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Cancer-associated fibroblasts in the single-cell era

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Cancer-associated fibroblasts (CAFs) are central players in the microenvironment of solid tumors, affecting cancer progression and metastasis. CAFs have diverse phenotypes, origins and functions and consist of distinct subpopulations. Recent progress in single-cell RNA-sequencing technologies has enabled detailed characterization of the complexity and heterogeneity of CAF subpopulations in multiple tumor types. In this Review, we discuss the current understanding of CAF subsets and functions as elucidated by single-cell technologies, their functional plasticity, and their emergent shared and organ-specific features that could potentially be harnessed to design better therapeutic strategies for cancer.

CAFs are a central component of the tumor microenvironment (TME) in solid tumors. In some cancer types, such as breast and pancreatic carcinomas, CAFs are the most prominent stromal cell type, and their presence is associated with worse prognosis[1]. CAFs are highly heterogeneous in their phenotypes, origins and functions[2]. They can originate from resident tissue fibroblasts reprogrammed by cancer cell-derived factors[3,4], from mesenchymal cells recruited to the TME from the bone marrow[5,6] or from adipocyte-derived precursor cells[7], endothelial cells[8], mesothelial cells[9–11] or pericytes[12] (Fig. 1). This heterogeneity is evident in the vast array of tasks that fibroblasts perform in tumor progression and metastasis[2,13], including promoting cancer cell growth, angiogenesis and extracellular matrix (ECM) remodeling[14–16]. Moreover, CAFs orchestrate tumor-promoting inflammation and modulate the immune microenvironment toward immunosuppression[17]. These functions are mediated by intricate reciprocal signaling interactions with cancer cells, matrix components and infiltrating immune cells. In some cancer types, such as pancreatic ductal adenocarcinoma (PDAC), CAFs were also suggested to have tumor-inhibitory functions[18,19].

The diversity of CAF functions, origins and markers has led to the notion that CAFs are composed of multiple subpopulations that only partially overlap (Fig. 2). For example, studies with the Rip1Tag2 mouse model of progressive pancreatic cancer and the orthotopic 4T1 breast cancer model revealed limited overlap between various fibroblast markers, suggesting unique subpopulations[20]. Analyses of human cancers and mouse models using immunostaining[6,21–23], in situ hybridization[24,25], flow cytometry and fluorescence-activated cell sorting of CAF subsets[26,27] and mRNA microarrays confirmed the existence of unique CAF subsets[6,20,22,25,26,28]. While these studies delivered crucial initial information regarding CAF diversity, the advent of single-cell RNA-sequencing (scRNA-seq) technologies has revolutionized the CAF field and revealed additional layers of complexity.

In this Review, we examine the current understanding of CAF heterogeneity and function, considering recent scRNA-seq studies of various cancer types. We portray shared and organ-specific features of CAF subpopulations and discuss promising areas for future research, in particular, the potential use of CAFs as a treasure trove of much-needed therapeutic targets.

Classification of CAF subsets through single-cell technologies

Although the heterogeneity of CAFs was previously recognized, dissecting their complexity and plasticity in an unbiased manner was not feasible before the development of scRNA-seq technologies. While this approach was first described more than a decade ago[29], it was scarcely used to explore CAF heterogeneity until recently[13,30]. Similar to all scientific tools, scRNA-seq has strengths and weaknesses, which greatly depend on the chosen technology[31]. Two such limitations are the loss of spatial information and under-representation of some cell types (for example, fibroblasts) due to the difficulty in isolating them from tissue[32,33]. Indeed, the relatively small number of CAFs ultimately analyzed in some studies (Table 1) may have hampered detection of the full spectrum of CAF subpopulations.

Nevertheless, scRNA-seq has substantially contributed to the expansion of knowledge of CAF biological diversity, as will be discussed in detail here.

ECM-remodeling/myofibroblastic CAFs

One of the crucial elements shaping the TME is the ECM, a complex three-dimensional network of extracellular molecules that form a tissue-supportive physical matrix and affect the structure and function of the stroma in primary tumors and metastases[21,34].

ECM remodeling is a tightly regulated physiological process (for example, in development and wound healing). However, tumors co-opt this process to create a supportive microenvironment. CAFs are central players in the deposition, modification and degradation of the ECM milieu (Fig. 3a). Dysregulated ECM remodeling by CAFs can lead to the desmoplastic reaction associated with poor outcome in breast, pancreatic and lung cancers[2]. Loss of an organized and stable matrix is often considered a hallmark of these tumors, leading to extensive efforts to develop therapies targeting tumor ECM[21,34].

CAFs were known to be related to myofibroblasts even before they could be characterized by RNA-seq, due to their activated state in which they acquire specialized contractile features (identified by elevated expression of α-smooth muscle actin (α-SMA)), similar to the phenotype of fibroblasts in wound-healing processes[35]. In this section, we discuss CAF subpopulations determined by scRNA-seq studies as ECM-remodeling/myofibroblastic CAFs in multiple organs. Some studies classified ECM remodeling and wound-healing-associated features (for example, contraction) as distinct CAF functional states[13,36–41]. However, other studies show that the same subpopulation of fibroblasts is highly enriched for both ECM-associated genes (such as those encoding collagens, *DCN* and *FBLN2*) and contractile proteins (such as those encoded

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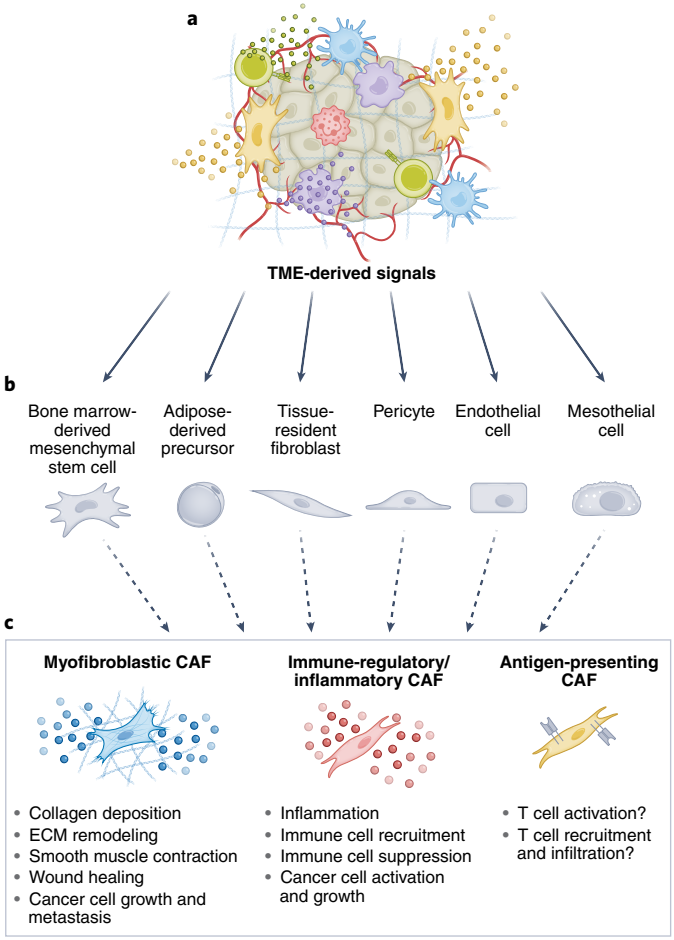

Fig. 1 | Primary CAF subsets and their potential origins. TME-derived signals (**a**) can reprogram a variety of proximal and distal healthy cells, including bone marrow-derived mesenchymal cells, adipocytes, resident fibroblasts, pericytes, endothelial cells and mesothelial cells (**b**), into CAFs. The major underlying CAF subgroups can be segregated into myofibroblastic CAFs, immune-regulatory and/or inflammatory CAFs and antigen-presenting CAFs (**c**) based on the tasks that they undertake in the TME, namely, reorganization of the ECM, inflammation and modulation of the immune system and direct effects on cancer cell proliferation and metastatic spread. Created with BioRender.com.

by *MYL9*, *TAGLN* and *ACTA2* (encoding α-SMA)), typical of wound-healing fibroblasts[42].

Pancreas. PDAC is characterized by a desmoplastic TME[43]. Cross-species analysis of human and mouse (KPC (LSL-*Kras*$^{G12D/+}$; LSL-*Trp53*$^{R172H/+}$;*Pdx1*^{Cre}) model) PDAC tumors by immunostaining[25] and scRNA-seq analysis[44] identified a subpopulation of myofibroblastic CAFs that was termed myCAFs (Fig. 4a). These cells express genes associated with smooth muscle contraction, focal adhesion, ECM organization and collagen formation and are located adjacent to neoplastic cells. myCAFs are characterized by high α-SMA expression; however, scRNA-seq analysis identified various other marker genes encoding contractile proteins (for example, *TAGLN*, *MYL9*, *TPM1*, *TPM2*, *MMP11*, *POSTN* and *HOPX*)[44]. In a xenograft model of human PDAC, one of these marker genes, *TPM1*, was used in addition to the gene encoding α-SMA to identify a myCAF subpopulation that differentiated from adipose-derived mesenchymal stem cells, demonstrating conservation of this marker between human and murine myCAFs[45]. Furthermore, POSTN$^+$ myCAFs were found to be the dominant subset specifically in dense-type PDAC tumors from human patients[46], and another scRNA-seq study of human PDAC identified a distinct CAF subpopulation marked by *POSTN*, in addition to a myofibroblastic CAF subpopulation marked as *ACTA2*$^+$ and *MYL9*$^+$ (ref. [47]). Myofibroblastic CAFs play a role from the early stages of tumorigenesis: scRNA-seq found that myofibroblastic CAFs were present in both samples derived from human intraductal papillary mucinous neoplasia (IPMN) (a common cystic precursor lesion of PDAC) and PDAC samples[48]. Myofibroblastic CAF heterogeneity can also be temporal, as CAFs were shown to acquire different phenotypes at different stages of PDAC mouse models: during late PDAC progression, one CAF subpopulation was lost to the dominance of two other major CAF subsets expressing genes linked to growth factor signaling, inflammation and the myofibroblast markers *Acta2* and *Tagln*[49]. Another study demonstrated that CAF subpopulations increase fibrillar collagens and cytokine secretion[50].

