroma to promote tumor growth and metastasis	(40)
Spaeth *et al*, 2009		BC, ovarian cancer, PDAC (primary)	CDX/IHC	/	FAP, FSP, Tenascin-c, Thrombospondin-1, Stromelysin-1	Produces HGF, EGF and IL-6 to promote tumor; produce VEGF to promote angiogenesis	(39)
Jung *et al*, 2013		Prostatic cancer (primary)	CDX/tumor tissue microarray staining	CXCR6/CXCL16	α-SMA, vimentin	Promotes tumor metastasis	(210)
Kidd *et al*, 2012		Ovarian cancer (primary)	CDX/RFP	/	a-SMA, NG2	Involved in angiogenesis	(211)

Table I. Continued.

First author/s, year	Source cells	Cancer species	Model/Method	Activation/ recruitment factors	Markers	Function	(Refs.)
Tang *et al*, 2020	AD-MSCs	Ovarian cancer (omentum metastases)	Co-cultivation/IHC	TGF-β1	α-SMA, FAP	Promotes tumor proliferation and progression, promotes omentum metastasis	(212)
Cho *et al*, 2012		BC (primary)	Co-cultivation/IHC, western blotting	BC Adipose-derived MSCs extracellular vesicles	α-SMA, SDF-1, VEGF, CCL5, TGFβ	/	(213)
Liu *et al*, 2019	BM-MSCs	BC (Bone metastases)	Co-cultivation/IFS, western blotting	mTORC2	α-SMA, E-cadherin	Secretes growth factors, IL-6, IL-10, IL-8, VEGF; modifies the extracellular matrix, supports angiogenesis and inhibit the anti-tumor immune response	(42)
Raz *et al*, 2018		BC (primary and lung metastases)	Genetically engineered mice, adoptive bone marrow transplantation /IHC	Tumor cell secretion factor	αSMA, PDGFRα^{-}, CD45^{-}	Promotes inflammatory, induce angiogenesis and promote tumor growth	(41)
Karnoub *et al*, 2007		BC (primary)	CDX/GFP	CCL5	GFP	Promotes tumor growth and lung metastasis	(214)
Zhang *et al*, 2016		XV2 (primary)	Co-cultivation/ western blotting	/	α-SMA, vimentin	/	(215)
Bochet *et al*, 2013	Adipocyte	BC (primary)	Co-cultivation/ quantitative PCR, western blotting CDX/GFP	Wnt3a/Wnt/ β-catenin	FSP-1, α-SMA-	Secretes FN and COL-1 to promote tumor migration	(216)
Jotzu *et al*, 2011		BC (primary)	Co-cultivation/IFS	TGF-β1/Smad3	a-SMA, tenascin-C	Secretes SDF-1 and CCL5 to promote tumor cell invasion	(217)
Kojima *et al*, 2010	RFs	BC (primary)	CDX/GFP	TGF-β/Smad, SDF-1/CXCR4	α-SMA	Secretes TGF-β to promotes tumor progression	(102)
Sharon *et al*, 2015		BC (primary)	Co-cultivation/ collagen contraction	OPN/CD44, OPN/αvβ3	α-SMA	Secretes SDF-1, promotes EMT and inflammation	(47)
Erez *et al*, 2010		Squamous skin carcinogenesis (primary)	CDX /IHC, IFS	NF-kB	α-SMA, PDGFRα	Recruits macrophage, neovascularize and tumor growth	(46)

Table I. Continued.

First author/s, year	Source cells	Cancer species	Model/Method	Activation/ recruitment factors	Markers	Function	(Refs.)
Baroni *et al*, 2016		BC (primary)	over-expressing miR-9 NFs/western blotting	miR-9	α-SMA, E-cadherin	Promotes tumor invasion and remodel ECM	(218)
Vu *et al*, 2019		BC (primary and lung metastases)	CDX/GFP	microRNA-125b/ TP53/TP53INP1	α-SMA, CAV1, TGF-β1, HGF	/	(48)
Gong *et al*, 2022		BC (lung metastases)	CDX/IFS, scRNA-seq	Neutrophil-derived IL-1	COX-2, PGE2	Promotes lung metastasis and immunosuppression	(49)
Garcia *et al*, 2020		PDAC (primary)	genetically engineered mice/ GFP	/	Gli1, α-SMA, PDGFRα	/	(76)
Arina *et al*, 2016		Fibrosarcoma (primary)	CDX/GFP	/	α-SMA	Produces COL-1	(219)
Vicent *et al*, 2012		Lung cancer (primary)	CDX/GFP	CLCF1-CNTFR, IL-6	α-SMA, FAP, vimentin	Promotes tumor growth	(220)
Nair *et al*, 2017	Cancer stem cell	BC (primary)	Co-cultivation/ Sphere-formation	TGF-β1	FSP1, α-SMA, SDF-1, TGF-β1, PDGFα, C1α1, vimentin	Forms fibroblast like cells surrounding CSC colonies	(221)
Sandoval *et al*, 2013	Mesothelial cells	Ovarian cancer (peritoneal metastases)	CDX/IHC	β1-integrin	α-SMA, cytokeratins, calretinin	Promotes angiogenesis and tumor invasion	(222)
Huang *et al*, 2022		PDAC (primary)	genetically engineered mice/ scRNA-seq	TGF-β, IL-1	Mesothelin, IL-6, SDF-1, PDGFα, α-SMA, Thy1, TGF-β1	Induces immature CD4+T cells to transform into regulatory T cells	(62)
Zeisberg *et al*, 2007	Endothelial cells	Melanoma (primary)	CDX/IFS	TGF-β1	α-SMA, FSP1	/	(223)
Potenta *et al*, 2008	Epithelial cells	PDAC (primary)	3D Co-cultivation/ Morphological identification, quantitative PCR, western blotting	hedgehog/Gli1/ Snail	vimentin, Gli1	Promotes tumor migration and invasion	(24)
Xin *et al*, 2020		BC (primary)	IHC	Fluid Shear Stress/ JNK	vimentin	Promotes the survival of suspended CTCs in shear flow	(224)

Table I. Continued.

First author/s, year	Source cells	Cancer species	Model/Method	Activation/ recruitment factors	Markers	Function	(Refs.)
Kan *et al*, 2020		ovarian cancer (ascites)	IFS	TGF-β/ZEB2	α-SMA, EpCAM-/ CD45-	Chemotherapy resistance, promotes tumor metastasis and invasion	(225)
Yoshimura *et al*, 2018		CRC (primary)	CDX/IHC	Wnt/β-catenin	vimentin	Changes cell morphology to promote tumor proliferation and invasion	(226)

PSC, pancreatic stellate cells; HSC, hepatic stellate cells; MSC, mesenchymal stem cells; AD-MSCs, adipose-derived MSCs; BM-MSCs, bone marrow-derived MSCs; PDAC, pancreatic cancer; CRC, colorectal cancer; LM, liver metastasis; BC, breast cancer; FN, fibronectin; CAFs, cancer-associated fibroblasts; myCAFs, myofibroblastic CAFs; iCAFs, inflammatory CAFs; IHC, immunohistochemistry; IFS, Immunofluorescence staining; CDX, cell derived xenograft; GFP, green fluorescence protein; scRNA-seq, single cell RNA sequencing; RFP, red fluorescence protein; PDGF, platelet-derived growth factor; PDGFR, PDGF receptor; FGF, fibroblast growth factors; FGFR, FGF receptor; TGF-β, transforming growth factor-β; TGF-βR, TGF-β receptor; SDF-1, stromal cell-derived factor 1; CCL, chemokine (c-c motif) ligand; CCR, C-C chemokine receptor; VEGF, vascular endothelial growth factor; OPN, osteopontin; CXCL, CXC chemokine ligand; mTORC2, mammalian target of rapamycin complex 2; α-SMA, α smooth muscle actin; PDPN, podoplanin; FAP, fibroblast activation protein; FSP1, fibroblast-specific protein 1; COL-1/3, collagen1/3; ECM, extracellular matrix; HA, hyaluronan; HGF, hepatocyte growth factor; MMP, matrix metalloproteinases; IL, interleukin; EGF, epidermal growth factor.

have positional memory characterized by gene expression patterns (72). LMs have unique and similar morphological characteristics in PDAC and CRC (35). This may be largely attributed to liver RFs, including HSCs (73), which have conserved transcriptional programs in different tumors and upon tumor progression (18). These persistent TMEs builders ultimately construct microenvironments that differ from the primary tumor in LMs.

Lineage-dependent heterogeneity of CRC pCAFs and LMs mCAFs is easily observable, however, in PDAC; PSCs and HSCs are known to be homologous and the primary tumors are characterized by abundant desmoplasia, constituting up to 90% of the total tumor volume (58), exceeding the proportion of stroma in LMs (74). One explanation for this is the presence of other stroma-producing cells in the pancreas (32). Helms *et al* (75). conducted fluorescence tracing on PSCs and found that they only produced a small portion of myCAFs and PSC ablation still generated a high abundance of α-S-adenosylmethionine (SAM) + CAFs, suggesting that PSCs are not the only source of myCAFs They speculated that other myCAFs in PDAC may originate from other RFs or the bone marrow (75). Through lineage tracing, Garcia *et al* (76) found that resident Gli1+ fibroblasts, which are distinct from PSCs in healthy pancreas, may a source of myCAF populations, providing clues for this hypothesis.

Main sources of pCAFs and mCAFs in lung cancer. The lung cancer pCAFs mainly originate from lung RFs and MSCs. Enhancing the expression of hypoxia inducible factor-1 in fibroblasts via hypoxia induced the conversion of normal fibroblasts into CAFs (77). Research indicates that fibroblasts activated by lung cancer cell conditioned media lead to an increase in interleukin (IL)-6 production, inducing epithelial-mesenchymal transition and cisplatin resistance in non-small cell lung cancer (NSCLC) cells (78). Treatment with NSCLC-derived factors induce a CAF phenotype in both normal lung resident MSCs and lung cancer-associated MSCs (79). One of the most common sites of metastasis in lung cancer is intrapulmonary metastasis to the ipsilateral or contralateral lung (80). A study showed that CAF extracellular vesicles (EVs) can activate lung fibroblasts and induce the formation of pre-metastatic niches in the lungs (81). Another study shows that the metastatic cells can bring stromal components from the primary site to the lungs (82).