Breast. Myofibroblastic subsets of CAFs were identified in most scRNA-seq studies performed on breast cancer (Fig. 4b). In a syngeneic mouse model of transplantable triple-negative breast cancer (TNBC), ECM-remodeling or wound-healing signatures were identified in two distinct CAF states, marked by *Fbn1* and *Mfap5* or *Acta2* and *Thbs2*, respectively[13]. Both functional states were derived from a larger population of CAFs termed pCAFs (PDPN$^+$), which consisted of four more subsets. At advanced tumor stages, the two myofibroblastic subsets dominated the pCAF population[13]. In two other studies, one using the same mouse model and the other using transgenic MMTV-PyMT (mouse mammary tumor virus–polyoma middle tumor antigen) mice, the relevant cells were collectively designated as myCAFs[51] or mCAFs[30] in the absence of longitudinal analysis. The spatial location of mCAFs was linked to their function as they were most abundant in the invasive part of the tumors, particularly within collagen-rich streaks. Despite differences that may result from the different model systems, these studies highlight the generality of ECM-remodeling and/or contractile CAF subpopulations.

One of the strongest risk factors of developing breast cancer is advanced age. scRNA-seq of cells isolated from human postmenopausal breast tissue identified two fibroblast subsets expressing various types of collagens. A comparison between the gene signatures of postmenopausal fibroblast subsets and the gene expression profiles of 1,100 breast tumors in the Cancer Genome Atlas dataset revealed significant overlap with a gene profile exclusively associated with luminal breast tumors, suggesting that this fibroblast subpopulation may contribute to breast carcinogenesis in the elderly[52].

Lungs. scRNA-seq of human non-small cell lung cancer (NSCLC; squamous cell carcinoma and adenocarcinoma) revealed five distinct CAF types based on the expression of a unique repertoire of collagens and other ECM molecules. Further characterization showed high expression of genes associated with myogenesis (including *ACTA2*) in one subpopulation, whereas another showed a strong expression of genes linked to epithelial–mesenchymal transition (EMT) and the ECM[37]. scRNA-seq of tissues obtained from patients with lung adenocarcinoma at different stages of the disease identified seven subpopulations of fibroblasts, three of which were functionally related to ECM modulation (Fig. 4c): *COL13A1*$^+$ matrix fibroblasts, *COL14A1*$^+$ matrix fibroblasts and myofibroblasts (*ACTA2*$^+$*TAGLN*$^+$). The matrix-related fibroblasts (*COL13A1*$^+$ and *COL14A1*$^+$) were the main subpopulations present in normal lungs and early-stage tumors. By contrast, myofibroblasts were dominant in advanced-stage tumors (including in metastatic lymph nodes), reflecting a gradual change of fibroblast states associated with tumor progression[36]. Intriguingly, an scRNA-seq study that compared ground glass nodule and solid lung adenocarcinoma showed that the fibroblasts of the former, which has low malignant potential and

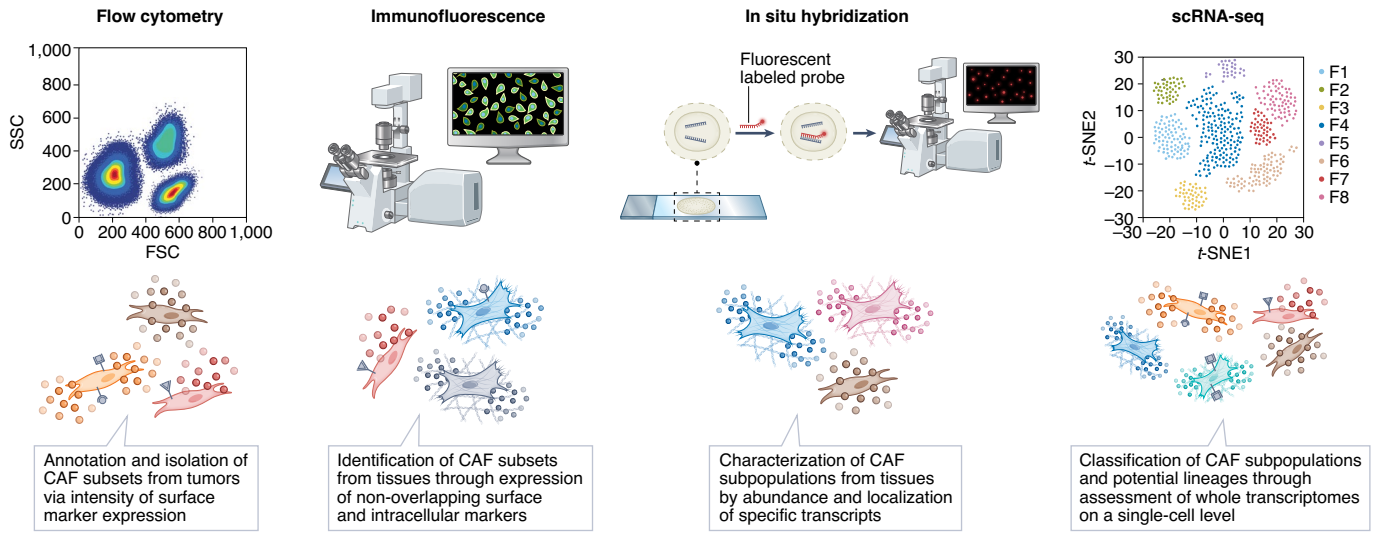

Fig. 2 | Identification of discrete CAF subsets achieved through different experimental systems. From left to right, flow cytometry has been employed to annotate and sort different CAF subgroups from tumors, also enabling CAF subset isolation for further experimentation into CAF phenotypes and tasks. Immunofluorescence and RNA in situ hybridization experiments revealed CAF subsets from tissue samples, based on discrete expression of surface and intracellular protein markers and transcripts; these methods also reveal important spatial information regarding CAF subtype localization in the TME. Finally, scRNA-seq has provided a breakthrough in stratification of a multitude of new CAF subtypes through high-resolution characterization of whole transcriptomes on a single-cell level, providing information on rare CAF subsets and potential information regarding CAF lineages. *t*-SNE, *t*-distributed stochastic neighbor embedding. Created with BioRender.com.

a better survival rate, expressed lower levels of collagens, emphasizing the impact of excessive ECM deposition on patient prognosis[53].

Liver. scRNA-seq analysis of human intrahepatic cholangiocarcinoma (ICC), an aggressive desmoplastic carcinoma with poor prognosis, identified a subpopulation designated as matrix CAFs (mCAFs), expressing low levels of *ACTA2* but high levels of ECM-associated genes including various collagens, *POSTN*, *FN1*, *LUM*, *DCN* and *VCAN*. In agreement with findings from breast cancer[30], these POSTN$^+$ mCAFs were found in the invasive front of the tumor, predominantly within collagen-rich streaks, suggesting a close association with tumor invasiveness[54]. A high proportion of ECM-modulating CAFs may be characteristic of highly desmoplastic TMEs. In a mouse model of desmoplastic liver metastasis induced by injection of PDAC and colorectal cancer (CRC) cell lines into the hemispleen, scRNA-seq revealed that myofibroblastic CAFs comprised more than half of the total analyzed CAFs[55]. The presence of such ECM-modulating CAFs was also confirmed in human CRC liver metastases, although, compared to mice, human CAFs appeared to largely originate from hepatic stellate cells and lacked a portal fibroblast and mesothelial cell signature, which may have distinct functions[55]. This was in agreement with functional studies suggesting that CAFs may also have both tumor-promoting and tumor-inhibitory functions: in a three-dimensional culture model, collagen type I acted as a mechanical barrier restricting tumor expansion and invasiveness, whereas hyaluronic acid promoted tumor growth and spread[55] (Fig. 4d).

Ovary. High-resolution scRNA-seq dissection of human ovarian tumors revealed a subset of CAFs, annotated as transforming growth factor (TGF)-β CAFs, which resembled a combination of the myCAF and TGFβ-driven CAF subsets described in PDAC[44,56] (Fig. 4e). The main markers of the TGF-β CAFs included genes associated with TGF-β-induced reactive stroma, as well as *POSTN*, *ACTA2*, *MMP11*, *TAGLN* and *FN1* (ref. [56]). In another scRNA-seq study of human serous ovarian cancer derived from primary tumors and metastatic omentum as well as the normal ovary, the fibroblasts were subgrouped into normal, primary and metastatic subsets. Collagen genes and matrix metallopeptidase (MMP)-associated genes were upregulated in the primary and metastatic tumor fibroblasts when compared to the normal subset, suggesting a role for ECM modulations in tumor progression[57].

Skin. scRNA-seq profiling of the stromal compartment in a transplantable model of murine melanoma at distinct time points of tumor development identified three functionally and temporally distinct subpopulations, referred to as S1 ('immune'), S2 ('desmoplastic') and S3 ('contractile'), which were also validated in human melanoma. The S2 cells (PDPN^{hi}PDGFRα^{hi}CD34^{lo}) upregulated genes encoding ECM components, including various collagens, whereas the S3 cells (α-SMA^{hi}) expressed genes involved in the regulation and rearrangement of actin cytoskeleton, indicating a contractile stromal subset. S3 cells also expressed some pericyte-associated markers, suggesting that this may be their cell of origin. Temporal analysis revealed dynamic changes whereby early tumors primarily comprised S1 ('immune') and S2 ('desmoplastic') cells, whereas mid and late tumors exhibited S2 ('desmoplastic') and S3 ('contractile') enrichment[38]. These detailed definitions further emphasize the question of whether CAF subpopulations are truly distinct or a 'snapshot' of plastic functional states.

ECM-remodeling/myofibroblastic CAFs in other organs. Single-cell studies in other organs confirmed the presence of ECM-remodeling/myofibroblastic CAF subtypes that may be defined as two separate states. Analysis of human prostate tumors unveiled two distinct clusters representing either myofibroblastic or ECM-associated phenotypes, which was supported by enrichment in the ECM-associated subpopulation of CREB3L1 and PLAGL1, transcription factors known to control ECM production and composition, compared to an increase in levels of the regulators HOXB2 and MAFB in the myofibroblastic subpopulation[39]. A study of human urothelial bladder carcinoma[58] identified a subset of CAFs termed mCAFs, with similar characteristics to the myCAFs described in PDAC[25,44], and marked by *RGS5* expression, suggesting

Table 1 | Summary of the main CAF subsets and features in each organ

	Organ	Organism	Central features	Signature/markers	Total number of analyzed fibroblasts	Ref.
Inflammatory	**Breast**	Mouse allograft model of TNBC (BALB/C-derived 4T1 mammary tumors)	Inflammation, immune trafficking and complement	*Ly6c1*, *Il6*, *Il33*, *Cxcl1*, *Cxcl12*, *Ccl2*, *Ccl7*, *C3*, *C4b*, *C1s1*, *C1s2*	535	51
		Mouse allograft model of TNBC (BALB/C-derived 4T1 mammary tumors), human TNBC samples	Inflammation	*PDPN*, subdivided into two populations characterized by *CXCL1* and *IL6*	8,033	13
		MMTV-PyMT model, human BC tissue samples	Inflammation and chemotaxis	*Cxcl14*	768	30
		Human BC	Inflammatory response, TNF signaling, cytokine pathway	*CXCL12*, *SOD2*. Additional pathways: detoxification (*ADH1B* and *GPX3*), response to stimuli (*RGMA* and *SCARA5*)	18,296	78
		PyMT/WT, PyMT/ELF5	Inflammatory response, complement activation, monocyte recruitment	Involuting CAF signature: *CXCL12*, *Ly6c1*, *C3*, *C4b*	2,255	80
		Human TNBC	Inflammation and chemotaxis	*IGF1*, *FIGF*, *PDGFD*, *CXCL12* and *CXCL13*. As opposed to myCAFs, iCAFs are characterized as $FAP^{lo}CD90^{lo}$.	1,409	79
	Pancreas	Human IPMNs and PDAC tissue	Inflammation	*VIM*, *FAP*, *COL3A1*, *DES*, *IL6* and *CXCL12*	≥267	48
		KPP, PRT, KIC, KPC and KPfC mouse models of PDAC; human PDAC	Chemoattraction, inflammation, immune trafficking, complement regulation	*Cxcl1*, *Cxcl2*, *Cxcl9*, *Cxcl10*, *Cxcl12*, *Cxcl1*, *Ccl2*, *Ccl7*, *Il6*, *Il8*, *Il1r1*, *Lif*, *Cfd*, *C7*, *C3*, *C1s*, *C1r*, *Pdgfra*	962 (human) and 4,012 (mice) (44); 8,439 (46); 5,802 (47); 2,143 (49); ~10,900 (mice) and 8,931 (human) (50); 12,239 (71); 1,753 (72)	44,46,47,49, 50,71,72
	Lung	Human NSCLC; murine lung adenocarcinoma model ($Kras^{LA1}$)	Inflammation, chemotaxis	*CXCL12*, *CXCL14*, *PDGFRA*	3,794 (36); 428 (83)	36,83
	Ovaries	Human ovarian cancer	Inflammation, complement regulation and chemotaxis	*IL1*, *IL6*, *IL10*, *CXCL1*, *CXCL2*, *CXCL10*, *CXCL12*, *CXCL14*, *CCL2*, *SOCS3*, *C3*, *C7*, *C1QA*, *C1QB*, *C1QC*, *CFD*, *CFB*, *SERPING1*	7,760 (56); 547 (57)	56,57
	Liver	Human ICC; murine ICC models (YAP/AKT, KRAS/p19)	Inflammation, chemotaxis, complement, HGF–MET signaling	*FBLN1*, *IGF1*, *CXCL1*, *CXCL12*, *IGFBP6*, *SLPI*, *SAA1*, *C3*, *C7*, *IL6*, *HGF*, *CCL21*	498 (54); 12,431 (mice) and 4,463 (human) (89)	54,89
	Bladder	Human urothelial bladder carcinoma	Inflammation and chemotaxis	*CXCL12*, *IL6*, *CXCL14*, *CXCL1* and *CXCL2*, marked by *PDGFRA*	N/A	58
	Skin	Murine melanoma	Immune trafficking and inflammation	Marked by *Pdpn*, *Pdgfra* and *Cd34*: *Cxcl12*, *Csf1* and *Ccl8*, *Il6ra* and *Il6st*, *C3*, *C2* and *C4b*	N/A	38
Immune regulatory	**Breast**	Mouse allograft model of TNBC (BALB/C-derived 4T1 mammary tumors)	Antigen presentation, MHC-II genes	*CD74*, *H2-Aa*, *H2-Ab1*, *H2-Eb*, *Cd74*	535	51

Continued

Table 1 | Summary of the main CAF subsets and features in each organ (continued)

	Organ	Organism	Central features	Signature/markers	Total number of analyzed fibroblasts	Ref.
		Mouse allograft model of TNBC (BALB/C-derived 4T1 mammary tumors), human TNBC samples	Immune regulation, chemotaxis	*PDPN* subpopulation subdivided into Ly6c$^+$, *Cxcl12*, *Saa3* subsets	8,033	13
		Mouse allograft model of TNBC (BALB/C-derived 4T1 mammary tumors), human TNBC samples	MHC-II presentation and immune regulation	*S100A4*, *CD73*, *H2-Aa*, *H2-Ab1* and *CD74*; *SLPI* and *SPP1*	8,033	13
		Human BC	IFN-γ, cytokine signaling, MHC-II presentation	*CCL19*, *CCL5*, *CD74*	18,296	78
		Human TNBC	Immune regulation	*CXCL12* hallmark gene, C5–C5AR1 signaling and TGFB1–TGFBR1 and TGFB2–TGFBR1 axes. CXCL12–CXCR4 and CXCL13–CXCR5 signaling.	1,409	79
	Pancreas	Human PDAC	Immune regulation via complement secretion	*C3*, *C7*, *CFB*, *CFD*, *CFH*, *CFI*	2,958	73
		KPP mice and human PDAC	Immunoregulatory module	*Pdgfc*, *Vegfa*, *Il33*, *Il18*	~10,900 (mice) and 8,931 (human)	50
		Mouse (KPC model) and human PDACs	AP, T cell regulation	MHC-II in mice (*H2-Aa* and *H2-Ab1*, *Cd74*) and humans (*HLA-DRA*, *HLA-DPA1*, *HLA-DQA*, *CD74*). Additional immunoregulatory genes: *BCAM*, *F11R*, *IRF5*	962 (human) and 4,012 (mice)	44
		PRT, KIC, KPC and KPfC mouse models of PDAC; human PDAC	AP, MHC-II genes	*CD74*, *H2-Aa*, *H2-Dmb1*, *HLA-DQA1*, *CD83*	2,143 (49); 12,239 (71); 1,753 (72)	49,71,72
	Lung	Human lung adenocarcinoma	Immune modulation and antigen presentation	*CFD*, *CXCL14*, *CXCL12*, MHC-II, *CD74*	2,257	84
	Prostate	Human prostate cancer	Inflammation, regulation of myeloid cell recruitment	*CCL2*, *CXCL12*, *CCL11*, *CXCL1*, *IL33*	3,321	86
	Liver	Mouse model of liver metastasis (PDAC/CRC tumor cell line injection)/human CRC liver metastases	Inflammation, IFN response, HGF–MET signaling, antigen expression	*HGF*, *H2-Q4*, *H2-Q7*, *Ifitm*	N/A	55
		Murine ICC model, human ICC	AP	*CD74*, *HLA-DRA*, *HLA-DRB1*, *H2-Q4*	498 (54); 12,431 (mice) and 4,463 (human) (89)	54,89
Antigen presentation	**Pancreas**	Mouse (KPC model) and human PDACs	AP; partial activation of CD4$^+$ T cells (by TCR ligation)	Mouse: *H2-Aa*, *H2-Ab1* and *Cd74*; *Saa3* and *Slpi*. Human: *HLA-DRA*, *HLA-DPA1*, *HLA-DQA1* and *CD74*; *SLPI*	962 (human) and 4,012 (mice)	44
		Mouse model of human PDAC (cell line-derived xenograft)	AP	*CD74* and *HLA-DRA*	699	45

Continued

Table 1 | Summary of the main CAF subsets and features in each organ (continued)

	Organ	Organism	Central features	Signature/markers	Total number of analyzed fibroblasts	Ref.
		Tamoxifen-inducible mouse model of PDAC (PRT)	AP	*H2-Aa*, *Cd74* and *Cd83*	12,239	71
		KPP mouse model of PDAC	AP	*H2-Ab1* and *Cd74*; *Saa3*	~10,900 (mice) and 8,931 (human)	50
		KIC, KPC and KPfC mouse models of PDAC	AP; partial activation of $CD4^+$ T cells (by TCR ligation); induction of naive $CD4^+$ T cells into regulatory T cells	*H2-Aa*, *H2-Ab1*, *H2-Eb1* and *Cd74*; *Msln*, *Upk3b*, *Lrrn4* and *Krt19* (mesothelial genes)	17,055 (derived from integrated data of three papers[44,49,50]	11
	Breast	Mouse allograft model of TNBC (BALB/C-derived 4T1 mammary tumors)	AP	*H2-Aa*, *H2-Ab1* and *Cd74*; *Slpi* and *Spp1*	8,033	13
			AP	*H2-Aa*, *H2-Ab1*, *H2-Eb1* and *Cd74*; *Krt8*, *Krt18* and *Fsp1*	535	51
	Liver	Human ICC	AP	*HLA-DRA*, *HLA-DRB1* and *CD74*	498	54
	Lung	Human and mouse (LLC model) lung tumors	AP; formation of functional spots within tumors that sustain $CD4^+$ T cells; priming $CD4^+$ T cells along with rescuing them from apoptosis	Human: *CD74* and *SLPI*; *IL6*, *CFD*, *C1QA* and *C1QB*. Mouse: *Cd74* and *Slpi*	798 (human), N/A (mice)	91
ECM-remodeling/ myofibroblastic CAFs	**Pancreas**	Mouse (KPC model) and human PDACs	Smooth muscle contraction, focal adhesion, ECM organization and collagen formation	Mouse: *Acta2*, *Tagln*, *Ifgbp3*, *Thy1*, *Col12a1* and *Thbs2*. Human: *ACTA2*, *TAGLN*, *MYL9*, *TPM1*, *TPM2*, *MMP11*, *POSTN* and *HOPX*	962 (human) and 4,012 (mice)	44
		Mouse model of human PDAC (cell line-derived xenograft)	ECM organization, cell adhesion and blood vessel development	*ACTA2*, *TAGLN*, *BGN*, *COL8A1*, *COL15A1*, *IGFBP7*, *TPM1* and *TPM2*	699	45
		Human PDAC	Focal adhesion and ECM–receptor interactions	*COL10A1* and *POSTN*	8,439	46
		Human PDAC	ECM remodeling	*ACTA2*, *MYL9* and *POSTN*	5,802	47
		Human IPMNs and PDAC tissue	ECM remodeling	*ACTA2*	≥267	48
	Breast	Mouse allograft model of TNBC (BALB/C-derived 4T1 mammary tumors)	ECM organization and wound-healing features	*Acta2*, *Thbs2*, *Fbn1* and *Mfap5*	8,033	13
			ECM remodeling	*Acta2*, *Tpm1*, *Tpm2*, *Myl9*, *Tagln*, *Cnn2*, *Cnn3*, *Igfbp3*, *Tnc*, *Tmem119*, *Tgfb1*, *Tgfb2*, *Ctgf*, *Pgf*, *Vegfa* and *Wnt5a*	535	51
		Transgenic mouse model of breast cancer (MMTV-PyMT)	ECM molecule production	*Dcn*, *Lum*, *Vcan*, *Col14a1*, *Fbln1*, *Fbln2*, *Smoc*, *Lox*, *Loxl1*, *Pdgfra* and *Cxcl14*	768	30
	Lungs	Human NSCLC	Myogenesis, NOTCH pathway and angiogenesis	*ACTA2*, *MEF2C*, *MYH11*, *ITGA7*, *COL4A1* and *COL10A1*	1,465	37
		Human NSCLC	ECM remodeling	*COL13A1*, *COL14A1*, *ACTA2*, *TAGLN*, *MYH11*, *MYLK* and *ACTG2*	3,794	36
	Liver	Human ICC	ECM and collagen fibril organization	*POSTN*, *FN1*, *LUM*, *DCN*, *VCAN*, *COL5A1*, *COL5A2* and *COL63A*	498	54