Main sources of pCAFs and mCAFs in BC. BC CAFs have a wide range of sources, primarily including RFs, BM-fibroblasts, MSCs (including BM-MSCs) and adipocytes (83,84). RFs and BM-MSCs are two more important sources of CAFs in BC. RNA-seq of pCAFs extracted from a mouse orthotopic transplantation model showed that podoplanin+ CAFs had a transcription pattern similar to normal mammary fat pad fibroblasts, while fibroblast-specific protein 1 (FSP1) was not expressed in normal fibroblasts. Therefore, FPS1+ CAFs may have been recruited from the periphery (85). A previous study on adoptive bone marrow transplantation confirmed that BC recruits large numbers of MSCs that do not express platelet-derived growth factor (PDGFRα) from the bone marrow into the primary tumor and lung metastases and found that CAFs derived from resident CAFs and BM-MSCs had different abilities in inducing angiogenesis and recruiting macrophages (41).

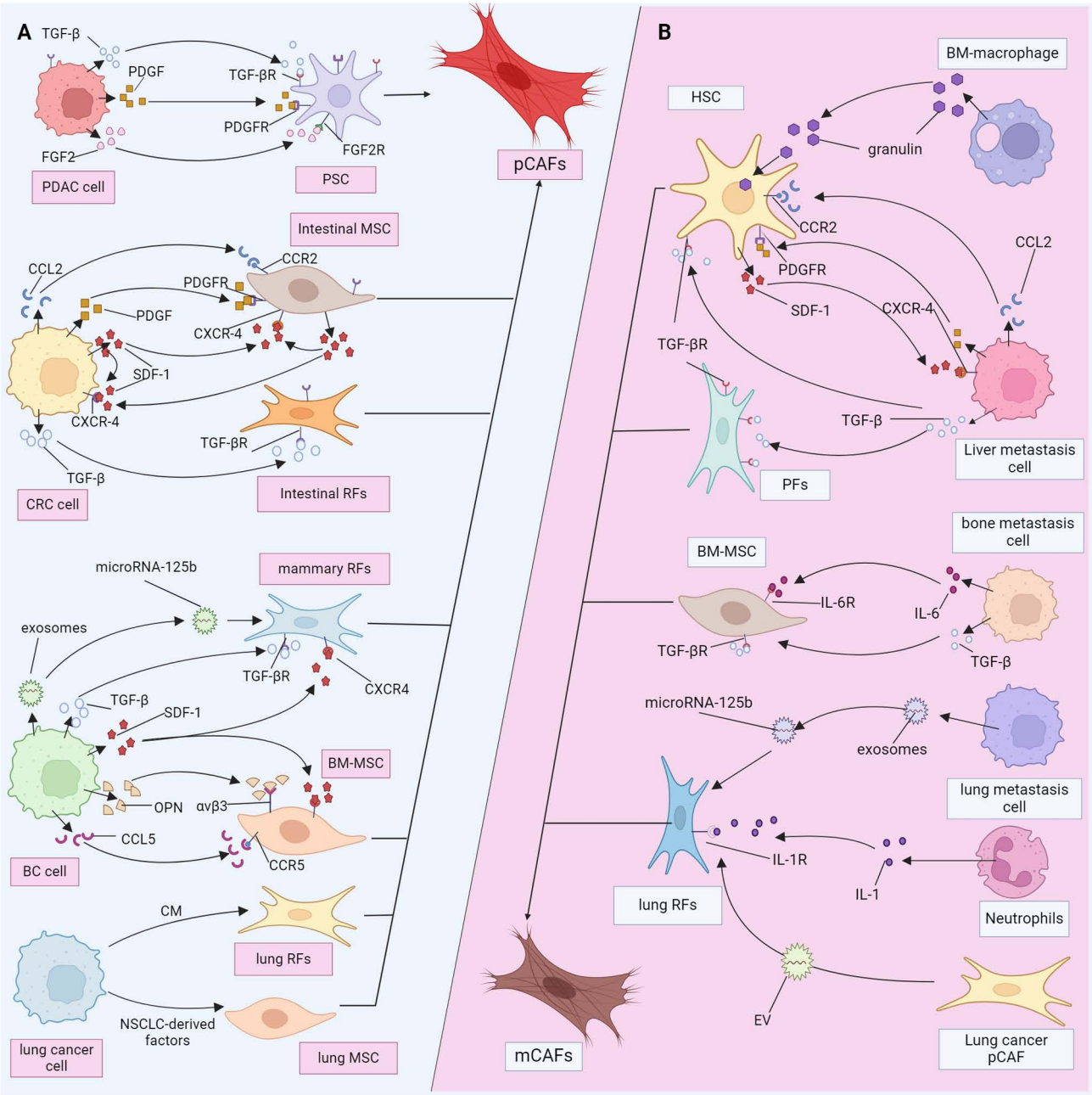

Figure 2. mCAFs and pCAFs are from different sources. (A) PDAC cells can activate PSCs into pCAFs by secreting TGF-β, PDGF and FGF2. CRC cells secrete TGF-β to activate intestinal RFs into pCAFs and simultaneously secrete CCL2 and PDGF to activate intestinal MSC and a SDF-1/CXCR-4 secretory loop is formed between CRC cells and pCAFs, which not only activates pCAFs but also promotes tumor progression. Mammary resident fibroblasts can be activated by TGF-β, SDF-1 and microRNAs secreted by BC cells. SDF-1 can activate BM-MSCs and BM-MSCs are also activated by OPN and CCL5. Conditional culture medium of lung cancer can activate lung resident fibroblasts and NSCLC-derived factors can activate lung MSC into CAF. (B) HSCs can be activated by macrophage-secreted granulin, as well as by HSC-secreted CCL2, PDGF and TGF-β; at the same time, HSCs also release SDF-1 to promote the secretion of TGF-β by LM cells. Bone metastasis cells secrete IL-6 and TGF-β to induce BM-MSCs into mCAFs. microRNAs from exosomes of lung metastatic tumor cells and IL-1 secreted by neutrophils can activate lung resident fibroblasts. Lung cancer pCAF-derived EVs can activate lung resident fibroblasts within the niche. CAFs, cancer-associated fibroblasts; mCAFs, metastatic CAFs; pCAFs, primary CAFs; PDAC, pancreatic cancer; PSCs, pancreatic stellate cells; TGF-β, transforming growth factor-β; PFs, portal fibroblasts; PDGF, platelet-derived growth factor; MSC, mesenchymal stem cells; SDF-1, stromal cell-derived factor 1; CXCR, C-X-C chemokine receptor; CRC, colorectal cancer; ; BC, breast cancer; BM-MSC, bone marrow-derived MSC; OPN, osteopontin; CCL, chemokine (c-c motif) ligand; NSCLC, non-small cell lung cancer; HSCs, hepatic stellate cells; IL, interleukin; EVs, extracellular vesicles; LM, liver metastasis; RFs, resident fibroblasts; TGF-βR, TGF-β receptor; BM-macrophages, bone marrow-derived macrophages; FGF, fibroblast growth factors; FGFR, FGF receptor; CCR, C-C chemokine receptor; IL-R, interleukin receptors.

BC bone metastasis mCAFs are mainly derived from BM-MSCs and primary tumor pCAFs. Specifically, pCAFs from triple-negative BC (TNBC) produce stromal cell-derived factor 1 (SDF-1) and IGF-1 to induce bone metastasis of cancer cells with high Src activity (86), where pCAFs are transferred to bone marrow together with BC cells under the mediation of osteopontin (OPN) (87). Tumor cells continue to evolve after metastasis, but they no longer depend on the primary tumor. They maintain a state of reduced information exchange due to physical and chemical barriers, especially in organs such

as the brain and bone (88). The influence of the bone marrow microenvironment on bone metastasis is undoubtedly great. BM-MCSs, as the most important stromal cells in the bone marrow, differentiate into different cell subsets such as osteoblasts, adipocytes, fibroblasts and pericytes (89), playing a key role in tumor cell homing, bone marrow colonization and tumor cell dormancy (90). The deleting of Rictor gene reduces the secretion of IL-6, receptor activator of nuclear factor-kappa B ligand (RANKL) and TGF-β and inhibits the transition from BM-MCSs to CAFs, resulting in lower chemotaxis and less proliferation in TM40D cells (42).

CAFs in BC lung metastases are mainly derived from lung RFs and BM-MSCs. One study found that the expression patterns of specific genes in the lung CAFs of mice with transgenic BC dynamically changed at different metastatic stages (91). Houthuijzen *et al* (71) reported that a RF population only found in the lungs was transformed into CAFs, promoting the lung metastasis of BC cells. Raz *et al* (41) used β-actin-GFP-PyMT double transgenic mice generated via adoptive bone marrow transplantation as donors, transplanted their bone marrow into non-transgenic control mice and found that GFP-labeled CAFs were specifically recruited in the primary BC tumors and lung metastases.

Lymph nodes are transit stations for tumor metastasis, but there have been few reports on the source of CAFs during lymph node metastasis (Met-LNs). Immunohistochemistry (IHC) assays showed that pCAFs in BC has similar biomarkers with mCAFs in matching Met-LNs (92) and only small differences were found in their transcription profiles (93,94). Such results suggest that Met-LN mCAFs were derived directly from pCAFs.

The experiments of Helms *et al* (75) revealed that different CAF precursor cells can differentiate into CAF cell lines of the same functional type under the action of multiple activation pathways. Notably, Han *et al* (95) had the opposite view, reporting that Tomato-labeled ISL1+ mesenchymal cells in mouse models of PDAC gradually expanded as the tumor progressed and eventually developed into a vast majority of CAFs. They hypothesized that CAFs in the PDAC stroma are more likely to have a single origin and their diversity comes from acquired stimulation from their complex microenvironment (95). This may pose a great challenge to the lineage-dependent heterogeneity of CAFs. Nonetheless, metastatic heterogeneity can still be explained by organ-specific microenvironments.

Differential activation patterns of pCAFs and mCAFs. CAFs exist in the TEMs as quiescent precursor cells and are abnormally activated to serve tumor proliferation, migration and drug resistance (Table II). In addition to lineage-dependent heterogeneity, the heterogeneity between pCAFs and mCAFs is also reflected in their mode of activation. First, fibroblasts in metastatic sites can be activated differently from CAFs in primary tumors (18). For example, macrophages promote the activation of HSCs into αSMA+ myCAFs which secrete high levels of periosteal proteins in the LMs of PDAC by producing granulin (96). CRC exosomal HSPC111 regulates the lipid metabolism of HSCs and promotes the formation of pre-metastatic liver niches (97). Furthermore, even identical CAF subtypes may exhibit different activation levels in primary and secondary sites. For example, the liver colonization of tumors is extremely dependent on TGF-β signaling in the liver stroma (98), which is compatible with the high ECM stress of the liver. This is because TGF-β is present in the ECM and binds to latent TGF binding proteins; its activation and release require mechanical forces acting on integrin-specific domains (99). TGF-β released under the high ECM stress in LMs participates in ECM remodeling and EMT through classical and non-classical pathways and plays a decisive role in cancer cell migration and invasion (100,101). The positive feedback loop of TGF-β secretion is established by TGF-β-activated CAFs through the SDF-1/C-X-C chemokine receptor 4 (CXCR4) signaling pathway (69,102). Some studies have shown that LMs express more CXCR4 than the primary tumor (103,104), which confirms the differential activation of pCAFs and mCAFs.

Notably, while the differences in activation modes between primary and metastatic CAFs are constantly being discovered, different CAF activation methods within the same site are also found in the complex microenvironment. In PDAC, IL-1 induces leukemia inhibitory factor expression and activates the downstream JAK/STAT pathway to transform PSCs into iCAFs, while TGF-β antagonizes this process by downregulating IL-1R1 expression and promoting myCAF generation (105). This indicates that there may be mutual antagonistic between different activation modes for CAFs, which may be the reason for the differential spatial distribution of these CAF subtypes in PDAC. The ability to dynamically change with different environmental stimuli reflects the extremely strong plasticity of CAFs, which may be one of the reasons why the source of CAFs so far remains elusive (106).

Differential expression levels of biomarkers in pCAFs and mCAFs. In most cases, CAF markers used are not considered to be representative of their functional heterogeneity and this is because CAFs lack unique markers, as most of them are also expressed in other cells (107). The classification of CAFs with known markers only compensates for the current lack of understanding on this topic, although combinatorial labeling improves the sorting accuracy of CAFs in complex environments (20). It should be noted that the classification of functional CAF subsets using markers is not as clear as that of immune cells. Additionally, due to the presence of different functional subtypes of CAFs, a single marker is not sufficient to distinguish them effectively. When attempting to generalize the prognostic value of the complete CAF population without distinguishing the effects of the heterogeneous CAF subgroups, contradictory results may be obtained (106). Nevertheless, a number of studies have investigated the prognostic value of commonly used CAF biomarkers in various types of cancer (108). Similarly, the differential expression of common markers in mCAFs and pCAFs has also been reported (8,109,110).

Different in fibroblast activation protein (FAP). FAP is a type II transmembrane serine protease with both dipeptidyl peptidase and endopeptidase activity. It is expressed in ~90% of CAFs and is a hallmark of CAF activation (111). Studies have shown that FAP+ CAFs promote tumor progression, ECM degradation, tumor invasion, angiogenesis and immune suppression (112,113). Brain metastases from multiple types of

Table II. Different activation methods of CAFs.

First author/s, year	Activation method	Cancer type	Precursor cells	Secretory factors and pathways	Function	(Refs.)
Tan *et al*, 2020	TGF-β/Smad	CRC	HSC	SDF-1α/CXCR4/TGF-β1	Promotes liver metastasis	(69)
Tang *et al*, 2020		Ovarian cancer	ADSCs	MMP2, MMP9	Promotes tumor proliferation and progression, promote omentum metastasis	(212)
Quante *et al*, 2011		Gastric cancer	BM-MSCs	SDF-1α/CXCR4	Promotes tumor proliferation	(25)
Kojima *et al*, 2010		BC	NFs	SDF-1/CXCR4/TGF-β	Promotes tumor proliferation	(102)
Ringuette Goulet *et al*, 2018		Prostate cancer, Bladder cancer, CRC, BC	NFs	SDF-1/CXCR4	Promotes tumor proliferation	(227)
Erez *et al*, 2010	IL-1/NF-κB	Skin carcinoma	NFs	COX-2, IL-1b, IL-6, CXCL1, CXCL2,	Generates inflammatory fibroblasts to promote tumor proliferation, vascularization and macrophage recruitment	(46)
Zhang *et al*, 2023		Diffuse-type gastric cancer	NFs	IL-6, IL-24, LIF, GM-CSF	Promotes the malignant phenotype of tumors and maintain the M1/M2 macrophage ratio	(228)
Wei *et al*, 2019		Oral cancer	NFs	CXCL1/CXCR2, MMP-1	Promotes tumor invasion	(229)
Goulet *et al*, 2019	miRNA	Bladder cancer	NFs	IL-6	Induces tumor cell EMT	(230)
Scognamiglio *et al*, 2022		BC	NFs	MMPs, ITGs, FAK phosphorylation	Promotes collagen production, CAF contraction and tumor cell migration	(231)
Qin *et al*, 2021		CCA	NFs	miR-34c/Wnt1	Promotes tumor proliferation and migration	(232)
Zhou *et al*, 2018		Melanoma	NFs	miR-155/SOCS1/ JAK2/STAT3/VEGFα, FGF2, MMP9	Promotes angiogenesis	(233)
Ye *et al*, 2023	Hypoxia	Neck squamous cell carcinoma	NFs	miR-21/YOD1	Promotes tumor lymph node metastasis	(234)
Xu *et al*, 2021		CRC	NFs	IL-6/STAT3/HIF-1a/PKM2	Promotes tumor growth	(235)
Zhang *et al*, 2021		Lung cancer	NFs	HIF-1a/NF-κB/CCL5	Promotes tumor growth	(77)
Butti *et al*, 2021	OPN/CD44, αvβ3	BC	NFs	Twist1/SDF-1/EMT	Promotes tumor proliferation and migration	(236)
Sharon *et al*, 2015		BC	NFs	COX-2,CXCL2, CXCL1, IL6	Promotes tumor proliferation and inflammation	(47)
Calvo *et al*, 2013	Mechanical force	BC	NFs	YAP/TAZ	Promotes matrix sclerosis, tumor invasion and angiogenesis	(237)
Foster *et al*, 2017		BC	NFs	MRF/SRF, TGFβ/ YAP/TEAD	Regulates CAF contraction positive feedback loop and invasiveness	(238)
Cadamuro *et al*, 2013	PDGF/PDGFR	CCA	NFs	Rac1, Cdc42 Rho GTPase, JNK	Promotes tumor migration	(239)
Pietras *et al*, 2008		Cervical carcinoma	NFs	FGF-2, FGF-7	Promotes tumor proliferation and angiogenesis	(240)

Table II. Continued.

First author/s, year	Activation method	Cancer type	Precursor cells	Secretory factors and pathways	Function	(Refs.)
Scherz-Shouval *et al*, 2014	HSF1	BC	NFs	TGFβ, SDF-1	Promotes tumor growth	(241)
Ferrari *et al*, 2019		BC, CRC and Ovarian cancer	NFs	DKK3/β-catenin, YAP/TAZ	Promotes ECM remodeling, tumor proliferation and invasion	(242)
Guo *et al*, 2023	Radiotherapy	BC	Resident CAFs	IL-6/STAT3	Promotes proliferation and radiation resistance	(243)
Shintani *et al*, 2016	Cisplatin	NSCLC	Resident CAFs	IL-6	Induces EMT and cisplatin resistance in NSCLC cells	(78)

CRC, colorectal cancer; BC, breast cancer; HSC, hepatic stellate cells; MSC, mesenchymal stem cells; AD-MSCs, adipose-derived MSCs; BM-MSCs, bone marrow-derived MSCs; NFs, Normal fibroblasts; RFs, resident fibroblasts; CCA, Cholangiocarcinoma; TGF-β, transforming growth factor-β; SDF-1α, stromal cell-derived factor 1; CCL, chemokine (c-c motif) ligand; CXCL, CXC chemokine ligand; CXCR, C-X-C chemokine receptor; MMP, matrix metalloproteinases; COX-2, cyclooxygenase-2; IL, interleukin; LIF, leukemia inhibitory factor; GM-CSF, colony stimulating factor 2; NF-kB, Nuclear Factor-κB; miRNA, messenger-RNA-interfering complementary RNA; OPN, osteopontin; PDGF, platelet-derived growth factor; PDGFR, PDGF receptor; HSF1, heat shock factor 1; FAK, Focal adhesion kinase; Wnt, wingless-type MMTV integration site family; SOCS1, cytokine signaling 1; JAK2, Janus kinase 2; STAT3, signal transducer and activator of transcription 3; VEGF, vascular endothelial growth factor; FGF2, fibroblast growth factors; PKM2, Pyruvate kinase M2; YAP, Yes-associated protein; TAZ, WW domain containing transcription regulator 1; Twist1, twist family bHLH transcription factor 1; EMT, epithelial-mesenchymal transition; MRF, myocardin-related transcription factor; SRF, serum response factor; TEAD, transcriptional enhanced associate domain; Rac1, RAS-related C3 Botulinum Toxin Substrate 1; JNK, c-Jun N-terminal kinase; DKK, Dickkopf-3.

primary tumors uniformly show high levels of FAP expression and no association of histological type (114), therefore, FAP may serve as a broad therapeutic target for brain metastasis.

As the most promising therapeutic target and radiotracer, FAP has become the focus of CAFs research in recent years (115,116). FAP inhibitors (FAPi) based on quinoline have been used as tracers for positron emission tomography and computerized tomography (PET-CT) diagnosis. FAPi can specifically bind to FAP and be internalized by CAFs. 68Ga, 177LU or 18F can chelate it and be fed back as image information through imaging systems for the diagnosis and identification of tumors (111). FAPi is characterized by high intratumoral uptake and rapid *in vivo* clearance. Various types of FAPi have since been developed and compared head-to-head with fluorodeoxyglucose (FDG) PET-CT, showing that FAPi tracers are more advantageous in discovering some primary, lymph node, or metastatic tumors (112,115,117). However, the sample sizes of these studies are very limited and there is still no convincing evidence of which tumor types or which metastatic organs are more appropriate to FAPi. However, it is important to improve the detection rate of early tumors and accurately determine the tumor stage to positively influence clinical decisions. Serfling *et al* (118) established a positive correlation and significance between FAP targeted expression and FAPi PET standardized uptake values (SUVs). In their study, 15 patients underwent 68Ga-FAPi-46 PET-CT scans to determine the biodistribution of 68Ga-FAPi-46 in various tissues (117). After subsequent surgical treatment, FAP expression was scored on the excised samples and the results showed a positive correlation between FAP IHC scores and the 68Ga-FAPi-46 SUVmax and SUVmean (119). In addition, Serfling *et al* (118) suggested that FAPα Met-LNs expression was correlated with lesion size. Sollini *et al* (120) reasoned that the relatively low performance of 68Ga-FAPi in detecting Met-LNs reported in some studies may be related to the low enrichment of CAFs within the lymph nodes. The SUVs of FAPi PET/CT reflects the expression of FAP in CAFs to a certain extent. This is an important means to compare marker heterogeneity between pCAFs and mCAFs. Data on the SUVs of primary tumors and metastatic tumors regarding FAPi PET-CT were collected and compared in order to find partial correlations. Disappointingly, research on FAPi tracers is still in its infancy. Moreover, factors such as their wide variety, insufficient sample size and the majority of studies being pan-cancer samples prevent the comparison of the data comprehensively. Although some meta-analyses have demonstrated the diagnostic advantages of FAPi tracers (120), this is not the focus of the present study. However, the present study still showed some results suggesting the possibility of differential expression of FAP between pCAFs and mCAFs (Table III).