Continued

Table 1 | Summary of the main CAF subsets and features in each organ (continued)

	Organ	Organism	Central features	Signature/markers	Total number of analyzed fibroblasts	Ref.
		Mouse model of liver metastasis (PDAC/CRC tumor cell line injection)/human CRC liver metastases	ECM remodeling/opposing effects on tumor growth	Mouse: *Acta2*, *Col1a1* and *Col3a1*. Human: *COL1A1*, *COL3A1* and *COL63A*	N/A	55
	Ovary	Human ovarian cancer	TGβ-induced reactive stroma	*ACTA2*, *POSTN*, *COMP*, *COL10A1*, *COL11A1*, *MMP11*, *TAGLN* and *FN1*	7,760	56
	Skin	Melanoma mouse model	Desmoplastic reaction/contraction of actin stress fibers	*Pdpn*, *Pdgfra*, *Postn* and *Tnc*/*Acta2*, *Rock1*, *Mlc2* and *Mlck*	N/A	38
	Bone	Human osteosarcoma	ECM remodeling	*COL14A1*, *ACTA2*, *MYL9* and *LUM*	N/A	40
	Colon	Human CRC	Cytoskeleton/ECM remodeling	*ACTA2*, *TAGLN* and *PDGFA*, *MMP2*, *DCN* and *COL1A2*	26	41
	Urinary bladder	Human urothelial bladder carcinoma	Focal adhesion and contraction	*RGS5*, *MYL9* and *MYH11*	N/A	58

Numbers in parentheses in penultimate column indicate reference cited in far right column. AP, antigen presentation; BC, breast cancer; CXCR, C–X–C motif chemokine receptor; WT, wild-type; N/A, not available; MHC-II, MHC class II; KPP, *Pdx1*$^{cre/+}$;LSL-*Kras*$^{G12D/+}$;*p16/p19*$^{flox/flox}$; PRT, *Ptf1a*^{CreER};LSL-*Kras*^{G12D};LSL-tdTomato; KIC, LSL-*Kras*$^{G12D/+}$*Ink4a*$^{fl/fl}$*Ptf1a*$^{Cre/+}$; KPfC, LSL-*Kras*$^{G12D/+}$;*Trp53*$^{fl/fl}$;*Pdx1*$^{Cre/+}$; YAP, Yes-associated protein 1; LLC, Lewis lung carcinoma; NOTCH, transmembrane receptor; AKT, serine–threonine kinase.

a potential pericyte origin, as shown in breast cancer and melanoma studies[26,30]. Accumulation of mCAFs in the tumor correlated with poor patient survival[58]. To explore the intratumoral heterogeneity of osteosarcoma, the most frequent primary bone tumor, scRNA-seq was performed on human primary, recurrent and lung metastatic lesions. Three CAF subclusters were identified: (1) *COL14A1*$^{+}$ matrix fibroblasts, (2) *DES*$^{+}$*ACTA2*^{lo}*COL14A1*^{lo} fibroblasts (indicative of smooth muscle-like cells) and (3) *ACTA2*$^{+}$*MYL9*^{hi}*LUM*^{hi} fibroblasts (lacking expression of *COL14A1* and *DES*). Although subcluster 3 resembled myofibroblasts, it also showed high expression of osteoblast markers (*IBSP* and *SPP1*), once again highlighting the plasticity of mesenchymal cell populations. Moreover, this subcluster was the major source of CAFs both in primary and recurrent lesions, whereas subcluster 2 was the main component in lung metastases[40]. An scRNA-seq study in human primary colorectal tumors and matched normal mucosa used a method termed reference component analysis to identify two distinct subtypes of CAFs (CAF-A and CAF-B). CAF-B cells expressed markers of myofibroblasts such as *ACTA2*, *TAGLN* and *PDGFA*, and these markers were downregulated in CAF-A cells, which expressed ECM-related genes such as *MMP2*, *DCN* and *COL1A2*. Activation of the upstream regulators TGF-β1 and SMAD3 in both CAF subtypes suggests common regulation of these two functional states[41]. In gastric cancer, a stage-dependent increase in three CAF subsets belonging to a stromal meta-cluster with endothelial cells and pericytes was demonstrated: two subsets exhibited expression of myofibroblastic genes such as *ACTA2* and *TAGLN*, whereas a specific cluster induced expression of collagen-associated genes that were correlated with inhibin subunit βA (INHBA) signaling[59], confirming previous findings regarding CAF-mediated INHBA signaling in gastric cancer[60].

Tissue-damage myofibroblasts versus ECM-remodeling/myofibroblastic CAFs. Comparing the transcriptional landscape of fibroblasts that function in physiological wound healing with ECM-remodeling/myofibroblastic CAFs yields interesting conclusions. For example, scRNA-seq of stromal cells in healthy and myocardial infarct-injured mouse hearts revealed multiple different fibroblast subpopulations[61]. Normal fibroblasts were characterized by platelet-derived growth factor (PDGF) receptor (PDGFR) α and Ly6a (SCA1) expression, whereas activated fibroblasts in injured heart tissue upregulated fibrogenic (for example, periostin (POSTN), collagens) and/or contractile proteins (for example, α-SMA), similar to myofibroblastic CAFs. This analysis also identified a subpopulation of fibroblasts termed F-Wntx in both normal and myocardial infarction-associated tissue, which expressed Wnt signaling inhibitors and had an anti-fibrotic phenotype. Single-cell analysis of fibroblasts isolated from murine skin wound-healing samples[42,62] or from human fibrotic skin[63] identified multiple heterogeneous fibroblast states expressing PDGFRα and pro-fibrogenic gene signatures (POSTN, collagens, TGF-β pathway) reminiscent of myofibroblastic CAFs. Similar analysis of fibroblasts isolated from murine lung[64] and liver fibrosis[65] identified subpopulations of activated myofibroblasts with collagens and matrix-remodeling gene signatures and a population of lipofibroblasts expressing lipid-synthesis and lipid-transport genes, in addition to common fibroblast genes[64]. Contrary to CAFs, these wound-healing and fibrosis studies did not identify distinct subpopulations of inflammatory and/or immune-regulatory fibroblasts. However, single-cell analysis of fibroblasts isolated from the colon mucosa of patients with ulcerative colitis (UC)[66] or from the ileal biopsies of patients with Crohn's disease[67] identified both inflammatory fibroblasts and myofibroblasts. Additionally, UC samples contained several subpopulations of Wnt ligand-expressing fibroblasts that were also expressed in the normal colon, in a spatial-specific manner. Inflammatory fibroblasts isolated from UC patient samples also expressed CAF-like markers[66] and stromal genes shown to be associated with poor prognosis in CRC[68]. Thus, although CAF-like subpopulations are also found in wound-healing contexts, their heterogeneity may be more reminiscent of chronic inflammatory conditions than of fibrotic tissue repair.

Pro-inflammatory and immune-regulatory CAFs

Under normal homeostatic conditions, resident tissue fibroblasts play important roles in maintaining tissue integrity. Fibroblasts can sense mechanical changes and tissue-damage signals and react by orchestrating tissue repair, mediated by ECM synthesis and remodeling, but also by regulating immune cell responses. This orchestration of immune cell activity appears to be both organ- and

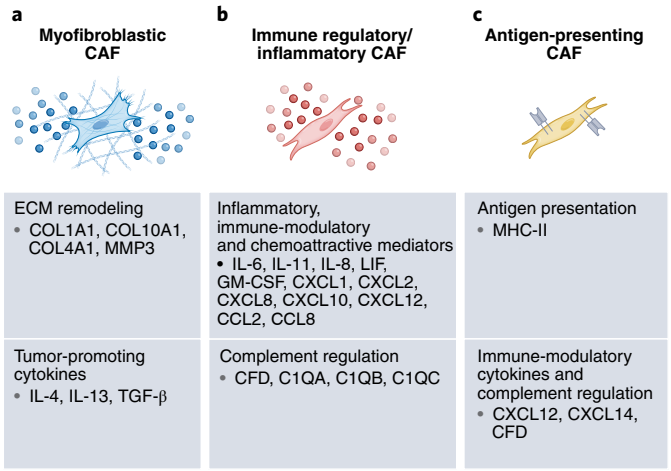

Fig. 3 | scRNA-seq reveals multiple tasks undertaken by discrete CAF subtypes. The major tasks performed by the three central CAF subtypes (ECM reorganization (**a**), immune regulation (**b**) and antigen presentation (**c**)) are mediated through expression of various cell surface receptors and secreted factors that influence tumor progression. The secreted factors and receptors listed in the figure generally vary according to disease type and organ and are designated accordingly in Fig. 4. COL, collagen. Created with BioRender.com.

context-dependent and is co-opted in tumors. The role of fibroblasts as central mediators of tumor-promoting inflammation was first suggested over a decade ago[69]. CAFs promote cancer growth, immune escape and metastatic dissemination by modulating the immune landscape in the TME. This is achieved through secretion of cytokines and chemokines, recruitment of suppressive myeloid and regulatory T cells (T_{reg} cells), suppression and exclusion of cytotoxic lymphocytes and dendritic cells and promotion of M2 and type 2 helper T (T_H2) cell polarization of macrophages and T cells, respectively[17] (Fig. 3b). Until recently, it was not clear whether diverse immune-regulatory activities of CAFs, such as promoting inflammation and immune suppression, are performed by a homogeneous group and whether these tasks represent a global feature shared by different cancer types and organs. A surge of studies employing scRNA-seq or using different markers to segregate CAF subpopulations have addressed the heterogeneity of immune-regulatory CAFs and the disease- and organ-specific nature of their activities. Most of these studies were conducted on highly desmoplastic carcinomas of the breast and pancreas.