Differences in α-SMA. α-SMA is a marker used to distinguish activated fibroblasts (95). Fibroblasts are activated to express α-SMA under inflammatory conditions, exhibit a hypercontractile phenotype and play a central role in wound healing (121). CAFs express high levels of α-SMA and are considered to be 'wounds that do not heal'. In tumors, α-SMA+ CAFs have been proven to be the main players in ECM modification, which is closely related to the integrin signaling cascade (9). Although previous studies have reported α-SMA+ CAFs secretion as a key regulator of cancer progression, therapeutic resistance and immunosuppression (83,122,123), the deletion of α-SMA-related genes and the targeted pharmacological inhibition can lead to a low reduction in survival and tumor differentiation and to an increase in angiogenesis and cachexia in mouse model of PDAC (18). Gui *et al* (8) found that BC metastases have a high expression of α-SMA. This is consistent with the results reported by Kwak *et al* (124), who found that patients with a high expression of CAF markers in their primary tumors also showed an tendency of higher of it in metastases, suggesting the possibility that mCAFs came from pCAFs. Notably, similar to the report of Serfling *et al* (118) on FAP, Itou *et al* (125) found that the distribution of α-SMA is also related to the size of LNs in Met-LN samples of intrahepatic cholangiocarcinoma, which is rare in micro-Met-LNs α-SMA, conversely, abundant α-SMA+ cells were found in macro-Met-LNs (118).

Differences in FPS1, PDPN and PDGFR. FPS1, PDPN and PDGFR are also commonly used as markers of CAFs (84). FSP1, also known as S100A4, is a common CAF marker. αFAP+ FSP1+ CAFs in human neuroblastoma were associated with M2-type macrophage, which enhances the proliferation and survival of neuroblastoma cells *in vitro* and stimulates implantation and growth of neuroblastoma tumor *in vivo* (126). FSP1+ fibroblasts accumulate around the carcinogen where they produce COLs, encapsulating carcinogen methylcholanthrene and protecting epithelial cells from DNA damage (127). Compared with FSP1, the expression of PDPN in various tumors is more consistent, as PDPN+ CAFs predict unfavorable prognosis in patients with various types of solid tumors, including stage IV lung cancer, bladder cancer and PDAC (128-130). PDGFRα/β+CAFs are able to induce polarization of M2 macrophages (29) and a PDGFR inhibitor called crenolanib has been shown to inhibit the growth of lung cancer cells *in vivo* (131). Research on PDGFR as a possible target for cancer therapy continues.

Among 64 patients with lung squamous cell carcinoma, 47 had a high level of PDPN expression in the primary stroma but 27 patients had a high level of PDPN in Met-LNs; univariate analysis found that only high PDPN expression in Met-LNs was significantly associated with prognosis (132). Koo *et al* performed immunohistochemical staining on different metastatic sites of BC and observed that FSP1 expression was significantly elevated in bone metastases while it was significantly reduced in LMs. The expression of PDPN was significantly elevated in bone metastasis and PDGFR expression was elevated in bone and lung metastases, but significantly reduced in LMs (109). These results prove that markers are expressed in different proportions in different metastatic sites. In one study, TNBC 4T1 cells were injected in situ into the breast fat pad of immunocompetent BALB/c mice, at week 4, it was observed that the proportion of PDPN+ CAFs, which originally accounted for 70% of the total CAFs, was reduced to 23% in the primary tumor, while FSP1+ CAFs, which originally accounted for 30%, increased to 77%. Notably, two FSP1+ CAF subtypes that were not observed in the primary tumor appeared in lung metastases and were shown to express IL6 and CXC chemokine ligand 1 (CXCL1), respectively (85).

Table III. SUVs of different FAPi tracers in primary and metastatic tumors.

		Primary tumor		Metastatic tumors				
First author/s, year	Tracer type	Cancer type	Sample size	SUV_{mean}	Metastatic organ	Sample size	$SUV_{mean/max}$	(Refs.)
Koerber *et al*, 2020	68Ga-FAPI	Pan-carcinoma	6	7.7±4.0)	Local recurrence	2	2.8±1.4	(110)
					lymph node	27	4.2±2.3	
					Lung	19	3.3±1.5	
					Tissue	12	3.5±1.7	
					Liver	14	4.9±2.1	
Mona *et al*, 2022	68Ga-FAPI-46	Pan-carcinoma	15	7.2±4.4	Lymph node	1	8.8	(119)
					Lymph node	1	4.6	
					Lymph node	1	5.5	
					Lymph node	1	6.3	
					Liver	1	2.1	
					Liver	1	3.0	
					All the Metastases	6	4.3±2.9	
Çermik *et al*, 2022	68Ga-FAPI	Pan-carcinoma	35	/	Lymph node	17	10.7 (3.2, 23.1)	(244)
					Bone	12	9.5 (2.4, 45.3)	
					Liver	9	7.5 (5.2, 12.6)	
					Peritoneum	7	16.5 (7.5, 29.4)	
Pang *et al*, 2023	68Ga-FAP-2286	Pan-carcinoma	46	11.1 (2.5, 28.9)	Local recurrence	9	5.8 (2.9, 16.5)	(245)
					Lymph node	107	10.6 (3.0, 20.1)	
					Lung	21	3.4 (0.6, 10.2)	
					Liver	27	6.9 (2.4, 12.2)	
					Peritoneum	86	6.7 (1.8, 27.0)	
					Subcutaneous	12	9.3 (6.3, 20.4)	
					Bone	38	6.6 (3.8, 13.3)	
Pang *et al*, 2023	68Ga-FAP-2286	Pan-carcinoma	13	13.6 (2.5, 25.8)	Local recurrence	4	11.2 (2.7, 14.4)	(245)
					Lymph node	35	8.3 (3.4, 15.6)	
					Lung	2	4.0 (3.8, 4.2)	
					Liver	6	4.6 (2.7, 7.2)	
					Peritoneum	10	8.1 (7.4, 10.3)	
					Subcutaneous	22	9.8 (6, 15.4)	
					Bone	10	6.9 (3.9, 12.2)	
	FAPI-46			13.3 (2.4, 21.8)	Local recurrence	4	9.6 (2.9, 13.6)	
					lymph node	35	8.2 (4.0, 15.4)	
					Lung	2	3.9 (3.6, 4.2)	

Table III. Continued.

First author/s, year	Tracer type	Primary tumor		Metastatic tumors				(Refs.)
		Cancer type	Sample size	SUV_{mean}	Metastatic organ	Sample size	$SUV_{mean/max}$	
					Liver	6	4.4 (2.9, 8.5)	
					Peritoneum	10	6.0 (3.6, 8.6)	
					Subcutaneous	22	11.4 (7.4, 19.2)	
					Bone	10	5.8 (2.9, 11.4)	

SUVs, standardized uptake values; FAPI, fibroblast activation protein inhibitor; FDG, fluorodeoxyglucose; SUV, standard uptake value.

The heterogeneity of CAFs from the perspective of scRNA-seq CAFs subtypes distinguished by transcriptional characteristics. Recent advances in scRNA-seq have allowed for a comprehensive profiling of the complexity and heterogeneity within the CAF subpopulations across various tumor entities (Table IV). Some reviews have examined the organ-specific features of CAFs and summarized the transcriptomic information of CAFs in different organs with ECM-remodeling, inflammation and immunity and antigen presentation (133,134). However, CAF subtypes do not exhibit universality across different tumor types, even though the classical myCAF and iCAF subtypes have been described in PDAC, BC and cervical cancer (59,135,136). scRNA-seq technology has the drawback of losing spatial information, resulting in incomplete information on the correlation between different CAF subtypes and the anatomical location of tumors. At the same time, the loss of temporal information makes it difficult to present the evolutionary trajectory of CAF subtypes. The integration of scRNA-seq and microarray-based spatial transcriptomics methods and pseudotime inference might be able to partially solve these issues (137,138). It is hypothesized that a more comprehensive understanding of the heterogeneity of CAFs will provide innovative solutions for cancer treatment and enable clinical applications.

Heterogeneity in the transcriptional features between pCAFs and mCAFs. The differences in transcriptional levels between mCAFs and pCAFs are being described as RNA-seq technology matures (23,139). The transcription profiles of MSCs obtained from bone metastases in BC are significantly different from those of CAFs obtained from the primary site (93). RNA-Seq for BC primary tumors and paired brain metastases yielded 48 differential gene expression signatures, most of which were those of immunity and fibroblasts (139). Similarly, another study noted the differential upregulation of genes associated with ECM remodeling and BM-derived cell recruitment in lung mCAFs compared with BC pCAFs (41). Within PDAC samples, Liu *et al* (140) found large changes in fibroblast subclasses at succeeding stages of PDAC progression, with the emergence of specific subclasses when cancer trespasses stroma to metastasize to proximal lymph nodes (stage IIA to IIB) and gene expression analysis showed increased expression of cytoskeletal protein and inflammatory cytokines when transition to IIB, indicating that tumor growth and metastasis are strictly regulated by genes.

Heterogeneity in microRNA profiles between pCAFs and mCAFs. MicroRNAs (miRNAs or miRs) are non-coding RNAs which can disrupt mRNA expression (141). Tumor cells can deliver miRNAs to CAFs through exosomes, promoting the malignant phenotype of CAFs (142). As expected, there are also differences between the miRNA profiles of pCAFs and mCAFs. Upon exposure to estrogen, the number of miRNAs upregulated or downregulated in skin mCAFs in BC is three times that in pCAFs (143), but the biological effects of these differentially expressed miRNAs need further verification. In advanced CRC, the differential expression of miR-21 between the center of the primary tumor and distant metastases is common (144). There were five upregulated and six downregulated miRNAs in the exosomes of mCAFs with peritoneal metastasis in ovarian cancer, among which

Table IV. Transcriptomic heterogeneity of CAFs across different cancer types.