Pancreas. Inflammatory CAF subtypes were identified in PDAC before single-cell studies of human and murine pancreatic cancer. A subset termed iCAF was distinguished from another subset, myCAFs, based on its low expression of the myofibroblastic α-SMA marker[25]. iCAFs were shown to be spatially separated from myCAFs and from the cancer cells, secreted inflammatory mediators such as interleukin (IL)-6, IL-11 and LIF and had a unique transcriptomic profile driven by IL-1 signaling[70]. The two subsets could interconvert, at least in vitro, depending on growth conditions, further raising the question of whether CAF subpopulations are transient functional states. A later study that included scRNA-seq of mouse and human PDAC samples[44] provided additional iCAF markers such as *IL6*, *CXCL8*, *CXCL1*, *CXCL2*, *CXCL12* and *CCL2* (Fig. 4a).

Differences between mouse models and humans can affect interpretation of CAF activity in the context of immune cell regulation. For example, the murine KPC model is extremely sparse in T and B cells compared to human samples[44], which can affect conclusions regarding CAF effects on immune exclusion. Nevertheless, similar inflammatory CAF subtypes were found (and given various designations) in multiple scRNA-seq studies of human and murine PDAC[47,49,71,72]. These studies support the inflammatory and immune-regulatory modalities among CAF subtypes, potentially also segregating iCAFs based on expression of inflammatory and chemotactic mediators such as C–X–C motif chemokine ligand (CXCL)14, IL-6, CXCL1, CXCL12, C–C motif chemokine ligand (CCL)7, CCL11 and different immune-modulatory hubs such as Ly6c1 and SCARA3, in addition to antigen-presenting modalities[49,71]. Another immune-regulatory function identified through scRNA-seq of human pancreatic CAFs is complement regulation[73] by csCAFs, a specialized subpopulation confirmed by weighted gene coexpression network analysis. CAFs expressing complement regulatory factors (C3, C7, complement factor (CF)B, CFD, CFH and CFI) that can promote inflammation and immune activity in the TME appear in close proximity to pancreatic cancer cells in stage I PDAC and decrease during tumor progression[73]. Early expression may suggest a tumor-repressive role, but it is also possible that their early inflammatory complement-mediated activities pave the way for PDAC development, given that complement activation is positively associated with cancer progression[74]. Only some of the PDAC CAF subsets designated as 'immune regulatory' based on gene expression were mechanistically connected with immune-modulating tumor-regulatory functions. For example, inflammatory mediators secreted from iCAFs, such as IL-6, IL-11, granulocyte–macrophage colony-stimulating factor (GM-CSF) and LIF, promoted activation of cancer cells to enhance tumor growth and survival[25,75]. In addition, cytometry by time-of-flight analysis of murine pancreatic tumors demonstrated that Hedgehog pathway inhibition increases the proportion of iCAFs versus myCAFs, sequesters repressive myeloid immune cells and inhibits $CD8^+$ T cell tumor infiltration, while promoting $FOXP3^+$ T_{reg} accumulation, thus generating an immunosuppressive TME[76].

Single-cell analysis using mass cytometry of murine and human PDAC samples unveiled fibroblast subsets with high versus low CD105 (ENG) expression that had opposing effects on immune activity and tumor growth[77]. While $CD105^{lo}$ CAFs were characterized by antigen-presentation modules (major histocompatibility complex (MHC) class II and CD74) and correlated with proliferation of T cell subsets, $CD105^{hi}$ CAFs were inversely correlated with certain $CD4^+$ and $CD8^+$ T cell subsets. The suppressive effects of $CD105^{lo}$ CAFs depended on proper immune activity that was abrogated in immune-deficient animals but did not rely on their antigen-presenting capabilities[77].

The iCAF phenotype was shown to be driven by IL-1 receptor 1 (IL-1R1)–Janus kinase (JAK)–signal transducer and activator of transcription (STAT) and nuclear factor (NF)-κB signaling in human PDAC organoids and murine models[75]. However, scRNA-seq from mouse and human PDAC revealed that iCAFs also express highly activated TGF-β receptor (TGF-β-R)2 and TGF-βR3 (ref. [44]), which can induce the myCAF phenotype, suggesting that the two CAF archetypes may affect each other. The dichotomy of IL-1 and TGF-β signaling in driving iCAF and myCAF subpopulations, respectively, was questioned in another scRNA-seq study of murine pancreatic cancer models[50]: $PDPN^{hi}$ CAFs were shown to play an immune-regulatory role in PDAC, where they diverged into two subpopulations through IL-1 and TGF-β signaling. The TGF-β-driven CAFs that accumulated in PDAC were identified by leucine-rich repeat-containing 15 (LRRC15) expression and correlated with poor response to anti-programmed death ligand 1 (PD-L1) therapy (Fig. 4a). Thus, although $LRRC15^+$ CAFs are mainly characterized by a myCAF-like signature, they may also play an active immunosuppressive role[50].

Breast. Inflammatory and immune-regulatory CAFs in breast cancer can be clustered as one population marked by both

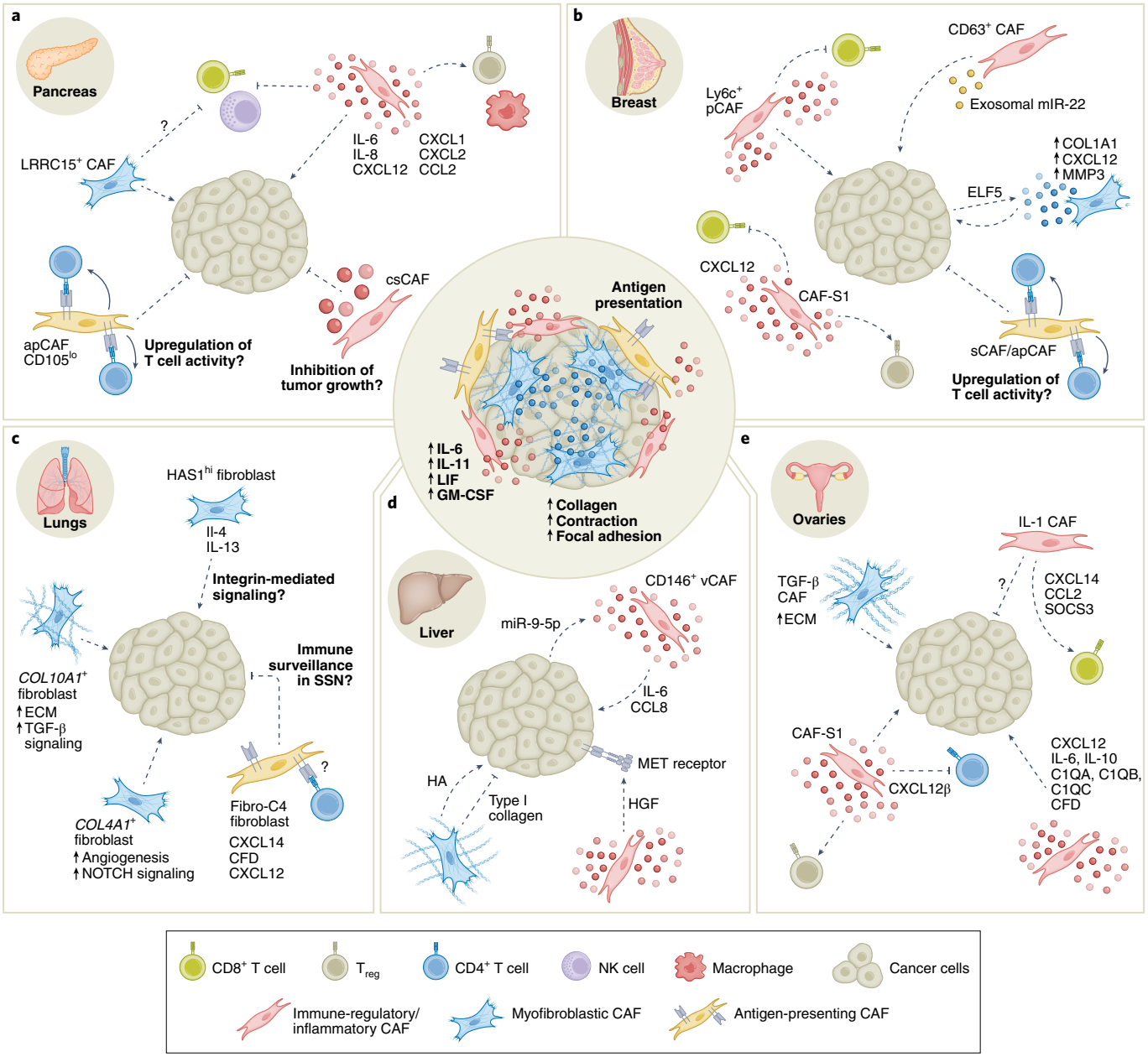

Fig. 4 | scRNA-seq analyses reveal universal as well as organ-specific CAF subsets. The main CAF subsets identified in the TME of most organs are inflammatory CAFs, antigen-presenting CAFs and myofibroblastic CAFs (center circle). Immune-regulatory (cytokine and chemokine secretion, crosstalk with immune cells) and myofibroblastic CAF activities (ECM modulation, collagen deposition, contraction and adhesion) are prevalent in all organs and across cancer types. Antigen-presenting activities of CAFs were predominantly reported in pancreatic and breast cancers. Organ-specific tasks and pathways identified in CAFs are depicted for the pancreas (**a**), breast (**b**), lungs (**c**), liver (**d**) and ovaries (**e**). Arrows indicate promotion of cancer progression and immune cell recruitment and/or activation. Inhibitory arrows indicate suppression of cancer progression or immune cell activity. Speculative or unverified pathways are labeled with question marks. NK, natural killer; SOCS3, suppressor of cytokine signaling 3; HA, hyaluronic acid; SSN, subsolid nodules; fibro-C4, fibroblast cluster 4. Created with BioRender.com.