First author/s, year	Organ	Organism	Subtypes	Signature/Markers	Central features	(Refs.)
Elyada *et al*, 2019	Pancreas	Human PDAC	myCAF	αSMA	ECM components, Contractility factors	(59)
			iCAF	αSMA, IL6, SDF-1	Inflammation	
			apCAF	Saa3	Antigen presentation	
Hosein *et al*, 2019		PDAC GEMMs	FB1	IL6, SDF-1, CCL2	Inflammation	(246)
			FB2	Ly6a, Ly6c1, Nov, Pi16	Physiological functions of fibroblasts	
			FB3	Acta2, Tagln	Contractility factor, Antigen presentation	
Neuzillet *et al*, 2019		Human PDAC	Subtype A	POSTN	Inflammation, ECM components	(247)
			Subtype B	MYH11	ECM components	
			Subtype C	PDPN	Inflammation	
			Subtype D	-	ECM components	
Lin *et al*, 2020		Human PDAC	Cluster 0	POSTN, MMP11	ECM components	(248)
			Cluster 1	-	Quiescent (or normal) CAFs	
			Cluster 2	RGS5, NOTCH3, CSRP2	Resembles smooth muscle cells	
Sebastian *et al*, 2020	Breast	BALB/c-derived 4T1 mammary tumors	Cluster 0	Ly6c1	ECM components, Immune responses	(249)
			Cluster 1	α-SMA	Cell proliferation, Migration, Invasion, Adhesion, EMT, Cancer stem cell activation	
			Cluster 2	Cdk1, Cenpa, Cenpf	Dividing cells	
			Cluster 3	Cd53	Cell migration, Angiogenesis, Apoptosis, Proliferation, EMT	
			Cluster 4	Crabp1	ECM components	
			Cluster 5	Cd74	Antigen presentation	
Friedman *et al*, 2020		TNBC syngeneic mouse model	PDPN+ CAF	PDPN	Immune regulation, wound-healing, cell migration, inflammatory, fiber organization	(85)
			S100A4+ CAF	S100A4	Antigen presentation, ECM remodeling, Protein-folding, metabolic	
Bartoschek *et al*, 2018		MMTV-PyMT mouse model	Vascular CAF	Notch3, Epas1,Col18a1, Nr2f2	Vascular development, Angiogenesis	(23)
			Matrix CAF	Dcn, Lum, Vcan, Col14a1, Fbln1, Fbln2, Smoc, Lox, Loxl1	ECM components and EMT	
			Cycling CAF	-	Cell cycle	
			Develop-mental CAF	Scrg1, Sox9, Sox10	Development, Morphogenesis of tissues	

Table IV. Continued.

First author/s, year	Organ	Organism	Subtypes	Signature/Markers	Central features	(Refs.)
Li *et al*, 2022	Gastric	Human gastric cancer	Inflammatory CAF	IL-6, SDF-1	Interaction with T cells	(136)
			extracellular matrix CAF	POSTN	Correlation with M2 macrophages	
Li *et al*, 2022	Cervical	Human cervical cancer	iCAF	IL6, IL8, CXCL1, SDF-1, CCL2,	Immune-related biological processes	(135)
			myCAF	αSMA	-	
Li *et al*, 2017	Colorectal	Human CRC	CAF-A	MMP2, DCN, COL1A2	ECM remodeling	(250)
			CAF-B	ACTA2, TAGLN, PDGFA	Cytoskeletal	
Peng *et al*, 2022		Human CRC	Myocancer CAF	COL1A2, COL1A1, SPARC, COL3A1, RGS5	ECM components, Focal adhesion, proteoglycans	(251)
			Inflammatory CAF	PDGFRA, RGS5 ACTA2 mCAFs, SDF1	EMT, Cholesterol homeostasis, Bile acid metabolism, Fatty acid metabolism	
Lambrechts *et al*, 2018	Lung	Human NSCLC	Cluster 1	COL10A1, TGF-β, FOXO1, HOXB2,	EMT, hypoxia, fission	(252)
			Cluster 2	COL4A1, ACTA2	Myogenesis, NOTCH pathway, angiogenesis	
			Cluster 5		Glycolysis, mTOR	
			Cluster 6	-	Nonmalignant tumors	
			Cluster 7		Glycolysis, mTOR	
Hornburg *et al*, 2021	Ovarian	Human Ovarian cancer	TGF-β CAF	TGF-β, POSTN, ACTA2, COL11A1, COL10A1, FN1, MMP11, TAGLN, COMP	ECM components	(253)
			IL1 CAF	CXCL14, CCL2, SOCS3	Inflammation	
Izar *et al*, 2020		Human HGSOC		Complement factors, CFB, CXCL, IL6, IL10		(254)
Zhang *et al*, 2020	Liver	Human ICC	vascular CAF	CD146, MYH11, GJA4, RGS5, IL-6, CCL8	Transcriptomic signature	(255)
			matrix CAF	COL5A1, COL5A2, DCN, COL6A3, POSTN, FN1, LUM, VCAN	ECM components	

Table IV. Continued.

First author/s, year	Organ	Organism	Subtypes	Signature/Markers	Central features	(Refs.)
			Inflammatory CAF	FBLN1, IGFI, CXCL1, IGFBP6, SLPI, SAA1, Complement factors	Inflammation	
			Antigen-presenting CAF	CD74, HLADRA, HLA-DRB1	Antigen presentation	
			EMT-like CAF	KRT19, KRT8, SAA1	EMT	
Davidson *et al*, 2020	Skin	Melanoma mouse model	Stromal 1	PDPN, PDGFRA, CD34	Immune responses	(256)
			Stromal 2	PDPN, PDGFRA	ECM components	
			Stromal 3	Acta2	Cytoskeleton	
Chen *et al*, 2020	Bladder	Human Bladder carcinoma	Inflammatory CAF	PDGFRA, SDF1, IL6, CXCL14, CXCL1, CXCL2	ECM degradation, Migration, Angiogenesis	(257)
			Matrix CAF	PGC1A, RGS5	Muscle system, focal adhesion, ECM associated pathways	
Del Valle *et al*, 2014	Head and neck	Human oral cavity cancer	CAF1	ACTA2, MYLK, MYL9	Wound healing, contracture	(93)
			CAF 2	FAP, PDPN, CTGF	ECM components	
Kurten *et al*, 2021		Human HNSCC	innovative elastic CAF	ELN, FBLN1, MFAP4	Secretion, adhesion, cell proliferation	(258)
			Classic CAF	FAP, PDGFRA, LOX, MMP	ECM components	

CAFs, cancer-associated fibroblasts; iCAFs, inflammatory fibroblasts; myCAFs, myofibroblastic CAFs; IL, interleukin; SDF-1, stromal cell-derived factor 1; Saa3, serum amyloid A3; EMT, epithelial-mesenchymal transition; ECM, extracellular matrix; GEMMs, genetically engineered mouse models; POSTN, periostin; MYH11, myosin-11; PDPN, podoplanin; Ly6c1, lymphocyte antigen 6 complex locus C1; Cdk1, cyclin-dependent kinase 1; Crabp1, cellular retinoic acid-binding protein 1; POSTN, periostin; CCL2, chemokine (c-c motif) ligand 2; CRC, colorectal cancer; MMP, matrix metalloproteinases; COL, collagen; ACTA2, smooth muscle alpha-actin 2; TAGLN, transgelin; PDGFA, platelet-derived growth factor A; FN, fibronectin; SOCS3, cytokine signaling 3; ICC, Intrahepatic cholangiocarcinoma; IGFBP7, insulin-like growth factor binding protein 7; COMP, cartilage oligomeric matrix protein; HGSOC, high-grade serous ovarian cancer; CTGF, connective tissue growth factor; HNSCC, head and neck squamous cell carcinoma; ELN, tropoelastin; FBLN1, fibrillin1; MFAP4, Microfibril Associated Protein 4.

miR-29c-3p downregulation was the most significant and positively correlated with patient overall survival (OS) (145).

Different secretomes of pCAFs and mCAFs. CAFs continuously release soluble molecules into the ECM to provide information feedback and functionally regulate the microenvironment (18,20). The biological characteristics of primary tumors and metastases are inseparable from cytokines secreted by pCAFs and mCAFs. CXCR4 is a G-protein-coupled receptor, which is little expressed if at all in normal cells, but dysregulated and aberrant in a number of tumors (146). The best characterized ligand that binds and activates CXCR4 is stromal SDF-1 (147). SDF-1/CXCR4 signaling has serious consequences on cancer cell differentiation, proliferation, invasion, metastasis and angiogenesis (148). Several studies have found that high expression of SDF-1/CXCR4 signaling is associated with high density of CAFs in tumor stroma (103,149). Tan *et al* (69) observed that there were more CXCR4+ cells at the LMs tissues Compared with the CRC primary sites. Maintenance of the SDF-1 gradient by the BC primary tumor is independently controlled by both miR-126 and miR-126*, which show a significantly lower expression in metastatic tissue compared with primary tumor tissue (150). Dai *et al* (149) detected a higher CAFs density in metastatic lesions than those in primary tumor site from human ovarian cancer tissues, however, no significant difference of SDF-1α production from CAFs was found between primary and metastatic lesions. This may require further validation of CXCR4 expression and regulation through methylation and acetylation (146). CAFs secrete leucine-rich α-2-glycoprotein 1 (LRG1) through the IL-6/Janus tyrosine kinase 2/signal transducer and activator of transcription 3 (IL-6/JAK2/STAT3) pathway (151). The expression of LRG1 is significantly upregulated in CRC LMs, making LMs more aggressive (151). In another study, increased aggressiveness was also found to be associated with high phosphoribosyltransferase expression in LMs (152). The secretome of mCAFs in peritoneal metastases of CRC mainly comprises insulin-like growth factor binding protein 2, CXCL2 and SDF-1, while pCAFs secrete higher levels of matrix metalloproteinases (MMP), chemokine (c-c motif) ligand 8 (CCL8) and CCL11 (153). Proteomic analysis showed that, compared with primary ovarian cancer tumors, 62 proteins in omentum metastases were significantly up- or downregulated, among which the expression of N-methyltransferase (NNMT) was significantly altered (104). NNMT transfers an active methyl group from SAM to nicotinamide to produce S-adenosylhomocysteine, the loss of which leads to decreased histone methylation, which affects gene expression (104). In another experiment, the difference between ovarian cancer primary tumors and metastases was also validated, where mCAFs were hypothesized to express higher levels of Jagged1 and cause peritoneal metastases to produce more vascular endothelial growth factor (VEGFA) and cyclin-dependent kinase inhibitor p21 (CDKN1A) (154). The methylation of metabolic genes NQO1 and ALDH1a3 induced in LMs downregulate the mRNA expression of metabolic genes in CAFs, however, compared with normal lung fibroblasts, the gene methylation levels of NQO-1 and ALDH1a3 in fibroblasts isolated from lung metastases remained at baseline levels (155).