inflammatory and immune-regulatory genes (for example, *IL6*, *IL8*, *CXCL1*, *CXCL2* and *CXCL12* (ref. [44])), similar to annotations in PDAC, or subclassified further based on the inflammatory cytokine gene signatures that they express (such as IL-6-secreting inflammatory CAFs) or by the immune cell activity that they modulate (for example, immune-regulatory CAFs may regulate leukocyte homing via CXCL12)[13]. Immune-regulatory CAFs may also be subcategorized according to unique tasks such as detoxification (alcohol dehydrogenase 1B (ADH1B) and glutathione peroxidase 3 (GPX3)) or interferon (IFN)-γ and cytokine response (expression of genes such as *CCL19* and *CCL5*) as was shown in human breast cancer[78]. Multiple studies revealed compartmentalization of immunomodulatory activities by CAF subsets in breast cancer. scRNA-seq in a mouse model of TNBC revealed two pro-inflammatory and two immunomodulatory CAF subpopulations within a subset of pCAFs compared to S100A4+ CAFs (sCAFs). Both pCAFs and sCAFs were also distinguishable in cohorts of human patients with breast cancer[13]. The immunomodulatory pCAFs in TNBC diverged into Ly6c^{lo} and Ly6c^{hi} subpopulations, with the latter shown to inhibit T cell proliferation

and activation in vitro and their relative abundance drastically reduced during tumor development[13]. Single-cell analysis of the stromal compartment in human patients with TNBC revealed that high-inflammatory CAF signatures are associated with T cell dysfunction and poor survival in TNBC[79] (Fig. 4b). In addition to experimental mouse models and interspecies differences, cancer type and mutational landscape may also influence CAF activity. For example, PDPN^{hi} CAFs identified in a TNBC mouse model were significantly less abundant in BRCA-mutated than in BRCA-wild-type human breast cancer[13]. Moreover, a shift of CAF function toward an immunosuppressive phenotype in response to genetic changes in the cancer cells was also observed in the MMTV-PyMT mouse model following mammary-restricted expression of the transcription factor ELF5. This transcription factor drives lactation during pregnancy and is linked to a more aggressive phenotype in pregnancy-associated breast cancer. Work with wild-type or ELF5-induced MMTV-PyMT mice suggested a shift toward an inflammatory and/or immunoregulatory CAF profile resembling their role in mammary gland involution[80].

A separate study identified four CAF subsets (CAF-S1–CAF-S4, based on expression of CD29, fibroblast-activation protein-α (FAP), fibroblast-specific protein 1 (FSP1), α-SMA, PDGFRβ and caveolin 1 (CAV1)) in human breast cancer, of which CAF-S1 demonstrated immunomodulatory activities including recruitment of CD4$^+$CD25$^+$ T cells through CXCL12 and promotion of T_{reg} differentiation[26]. Subsequent scRNA-seq analysis conducted on the CAF-S1 subset highlighted eight subpopulations, three of which resembled the iCAFs discovered in PDAC, with the rest resembling the myCAF subset[78] (Fig. 4b). The three iCAF-like subsets were enriched in patients with TNBC compared to those with luminal breast cancer, which was more enriched in myCAFs, highlighting the specificity of CAF heterogeneity with disease subtype.

Ovary. Most single-cell studies in ovarian cancer described subsets of CAFs with immune-regulatory and/or inflammatory phenotypes. Fibroblasts isolated from patients with ovarian tumors expressed inflammatory factors such as CXCL12, CXCL14, IL-6, IL-1 and CCL2 (ref. [56]) and complement factors such as C3, CFB and serpin family G member 1 (SERPING1), suggesting that this subtype promotes cancer progression through inflammation and immune and complement regulation. Such inflammatory modules were also shown in a different scRNA-seq study conducted on human-derived ovarian tumors[57]. However, as emphasized above, the context and stage of disease affect CAF activity. Thus, in this study, CAFs in metastatic niches were shown to secrete inflammatory mediators contrary to primary CAFs[57]. Similar findings and two CAF subtypes termed CAF-S1 and CAF-S4 were reported in a study based on flow cytometry isolation of CAFs from patients with mesenchymal high-grade serous ovarian cancer (HGSOC)[81]. The CAF-S1 subset, marked by high expression of CD29, FAP and FSP1, was associated with immunosuppressive functions by increasing attraction, survival and differentiation of CD25$^+$FOXP3$^+$ T lymphocytes, via its expression of CXCL12β, and was associated with worse prognosis (Fig. 4e). Single-cell analysis of ascite samples from patients with HGSOC also identified several inflammatory and immunoregulatory CAF populations marked by expression of complement factors (C1QA, C1QB, C1QC and CFB), chemokines (CXCL1, CXCL2, CXCL10 and CXCL12) and cytokines (IL-6 and IL-10)[82]. Therefore, there is a conserved immune-regulatory network in HGSOC-associated CAFs that may be targeted for cancer inhibition and immune system reinvigoration.

Immunoregulatory CAF subsets in other organs. Studies employing scRNA-seq for lung malignancies have characterized lung fibroblasts in pre-invasive lesions[53] and NSCLC[36,37,83,84]. The identified CAFs were predominantly myofibroblast-like, whereas immunoregulatory subtypes were not as clearly defined as those in other organs. Single-cell analysis of subsolid nodules from patients with early-stage lung adenocarcinoma revealed enrichment of immunomodulatory fibroblasts, characterized by tumor necrosis factor (TNF) and IL-6–JAK–STAT signaling and enriched for CXCL12 and CXCL14, which decrease in lung adenocarcinoma and metastases[84]. Enrichment of specific CAF subpopulations was shown in patients with squamous cell carcinoma of the lung, relative to adenocarcinoma, and particular subclusters were inversely correlated with patient survival, depending on disease type[38]. Whether early immune-modulatory programs in subsolid nodule-associated fibroblasts contribute to lung adenocarcinoma progression remains to be determined. The inflammatory state of lung CAFs may also be regulated by EMT of resident epithelial cells: a mesenchymal program in epithelial lung adenocarcinoma cells from a transgenic mouse model was shown to favor enrichment of inflammatory CAFs in single-cell analysis[83], confirming the effects of carcinoma cell states on stromal heterogeneity.

In prostate cancer, although most scRNA-seq studies of human or mouse prostate tumor samples demonstrated CAF heterogeneity, one study identified mostly myofibroblastic-like signatures (human)[39], whereas others also identified immune-associated subsets (human and mouse)[85,86], possibly reflecting species-dependent signatures. A study of mouse prostate stromal cells found that SCA-1$^+$CD90^{lo} fibroblasts express ECM-related genes such as *Fn1* but also genes encoding cytokines, chemokines and complement components (*Ccl2*, *Ccl7*, *Ccl11*, *Cxcl1*, *Cxcl2* and *C3*)[85], whereas a study of cultured CAFs from human prostate cancer tissues showed a role for inflammatory prostate CAF subsets in recruiting macrophages[86].

scRNA-seq studies in liver metastases and ICC revealed fibroblast inflammatory and immune-regulatory activities including M2 polarization of macrophages, activation of T_{reg} cells and reduced activity of CD8$^+$ T cells, natural killer cells and dendritic cells[87]. Six CAF subpopulations were reported in human ICC[54]. The major subset among them was CD146$^+$ vascular CAFs (vCAFs), expressing inflammatory mediators such as IL-6 and CCL8, with other subsets expressing high levels of CXCL1 and complement factors C3 and C7. Ligand–receptor interaction analysis indicated that vCAFs may interact with carcinoma cells through IL-6 and/or IL-6 receptor (IL-6R), promoting tumor growth and stemness. A hepatocyte growth factor (HGF) signaling hub, characterized by expression of HGF in inflammatory CAFs and its receptor MET in cancer cells, was identified in multiple liver cancer studies[55,88,89] and may be conserved in mice and humans: scRNA-seq combined with ligand–receptor analysis in a mouse model of ICC demonstrated that the HGF–MET signaling axis promotes tumor growth and may also be relevant in patients[89]. This signaling module may be part of a cooperative axis mediated through CAFs and scar-associated macrophages in ICC; single-cell combined with spatial analysis of samples from patients with ICC and liver metastases demonstrated that HGF is expressed in both CAFs and scar-associated macrophages and may interact with cancer cells expressing MET[88] (Fig. 4d). Thus, it is interesting to consider targeting specific inflammatory CAFs and their signaling hubs in ICC, including the IL-6–IL-6R and HGF–MET axes.

Antigen-presenting CAFs

The ability to stimulate T cell activation is associated with MHC class II-expressing cells termed antigen-presenting cells (APCs). The classical or professional APCs are dendritic cells, macrophages and B cells. However, recent studies have identified additional cell types expressing MHC class II molecules, which may therefore be capable of antigen presentation. These atypical APCs include mast cells, granulocytes, endothelial cells, epithelial cells and lymph node stromal cells[90]. Whether these cells can supply the three signals required for full activation of naive T cells, namely antigen presentation, co-stimulation and regulatory cytokines, remains unclear[90].

The identification and characterization of diverse APC types in the TME (Fig. 3c) may improve immunotherapeutic strategies for cancer treatment.