Heterogeneity in the matreotype of pCAFs and mCAFs. In a cross-comparison analysis conducted via RNA-seq, the differential expression of ECM-related genes is the main feature of transcriptome heterogeneity in inter-organ fibroblasts (156). Different types of COL and glycoproteins crosslink with each other to form a stable ECM network. The post-translational modification of matrix components in various organs via hydroxylation, glycosylation, transglutamination, sulfation, crosslinking, cleavage and degradation further modulates these features (14), such changes happen dynamically on a time scale from seconds to minutes. In this way, the various matrix components expressed through time and space are called the matreotype of the tissue (157). In the PDAC mouse model, after the fibrogenic gene Sonic hedgehog was deleted, the tumor stroma was significantly reduced, but the tumor acquired enhanced angiogenesis and invasive capabilities (158). This suggests that in early stages of tumorigenesis, fibroblastic reactions orchestrated by CAFs within the TMEs envelop the tumor cells, inhibiting their growth and spread. However, as tumor stromal components continue to evolve during the course of tumorigenesis, the further modification of the behavior of CAFs helps their tumor-promoting properties (159). Notably, the tumor-promoting and tumor-restraining abilities of CAF at different stages of tumor progression can both be induced by TGF-β (9). In the early stages of tumors, TGF-β primarily promotes fibroblast proliferation to inhibit metastasis and as CAFs evolve, TGF-β can also induce CAFs to promote metastatic events such as EMT (160).

CAFs modify ECM differently in different metastatic organs (161), For example, pyruvate has previously been shown to promote hydroxylation in the ECM by enhancing the activity of the enzyme COL prolyl-4-hydroxylase, promoting the stability of the matrix in lung metastases of BC (162). Peptidylarginine deiminase 4 (PAD4) can modify arginine residues into citrulline in the presence of Ca^{2+} and proteomic analysis shows that PAD4 is more abundant in the LMs matrix, enhancing the colonization of CRC cells in the liver (163). In addition, tumor ECM drives the positive feedback of matrix deposition and hardening through TGF-β-mediated COL enrichment, lysine oxidase (LOX)-mediated COL hyper-crosslinking and the CAFs contraction-induced activation of the Yes-associated protein/WW domain containing transcription regulator 1 (YAP/TAZ) and myocardin-related transcription factor A (MRTF-A) pathways to transform the compliance, stiffness, porosity, viscoelasticity and biochemical properties of the ECM (14,164,165). Shen *et al* (12) used atomic force microscopy and observed that the tissue hardness of LMs was higher than that of CRC primary lesions. Large RNA-seq data show that CAFs were more abundant in primary CRC tumors than in LMs, but contractile cluster 3 was only expressed in LMs (166). Furthermore, some independent laboratories have obtained significantly different results in the determination of hyaluronic acid (HA) content in PDAC primary tumors and metastases (167,168).

However, several experiments by Ueno *et al* (169) and Ao *et al* (170,171) revealed similarities in the desmoplastic reaction of CRC primary tumors, LMs and Met-LNs and they are equivalent in prognostic evaluation. These studies yielded different results, possibly due to differences in the sample size, staining methods and the selection of different

metastatic sites. A technique called in situ tissue decellularization of tissues may help eliminate systematic errors and more objectively evaluate the ECM in various biological contexts. This method preserves the structural ECM of tissues while efficiently removing cells, preventing tissue collapse and using natural tissue and organ vasculature (172). Furthermore, the authors verify that the ECM obtained using this technique does not differ from that of fresh tissue in terms of distribution and orientation, fiber gaps, fiber integrity and fiber diameter (172). Using this technology, Mayorca-Guiliani *et al* (172) constructed a natural lung metastasis ECM structure map and reproduced the whole process of lung metastasis ECM remodeling in a mouse model.

The heterogeneity between pCAFs and mCAFs in the plasma membrane, cytoplasm, exosomes and the nucleus has been extensively characterized, deepening our understanding of the differences in the microenvironments of primary tumors and metastases. However, most of the data collected focused on the differences between NFs and CAFs or CAFs in metastatic and nonmetastatic primary tumors (85,173-175), which is conducive to highlighting metastasis-inducing factors. Pseudo-time RNA-seq analysis using matched normal tissue around the tumor, primary tumor and metastatic tumor to simulate pre-tumorigenesis, early-stage tumors and advanced-stage tumors, respectively, has shortcomings in effectively simulating the dynamic changes of CAFs during tumor evolution (18). However, it is an excellent model to describe the spatial heterogeneity of pCAFs and mCAFs after metastasis occurs, which helps to explore more about this in the future.

3. Differential therapeutic response mediated by pCAFs and mCAFs

Molecular mechanisms underlying the differential treatment outcomes. We have previously discussed the differences between pCAFs and mCAFs from multiple aspects of their biological characteristics and their abilities in shaping the microenvironment of primary and metastatic tumors. When facing treatment pressure, the microenvironment of metastatic tumors provides more efficient protection for the survival of tumor cells (8). mCAFs may make metastatic tumors relatively more drug-resistant through EMT or sustaining cancer stemness (Fig. 3B). Gui *et al* (8) isolated eight CAFs from normal tissues, primary tumors and multiple metastatic tumors of patients with BC Co-culture *in vitro* has shown that mCAFs can enhance the proliferation, migration and invasion of BC cell lines. The team further verified the resistance of mCAFs to treatment and their results showed that tumor cells co-cultured with mCAFs exhibited stronger doxorubicin resistance than pCAFs and observed that mCAFs induced tumor cells to undergo EMT and express more tumor stem cell markers (8), which may be one of the mechanisms underlying the stronger drug resistance in metastases (176,177). Comparison of the gene expression profiles of pCAFs and mCAFs revealed multiple significantly differentially expressed genes, of which IGF2 was the most significantly enriched (8). The overexpression of IGF2 was shown to play a key role in chemotherapy resistance (178). Mukherjee *et al* (154) discovered that when CAFs differentially overexpressing Jagged1 were co-cultured with ovarian cancer cells, the Notch3 signal increased with the increase in the expression level of Jagged1. Peritoneal metastasis mCAFs obtained from ascites have been shown to express higher levels of Jagged1 than the primary tumor (154). Further experiments show that CAFs affect the expression of VEGFA and CDKN1A through Jagged1/Notch3, increasing the proportion of tumor stem cells and resistance to cisplatin (154).

In addition, CAFs and immune cells are the main supporting cell populations in solid tumors and there is extensive functional interaction between them (7). Although pCAFs and mCAFs can crosstalk with immune cells in various ways, affecting their chemotaxis and polarization, and regulating the immune response in the TMEs (60,179), mCAFs may achieve protective effects against metastases through stronger immunosuppressive effects (Fig. 3C). A RF population that highly expresses cyclooxygenase-2 has been shown to exist only in the lung, where it promotes the lung metastasis of BC cells and inhibits the antigen presentation function of BM dendritic cells (71). CRC peritoneal metastasis mCAFs have a different secretome from pCAFs as the macrophages displayed expression profiles associated with T cell biology with a pronounced shift to a type 2 immune response and T cell tolerance (153). Similarly, peritoneal metastases mCAFs have been found in mouse models of gastric cancer to induce macrophage M2 polarization, resulting in low infiltration of CD8+ T cells (16).

Heterogeneity of matreotype affects the drug response. Most anti-tumor resistance studies focus on exploring the underlying molecular mechanisms. However, the limited distribution of drugs in tumors is often ignored. Drug penetration efficiency directly can affect drug efficacy and its influencing factors involve the ECM, vascular structure and hemodynamics (180). It is well known that the ECM forms a physical barrier that greatly impedes drug delivery (180). Some studies have described the heterogeneity in physical properties between the primary and metastatic stroma and the difference in the matreotype may be the key to the differences in the response of different metastatic organs to anti-tumor treatment (12,74,181) (Fig. 3A).

Studies have proven that stromal mechanical stress can guide the directed differentiation of naïve MSCs (182). For example, vascular progenitor cells with low and high stiffness differentiate into endothelial and smooth muscle cells, respectively, under the mediation of integrin (181). In another study, the effects of load initiation time, magnitude and mode of mechanical force on the formation of microvascular networks were also simulated (183). This means that primary tumors and metastases produce different the microvascular system in different matreotypes. For example, despite tumor vascularization, irregular vascular networks enhance blood fluid resistance in mouse models of LMs, leading to capillary collapse within metastases and limiting the tumor perfusion of drugs (13). Furthermore, stromal hyperplasia and a lack of blood supply have been shown to lead to a series of malignant events, such as hypoxia, in the deep tumor (182). Hypoxia has been previously found to be an important cause of treatment resistance (184).

In addition, the deposition of ECM traps immune cells in the tumor stroma and increases their resistance to infiltration into the tumor parenchyma (14). Gertych *et al* (15) observed