Pancreas. A subpopulation of CAFs expressing MHC class II-related genes was first described in the mouse KPC model and human PDAC tumors using scRNA-seq, RNA in situ hybridization, immunohistochemistry and imaging mass cytometry[44] (Fig. 4a). These cells, termed apCAFs, were capable of partially activating CD4$^+$ T cells in vitro by T cell receptor (TCR) ligation in an antigen-dependent manner. However, they expressed low levels of the CD80, CD86 and CD40 co-stimulatory molecules required for full T cell activation. Injection of a human PDAC cell line and human adipose-derived mesenchymal stem cells into immunodeficient mice also led to the differentiation of the latter into CD74$^+$ and HLA-DRA$^+$ apCAFs[45]. These cells were also found at late and invasive tumor stages in a tamoxifen-inducible mouse model of PDAC[71]. By contrast, scRNA-seq on PDAC patient samples in another study did not identify a distinct apCAF subset[46,72]. These discrepancies may be caused by technical differences in the cell-sorting markers chosen, single-cell preparation methods, the possible scarcity of this population and CAF plasticity. A recent study reanalyzing published scRNA-seq datasets indicated that apCAFs may originate from mesothelial cells through the IL-1- and TGF-β-induced downregulation of mesothelial features and upregulation of fibroblastic ones[11]. A separate scRNA-seq analysis also reported transcriptional similarities between and clustering of apCAFs and mesothelial cells[50]. Although, in mice, apCAFs formed a distinct transcriptional subset, in human tumors, they may be admixed with other subpopulations such as iCAFs[44,50] and share the plasticity shown for other CAF subpopulations, as they were able to convert into myCAFs under certain culture conditions[44].

Breast. apCAFs were described in an scRNA-seq study of a mouse model of TNBC as a subset within a larger S100A4$^+$ sCAF population[13] (Fig. 4b). A high ratio of sCAFs to the other dominant CAF population, PDPN$^+$ pCAFs, correlated with BRCA mutations in the cancer cells and with improved disease outcome in patients with breast cancer, indicating that apCAFs may be tumor repressive[13]. Temporal analysis of CAFs during different stages of tumor progression demonstrated that apCAFs appeared predominantly at advanced stages of tumor progression and metastases[13]. A different study identified apCAFs also in healthy mammary and pancreatic murine tissues, suggesting a role for them in tissue homeostasis[51].

apCAFs in other organs. scRNA-seq of samples from patients with ICC revealed a subpopulation expressing MHC class II-related genes such as *CD74*, *HLA-DRA* and *HLA-DRB1* (ref. [54]). A recent study suggested that dense apCAF regions in human lung tumors define immunologically active regions with increased CD4$^+$ T cell infiltration[91]. Leveraging published scRNA-seq data[37] and a defined metric for assessing physiologically relevant MHC class II gene expression, this study defined a population of potential apCAFs in human lung cancer and in mice and proposed alveolar epithelial cells as their potential origin.

Although a functional antigen-presenting role has not been described in all these cases, the multiple studies describing apCAFs in different tumor types and models suggest that this is a robust CAF subtype in carcinomas. More work is required to establish apCAFs as an independent CAF subpopulation, understand their origin and define similarities and differences with professional APCs regarding their ability to mediate full activation of T cells.

Additional CAF subpopulations identified by scRNA-seq

Although the most prevalent CAF subpopulations identified are ECM-remodeling/myofibroblastic and immune-regulatory ones, CAFs appear to be much more diverse, based on their origins and functions[92]. In this section, we discuss unique and rare CAF subpopulations identified in various tissues by scRNA-seq approaches and associated with less-known fibroblast functions.

vCAFs. scRNA-seq of CAFs from the MMTV-PyMT transgenic mouse model of breast cancer revealed a subpopulation enriched for expression of angiogenic genes, which was associated with blood vessels and designated as vCAFs[30]. A study of human ICC classified the majority of CAFs as vCAFs, which expressed high levels of microvasculature-associated genes (for example, the gene encoding CD146), as well as genes encoding inflammatory chemokines, such as *CCL8* and *IL6* (Fig. 4d). Immunohistochemistry and multiplex immunofluorescence staining revealed that CD146$^+$ vCAFs mainly localized to the tumor core and microvasculature, suggesting extensive interactions with cancer cells presumably via the IL-6–IL-6R axis[54]. A strong connection between CAFs and the vasculature was also demonstrated through scRNA-seq of tumor samples from an orthotopic ICC mouse model cultured in vitro with a neutralizing antibody against placental growth factor (PIGF), a member of the vascular endothelial growth factor (VEGF) family, which indicated that CAFs were the major cell population affected[93].

Metabolic CAFs. scRNA-seq of tissues from patients with PDAC with different degrees of desmoplasia identified a new subtype of CAFs with a highly activated metabolic state, termed metabolic CAFs (meCAFs), which were found predominantly in loose-type (low desmoplasia) PDAC and used glycolysis as a major metabolic mode. Multiplex immunofluorescence staining of PLA2G2A$^+$ meCAFs on matched samples confirmed their distinct identity. meCAFs were strongly correlated with the presence of immune cells and thought to communicate with T cells and macrophages. Although patients with PDAC with abundant meCAFs had a higher risk of metastasis, they had a better immunotherapy response when treated with programmed cell death protein 1 (PD-1) blockade[46].

CD63$^+$ CAFs. scRNA-seq of the MMTV-PyMT mouse model identified CD63$^+$ CAFs predominantly in advanced stages of breast carcinoma[94] (Fig. 4b). This subset promoted breast cancer resistance to tamoxifen by secreting exosomal miR-22, thereby downregulating estrogen receptor α (ERα) and phosphatase and tensin homolog (PTEN) in cancer cells. These CAFs were also found in ERα^{lo} or ERα$^-$ human primary breast cancer. Co-culture of human primary breast CD63$^+$ CAFs with ERα$^+$ human breast cancer cells endowed tamoxifen resistance, and treatment with an anti-CD63-neutralizing antibody enhanced tamoxifen sensitivity in breast tumor-bearing mice[94]. CD63$^+$ CAFs were also identified by scRNA-seq of cultured CAFs derived from human prostate cancer tissue, suggesting that they are not limited to breast cancer[86].

Rare pericryptal *Ptgs2*-expressing fibroblasts. scRNA-seq analysis of the mouse intestinal mesenchyme unveiled a subset of *Pdgfra*^{lo} fibroblasts expressing high levels of *Ptgs2* (COX2)[95]. These cells, termed rare pericryptal *Ptgs2*-expressing fibroblasts (RPPFs), were found also in healthy human colons, near the stem cell zone at the bottom of the crypts, where intestinal tumors are primarily initiated[95]. Genetic ablation of *Ptgs2* in fibroblasts was sufficient to prevent tumor initiation in the *Apc*$^{Min/+}$ and azoxymethane models of intestinal cancer. RPPFs were suggested to promote tumorigenesis by prostaglandin E2 (PGE2)-mediated expansion of colon stem-like cells[95]. An additional scRNA-seq study of the normal murine colon also revealed a subset of crypt-bottom fibroblasts (CBFs) defined by low *Pdgfra* expression and high *Cd34* and *Thy1* expression[96]. CBFs maintained intestinal stem cell proliferation through expression of canonical Wnt ligands (encoded by *Wnt2* and *Wnt2b*), Wnt signaling potentiators (encoded by *Rspo3*) and BMP antagonists (encoded

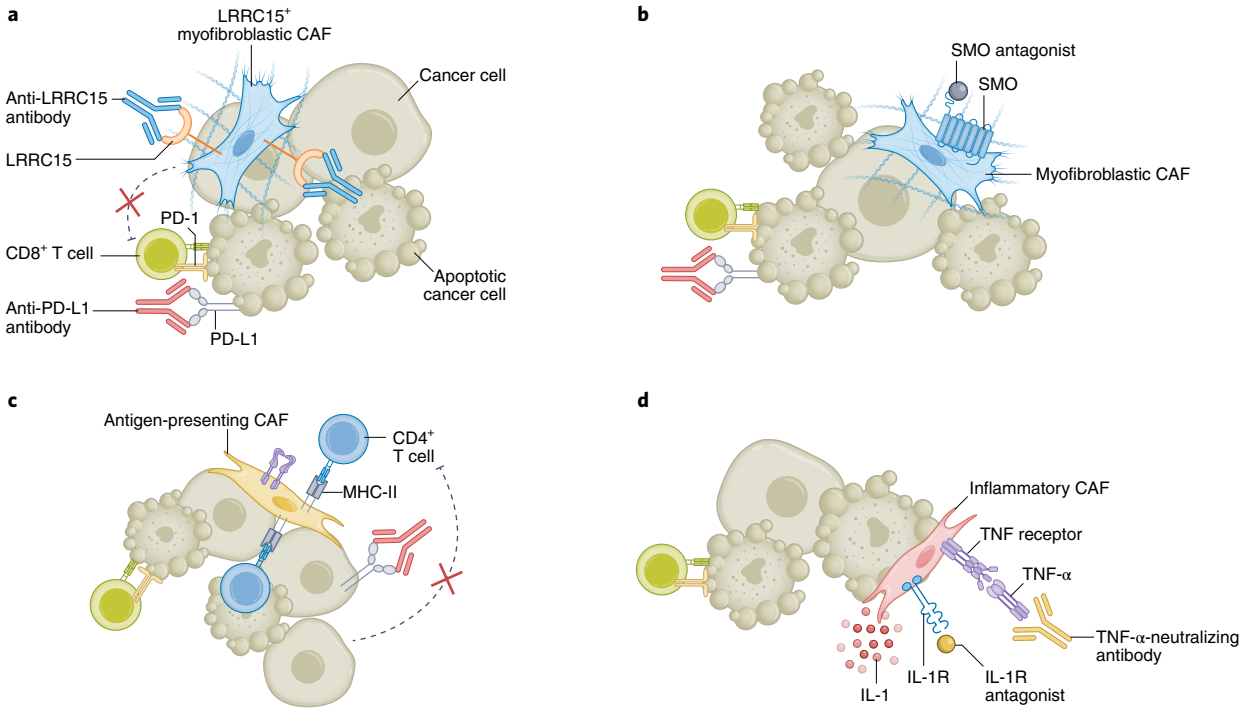

Fig. 5 | Examples of potential CAF targeting. a, Targeting LRCC15+ CAFs with antibodies or antibody–drug conjugates such as ABBV-085, in conjunction with conventional checkpoint inhibitor therapy (anti-PD-L1 antibody) may synergize to abolish their suppressive effect during immunotherapy[50,98]. **b**, Targeting Hedgehog signaling pathways via administration of SMO antagonists such as LDE225 can prevent their pro-tumorigenic activities[76]. Because such treatment promotes accumulation of immune-suppressive inflammatory CAFs, dual treatment with checkpoint inhibitors may provide important additive effects. **c**, Enhancing the activity of antigen-presenting CAFs and their ability to recruit CD4+ T cells[91] could promote anti-tumor immune activity. **d**, Targeting inflammatory CAFs with IL-1R antagonists (for example, anakinra) and anti-TNF-α antibodies could inhibit their immune-suppressive effects in the TME[75]. Created with BioRender.com.

by *Grem1*)[96]. It would be interesting to study whether RPPFs and CBFs share developmental trajectories in the colonic crypts.