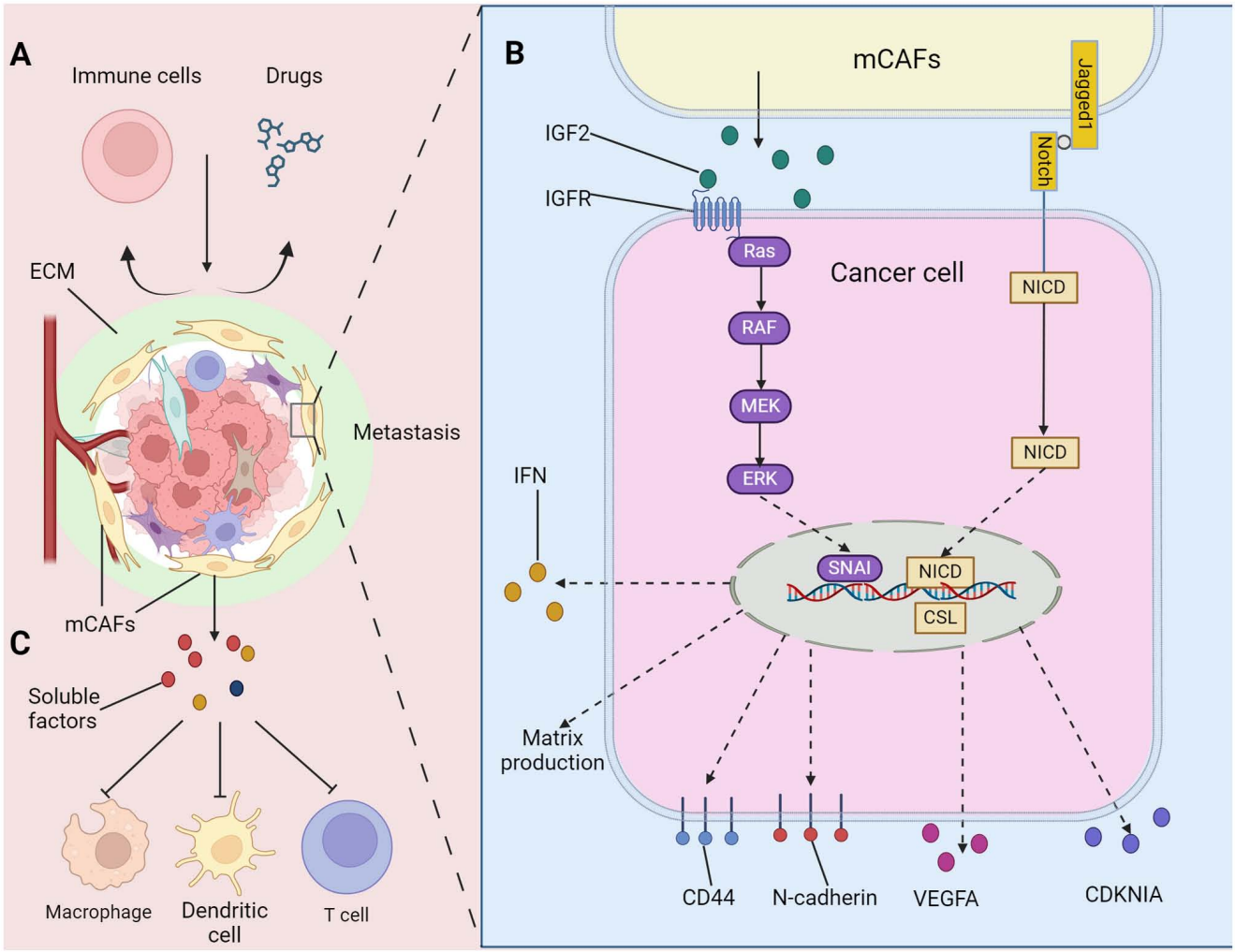

Figure 3. mCAFs provide an efficient protective environment for metastases. (A) The thickened ECM of metastases forms a physical barrier for drug penetration and immune cell infiltration. (B) mCAFs highly express IGF2 and Jagged1, activate downstream pathways, promote the secretion of IFN, VEGFA, CDKNIA and collagen and upregulate the expression of stem cell and mesenchymal markers in metastatic cells. (C) mCAFs secrete soluble factors to inhibit the polarization and chemotactic functions of immune cells. CAFs, cancer-associated fibroblasts; mCAFs, metastatic CAFs; pCAFs, primary CAFs; ECM, extracellular matrix; IGF, insulin like growth factor; CDKNIA, cyclin-dependent kinase; IGFR, IGF receptor; RAF, mitogen-activated protein kinase; MEK, mitogen-activated protein kinase kinase; ERK, extracellular regulated kinase; NICD, Notch intracellular domain; CSL, DNA-binding transcription factor; VEGFA, vascular endothelial growth factor A; IFN, interferon; SNAI, snail family transcriptional repressor.

that there was a difference in the proportion of CAFs in primary tumors and metastases and the differences in the matreotypes between the two were determined by COL11A1+ CAFs, although there is high CD8+ T cell infiltration in metastases, they are excluded by the proliferating ECM, resulting in a lower survival rate.

Notably, some studies have found that high intratumoral drug concentrations do not improve anti-tumor efficiency (185,186). Hence, the role of the TMEs as a physical barrier for drug delivery becomes worth re-examining. Hessmann *et al* (74) observed that KPC tumors had more CAFs and stromal hyperplasia than LMs and they found that CAFs captured 2',2'-difluorodeoxycytidine-5'-triphosphate (dFdCTP), an active metabolic component of gemcitabine, thereby reducing the chance of gemcitabine contact with tumors, resulting in PDAC primary tumors with lower sensitivity to chemotherapy than matched LMs. This demonstrates that the response of CAFs to the highly selective pressure exerted by chemotherapy is pluralistic, which may explain why successful treatments targeting the ECM are difficult.

4. Potential therapeutic opportunities targeting mCAFs

Metastases are almost incurable and are the underlying cause of mortality in most patients with cancer (10). The fact that mCAFs cause metastasis treatment resistance makes it a promising therapeutic target (17). Eliminating or balancing the differences between mCAFs and pCAFs may be an effective treatment method (8,154). As aforementioned, most of the differences between primary tumors and metastases are 'more and less' rather than 'presence or absence', which means that treatments targeting the differences between them not only improve the sensitivity of metastases also benefit primary tumors. Although targeting mCAFs in mouse models or cell experiments is emphasized, when these drugs are used in human trials, there is more hope of achieving simultaneous remission of both the primary and metastatic tumors.

Therefore, the present study did not emphasize whether the new therapeutic agents of clinical trials are mCAFs or pCAFs.

Restoration quiescence or apoptosis induction of mCAF precursor. Different metastatic organs have specific sources of CAF precursor cells; therefore, directly killing these precursor cells or restoration of their quiescence in the target organ can eliminate or attenuate their malignant effect on the metastatic microenvironment (10,187) (Fig. 4B). Bhattacharjee *et al* (35) depleted HSCs in triple transgenic mice through the injection of diphtheria toxin (DT) and the TdTomato reporter gene showed that 97% of the HSCs were depleted. In the PDAC and CRC mouse models, the depletion of HSCs led to a significant reduction in the area of LMs. The natural compound curcumin induces HSC senescence by activating peroxisome proliferator-activated receptor γ and promoting P53 expression (188). In a recent study, the targeted biomimetic nanoparticle-based delivery of all-trans-retinoic acid in resting HSCs improved artificially induced liver fibrosis in mice (189). Although numerous chemicals, herbs and their bioactive extracts have been proven to promote the apoptosis of HSCs, at present, no recognized HSC-depletion drugs have been approved for clinical use (187). Hence, there is still a long way to go in developing mCAF-derived treatments for metastases.

Targeting differentially expressed markers in mCAFs. Identifying the proportional expression of markers in primary tumors and metastases is a prerequisite for accurately targeting metastases. However, sufficient epidemiological evidence or clear molecular mechanisms regarding independent prognostic markers are necessary. FAP is probably the most reliable marker, as large studies have shown that the high expression of FAP is an independent prognostic marker for poor prognosis in ovarian cancer, lung cancer, PDAC, hepatocarcinoma and CRC (113,190). Therapeutic strategies for FAP, including FAP-activating drugs, DNA vaccines, anti-FAP chimeric antigen receptor redirected T cells, radionuclide-based approaches and FAP antibodies conjugated with toxins, have been reported to be effective in clinical and preclinical studies (111,113,115,191) (Fig. 4A). FAP-4-1BBL has bispecific antibody activity that can act on both FAP and the co-stimulatory molecule 4-1BBL and has been designed to provide costimulatory signals to immune effector cells selectively within the tumor (192). FAP-4-1BBL co-stimulates T cells in ex vitro in patient-derived tumor tissues, additionally the combination of carcinoembryonic antigen-targeted T cell bispecific antibody and FAP-4-1BBL in mouse models can induce the infiltration of CD8+ T cells and tumor regression (192). Subsequently, the first-in-human study of the FAP-4-1BB agonist RO7122290 was initiated in patients with advanced solid tumors, however, the study was not designed to demonstrate differences between single-agent and combination therapy (193). A case report described a patient with BC and brain metastases who experienced a decrease in the intensity of headaches after 4 weeks of FAPi targeted radiotherapy (194). In other studies, radiotherapy strategies based on the high expression of FAP and FAPi may create a new integrated tumor diagnosis/treatment model in the future. High FAPi expression on FAPi-46 PET-CT was a criterion of consideration for peptide-targeted radionuclide therapy following two cycles of ^{177}Lu-FAPi-46 targeted therapy. In the selected patients, 12 of the 18 advanced patients were stable disease with no significant change in clinical condition but the remaining six progressed (195). Another group conducted a similar study (196). Unfortunately, these studies did not provide the information on local response status in each patient, so it is unknown whether the therapeutic benefit was correlated to the SUVs and whether low FAP expression implies resistance to FAP-targeting therapy.

Targeting the matrix of metastases. The penetration efficiency of drugs in tumors directly affects the drug concentration in contact with tumors. The means of targeting the ECM mainly include adjusting the modification state to inhibit ECM deposition, reducing the production of COL to soften tumors and directly shearing the ECM (Fig. 4C). For example, inhibiting citrullination can reduce LMs growth in CRC (163). As an important active enzyme for ECM crosslinking, LOX can also achieve tumor anti-fibrosis by inhibiting it (197). Hepatic fibroblasts express angiotensin II (AngII), a component of the RAS system. AngII activates the AngII type I receptor (AT1R) to undergo liver fibrosis through downstream JAK2 signaling (198). Using patient samples and atomic force microscopy, Shen *et al* (12) found that tissue stiffness is higher in LMs than in primary CRC. Highly activated mCAFs increase tissue stiffness, which enhances angiogenesis and anti-angiogenic therapy resistance. Drugs targeting the LMs mCAFs RAS system inhibit fibroblast contraction and ECM deposition, thereby reducing LMs stiffening and increasing the anti-angiogenic effects of bevacizumab (12). The use of valsartan in the treatment of spontaneous lung metastases of BC in mouse models inhibits the production of fibronectin and vimentin and reduces the occurrence of lung metastases (199). Another phase II clinical trial of FOLFIRINOX in combination with losartan has also achieved promising results as a neoadjuvant therapy for locally advanced unresectable PDAC (200). These studies demonstrate the anti-tumor efficacy of reduction of ECM stiffness, however, there seems to be little therapeutic breakthrough in clinical trials involving the direct degradation of the ECM. PEGPH20, is a polyethylene glycol hyaluronidase and early research has found that it can increase the distribution concentration of antitumor drugs in primary and metastatic tumors by degrading HA in the ECM (201). However, PEGPH20 combined with standard regimens for advanced PDAC has failed in multiple clinical trials. Some hypothesize that using only one ECM-degrading enzyme may be the reason for not meeting the expected clinical outcomes (202). However, this does not explain the negative results of the phase IB/II trial of PEGPH20 in combination with FOLFIRINOX in patients with metastatic PDAC (203). This is more likely due to the fact that ECM degradation products have a similar structure to some growth factors and they can bind to the corresponding receptors and activate downstream signaling pathways (14). Another clinical trial investigated AG in combination with PEGPH20 in the treatment of advanced PDAC. Although the combination group showed an increased objective response rate, it did not show improved OS or progression-free survival (204). Meanwhile,

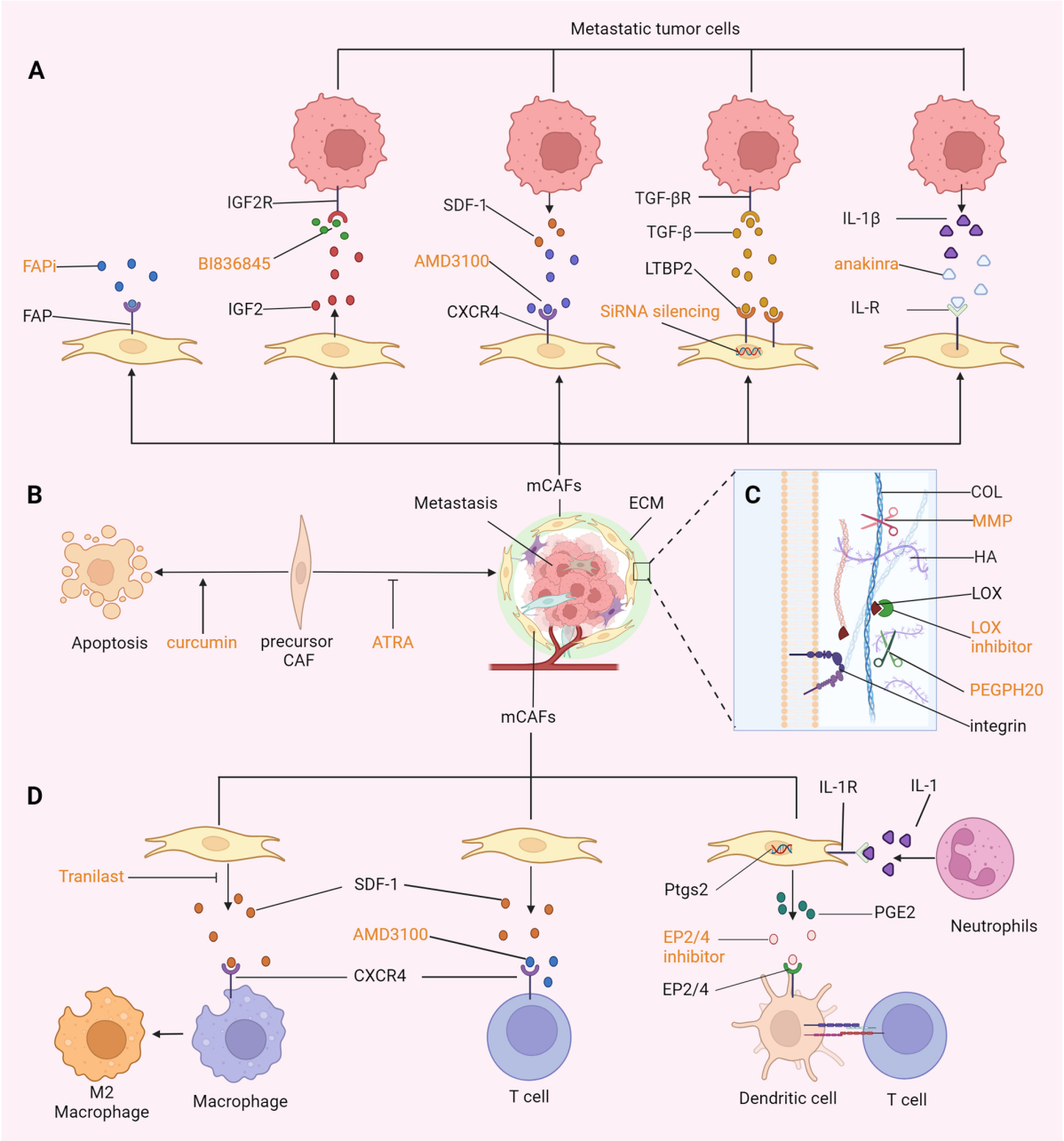

Figure 4. Potential therapeutic opportunities by targeting mCAFs. (A) Blockage of highly expressed signaling molecules or receptors in mCAFs. FAPi can directly target the FAP on the surface of CAFs and exert cytotoxic effects on CAFs by chelating radioactive isotopes and toxins. Using inhibitors such as BI836845, AMD3100 and anakinra to block the corresponding receptors can cut off the signaling communication between metastatic tumors and mCAFs; Similarly, silencing key genes such as LTBP2 can also achieve the objective. (B) Depletion or reversal of mCAFs; curcumin can induce HSC apoptosis and ATRA administration is able to convert PSC activation. (C) Degradation or softening of the ECM of metastasis sites. Using a LOX inhibitor to block ECM crosslinking for antifibrotic and antitumor effects or degradation of hyaluronan components by the PEGPH20 is able to reduce the interstitial fluid pressure of the tumor, leading to an increase in drug delivery. Although MMP is an endogenous collagenolytic enzyme, a number of studies have demonstrated its involvement in the degradation of the ECM, which provides a migratory path for tumor metastasis. (D) Relieving the immunosuppression effect of mCAFs and increasing T cell infiltration. Tranilast reduces M2 macrophage polarization by inhibiting SDF-1 secretion. The use of AMD3100 to competitively inhibit the SDF-1 receptor can increase the infiltration of CD8+ T cells into the ECM. IL-1b secretion from neutrophils can enhance Ptgs2 expression and PGE2 secretion in fibroblasts, leading to reduced anti-tumor immunity and enhanced metastasis to the lung. Genetic loss of Ptgs2 or EP2/4 inhibiting in fibroblasts reverses the immunosuppressive phenotype in BMDCs. mCAFs, metastatic cancer-associated fibroblasts; FAPi, FAP inhibitor; FAP, fibroblast activation protein; CAFs, cancer-associated fibroblasts; IGF2, insulin like growth factor 2; IGF2R, IGF2 receptor; SDF-1, stromal cell-derived factor 1; CXCR, C-X-C chemokine receptor; TGF-β, transforming growth factor-β; TGF-βR, TGF-β receptor; LTBP2, Latent TGF-β binding protein-2; CCL, chemokine (c-c motif) ligand; CCR, C-C chemokine receptor; ATRA, all-trans retinoic acid; COL, collagen; MMP, matrix metalloproteinases; HA, hyaluronan; LOX, lysyl oxidases; Ptgs2, prostaglandin-endoperoxide synthase-2; EP2/4, E prostanoid receptors 2 and 4; PGE2, Prostaglandin E2; IL, interleukin; IL-R, IL receptors.

it was shown to increase the risk of thromboembolism, which requires prophylaxis with heparin. Hence, from a number of perspectives, PEGPH20 is not an optimum treatment choice.

Therapies targeting the matrix can also enhance the invasion of immune cells in metastases, which can be achieved by blocking the SDF-1/CXCR4 signaling axis in the case of BC

metastasis (103) (Fig. 4D). Through modifications, some drugs with direct tissue penetration have also been developed (205), including lipophilic liposomes, albumin preparations, water-soluble prodrug preparations and nanocrystals (206). These modifications have been shown to enhance the precise delivery of drugs in the complex ECM environment of metastases.

Other treatment strategies. Directly targeting the up- or downstream pathways of mCAFs is another method to relieve the drug tolerance of metastases, which is related to the crosstalk between mCAFs and metastatic tumor cells (Fig. 4A). Gene silencing or receptor blocking of IGF2 can effectively inhibit the promoting effect of mCAFs on the growth of metastatic tumors (8). CXCR4 is highly expressed in LMs from BC and CRC and the CXCR4 inhibitor AMD3100 has been shown to alleviate desmoplasia in metastases (69,103). CXCR4 blocking has also been observed to sensitize the mBC tumors to immune checkpoint blockers (103). Similarly, silencing LRG1, which is highly expressed in LMs, can significantly reduce tumor migration and invasion (151). IL-1R knockout mice have demonstrated that IL-1β secreted by tumors can induce bone mCAFs to secrete SDF-1, promoting bone metastasis and this effect can be blocked by the IL-1 inhibitor anakinra (207). ECM-CAFs make up a high proportion in LMs, especially at the center of LMs, and promote vascular growth and tumor proliferation by secreting LTBP2; siRNA-mediated silencing of LTBP2 expression can regulate the phenotype of ECM-CAFs (68). Tranilast inhibits the production of SDF-1 in the myCAF cell line LmcMF in a mouse peritoneal metastasis model of gastric cancer, reducing the infiltration of M2 macrophages and leading to apoptosis of cancer cells by an immune response (208). However, the LmcMF cell line used in that study completed peritoneal implantation via intraperitoneal injection and may not represent the true source of mCAFs in peritoneal metastases. Although a large number of positive results have been obtained in laboratory studies, large-scale clinical trials are needed to provide direct evidence for blocking the upstream and downstream signals of mCAFs to improve the control rate of metastases and reveal a new avenue for advanced treatment in various tumors.

5. Conclusion

The reason why the present study emphasized the heterogeneity of pCAF and mCAF is because CAFs are the main cellular components in ECM and the frequent information exchange between different 'personalities'. CAFs with tumor cells result in great differences between metastasic and primary TMEs, ultimately showing different resistance in treatment. The present study described these differences in terms of origin, activation patterns, markers, matreotype, cytokines and transcriptome profiles.

In fact, there are a number of differences between pCAFs and mCAFs that may explain the low treatment responsiveness of metastases and some studies eliminated these differences to enhance the sensitivity of metastases to treatment options (8,12). However, one should pay attention to the fact that differences in the distribution of various CAF subsets exist even within the primary tumor, the best examples being the tumor center and the invasion front (124). Furthermore, CAFs have a very clear stage-dependent heterogeneity and the identity and prevalence of the various CAF subtypes present in a tumor or metastatic site change in response to normal, inflammatory, precancerous and malignant states, including anticancer treatment (18). These characteristics of CAFs also change as tumor growth progresses (85). The heterogeneity of pCAFs and mCAFs presented in the present review is only a cross-section at a certain point in time. Therefore, it is necessary to rely on new culture methods and observation methods to comprehensively and clearly describe the succession process and role of CAFs in the progression of the entire tumor.

Therapies targeting CAFs are currently being developed, including methods such as blocking ECM deposition and remodeling, directly targeting tumor-promoting CAFs, or using the plasticity of CAFs to engineer them into tumor-suppressing phenotypes (10). Most of these treatment strategies have failed because the heterogeneity of CAFs in different cancer types, tumor stages and metastasis sites makes these treatment methods one-sided (106), requiring a more comprehensive understanding of the role of CAFs within tumors. Future treatment options for advanced tumors may not only consider the molecular type of the tumor but also comprise more elaborate individualized treatment strategies which consider the heterogeneity of pCAFs and mCAFs. Some challenging questions lie ahead, such as the criteria for identifying the heterogeneity between mCAFs and pCAFs. Additional treatment for metastases will inevitably increase patient intolerance, so that screening for highly effective, low-toxicity sensitizers becomes particularly important. In addition, the timing of metastasis treatment and the choice of local or systemic treatment still needs to be solved urgently.

In conclusion, considering the biological heterogeneity of pCAFs and mCAFs, the present study provided a new perspective on the differential outcomes of primary and metastasis tumor treatment, revealing their key role in shaping different TMEs. It also explored possible means to improve the clinical treatment of metastases, providing new ideas for advanced anti-tumor treatments.

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Availability of data and materials

Not applicable.

Authors' contributions

CS and QZ conceived and designed the present study. ZK and CL contributed to data analysis and wrote the manuscript. WZ was responsible for the figures. LL supervised the study, aided by QZ and CS. Data authentication is not applicable. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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