Are the different CAF subtypes universal?

The wealth of scRNA-seq profiling of CAFs in diverse tumor types, mouse models and human patients raises the question of whether CAF subtypes are universal. The studies discussed here and summarized in Table 1 suggest that central features of different CAF subsets are conserved across organs, cancer subtypes and species. Similar hallmark genes were identified in multiple cancer types, with *IL6*, *Ly6c* and *PDGFRA* marking inflammatory CAFs; *Cxcl12* marking immune-regulatory CAFs; MHC class II (*H2-Aa*, *H2-Ab1* and *Cd74* in mice; *HLA-DRA*, *HLA-DPA1*, *HLA-DQA1* and *CD74* in humans) marking antigen-presenting CAFs; and *ACTA2*, *TAGLN* and *POSTN* marking myCAFs. Nevertheless, CAFs also possess organ-specific phenotypes, such as the HGF–MET signaling axis in the liver[89]. The colon, in which a unique segregation of the mesenchyme supports epithelial differentiation, also has specialized CAF subpopulations, such as the RPPFs and CBFs that promote epithelial stemness[95]. CAFs originating from bone marrow-derived mesenchymal cells appear to display similar phenotypes in organs such as the breast and pancreas that can be recapitulated in ex vivo co-culture experiments[6,23]. These bone marrow-derived CAFs display inflammatory and specialized programs that promote tumor progression and metastatic dissemination through increased vascularization, growth and migration. Such subsets can be identified in the TME through classical mesenchymal stromal cell markers (for example, *CD44* and *NT5E*) and additional markers that are upregulated in the TME, such as clusterin[6,13,23].

CAF composition may be heterogeneous even in different cancer subtypes within the same organ. Breast cancer luminal A tumors were found to contain a larger CAF-S2 subpopulation, HER2+ tumors promoted accumulation of CAF-S4 cells, and TNBC tumors were enriched in either CAF-S1 or CAF-S4 cells[26]. Similarly, in NSCLC, distinct enrichment of CAF subpopulations was observed in patients with squamous cell carcinoma compared to adenocarcinoma, which may have varying effects on patient outcome[38]. A recent cross-tissue analysis of fibroblasts from normal and perturbed disease states in human and mouse suggested that universal fibroblast states exist in normal tissues, serving as reservoirs that yield specialized and activated fibroblasts in disease[97]. Both universal and specialized CAF subtypes were similar between human and mouse.

An important element in addressing the universality of CAF subsets is their origin, which also presents a technical challenge when comparing different studies (Fig. 1). scRNA-seq has provided a wealth of data, revealing CAF clusters expressing genes that are classically used as markers of immune cells such as those encoding MHC class II or pericytes such as *RGS5*. Another confounding issue is the possibility of co-clustering of fibroblasts with cancer cells that have undergone EMT and therefore share mesenchymal markers. Several validation approaches can be conducted to avoid such misclassification, including injection of unlabeled cancer cells into reporter mouse models[39] (or labeled cancer cells into unlabeled mice), restricted expression of known oncogene mutations in tumor clusters[55], detection of large-scale copy number variations[80] and determination of cellular proliferation status[70]. The overlap of CAF features with those other cell types raises the question of how CAFs should be designated and whether they should be defined by origin or function. CAF plasticity also bears implications for classification and therapy. In vitro, iCAFs, myCAFs and apCAFs were shown to interconvert depending on culture conditions. As

RNA-seq provides a snapshot of the transcriptome, it is difficult to ascertain whether such data provide information about a continuous state transition of cells or a fixed cell type identity that undertakes a specific task. Time-resolved experiments, employing lineage tracing and pseudotime inference and careful assessment of the potential effects of in vitro growth conditions, can provide critical information about CAF subtype trajectories in the TME. For example, scRNA-seq in conjunction with pseudotime inference was used to identify two possible universal fibroblast populations marked as PI16$^+$ and COL15A1$^+$ (ref. [97]). A similar approach in a mouse model of TNBC inferred lineage trajectories during cancer development[13]. Both the potential origins and the plasticity of CAF subsets should be taken into consideration when categorizing CAFs from single-cell data and when developing potential treatments aimed at a specific subtype.

Establishing comprehensive nomenclature and annotation of CAF subpopulations is critical. The single-cell studies highlighted here support the notion of three major CAF subsets that can be parsed by myofibroblastic, inflammatory and/or immune-regulatory and antigen-presenting activities. These then further diverge into subpopulations with distinct markers that may differ between tumor types, organs and species. We therefore propose to hierarchically classify CAFs into one of these broad populations and annotate specialized functions through specific markers. For example, 'PDPN$^+$Ly6c$^+$ immune-regulatory CAFs' or 'PDPN$^+$LRRC15$^+$ myofibroblastic CAFs' are annotations that provide both a general understanding of CAF tasks as well as a precise and potentially targetable moiety.

Therapeutic approaches and future perspectives

Much work is still needed to map the full landscape of CAF subpopulations across human cancers. Better understanding of CAF plasticity, origin and interactions is required, especially in carcinomas other than those in the pancreas and breast. Nevertheless, with the wealth of data already accumulated, the field is ready to move to translating these emerging CAF atlases into therapeutic targets. Based on the insights detailed here, targeting specific signaling molecules and pathways, rather than a specific CAF subtype or cell of origin, may be a more viable therapeutic strategy, considering the plasticity and heterogeneity of the mesenchymal tumor-supporting milieu (Fig. 5). For example, TGF-β-driven LRRC15$^+$ CAFs correlate with poor response to anti-PD-L1 therapy in PDAC[50]. Reverting the LRRC15$^+$ phenotype[98] by combining anti-TGF-β therapies with anti-PD-1 and/or anti-PD-L1 treatment may have beneficial and additive effects. Such treatments were shown to revert the matrix-remodeling transcriptional profile of CAFs in breast cancer[99]. However, reversion of one pathway prevalent in a given CAF population (such as myCAFs) may promote dominance of a different CAF subtype that can hamper therapy. Indeed, inhibition of Hedgehog signaling was shown to impair myCAF activity and tumor growth, while promoting inflammatory CAF accumulation and an immune-suppressive TME[76]. Recent findings suggest, however, a limited plasticity in some CAF lineages that may serve as stable therapeutic targets, such as CD105$^+$ pancreatic CAFs[77] and pancreatic stellate cell-derived CAFs, which may be identified by a combination of α-SMA and the kinase TIE1 (ref. [100]). A common hallmark of inflammatory CAFs across tumor types is upregulation of IL-6–IL-6R signaling. Anti-IL-6 therapies such as siltuximab and tocilizumab are expected to target these CAFs[101,102]. Given that immunoregulatory CAFs can also drive JAK–STAT signaling in cancer cells[25], inhibition of JAK–STAT signaling may prove to be a promising arm of a combined therapeutic approach[82]. Another interesting therapeutic approach is targeting of CAF-derived ECM modifications. Immunotherapies have shown limited efficacy in highly stromal tumors, into which immune cells cannot infiltrate[103–105]. ECM normalization by modulating CAF activity may help restrict tumor growth and enhance the response to immunotherapy.

Deeper understanding of CAF plasticity and complexities in the coming years will reveal more therapeutic options that will instruct new CAF targeting strategies to formulate better cancer treatments.

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References

1. Liu, L. et al. Stromal myofibroblasts are associated with poor prognosis in solid cancers: a meta-analysis of published studies. *PLoS ONE* **11**, e0159947 (2016).
2. Sahai, E. et al. A framework for advancing our understanding of cancer-associated fibroblasts. *Nat. Rev. Cancer* **20**, 174–186 (2020).
3. Sharon, Y. et al. Tumor-derived osteopontin reprograms normal mammary fibroblasts to promote inflammation and tumor growth in breast cancer. *Cancer Res.* **75**, 963–973 (2015).
4. Kalluri, R. The biology and function of fibroblasts in cancer. *Nat. Rev. Cancer* **16**, 582–598 (2016).
5. Quante, M. et al. Bone marrow-derived myofibroblasts contribute to the mesenchymal stem cell niche and promote tumor growth. *Cancer Cell* **19**, 257–272 (2011).
6. Raz, Y. et al. Bone marrow-derived fibroblasts are a functionally distinct stromal cell population in breast cancer. *J. Exp. Med.* **215**, 3075–3093 (2018).
7. Kidd, S. et al. Origins of the tumor microenvironment: quantitative assessment of adipose-derived and bone marrow-derived stroma. *PLoS ONE* **7**, e30563 (2012).
8. Zeisberg, E. M., Potenta, S., Xie, L., Zeisberg, M. & Kalluri, R. Discovery of endothelial to mesenchymal transition as a source for carcinoma-associated fibroblasts. *Cancer Res.* **67**, 10123–10128 (2007).
9. Rynne-Vidal, A., Jimenez-Heffernan, J. A., Fernandez-Chacon, C., Lopez-Cabrera, M. & Sandoval, P. The mesothelial origin of carcinoma associated-fibroblasts in peritoneal metastasis. *Cancers* **7**, 1994–2011 (2015).
10. Sandoval, P. et al. Carcinoma-associated fibroblasts derive from mesothelial cells via mesothelial-to-mesenchymal transition in peritoneal metastasis. *J. Pathol.* **231**, 517–531 (2013).
11. Huang, H., Wang, Z., Zhang, Y. & Brekken, R. A. Mesothelial cell-derived antigen-presenting cancer-associated fibroblasts induce expansion of regulatory T cells in pancreatic cancer. *Cancer Cell* **40**, 656–673 (2022).
12. Murgai, M. et al. KLF4-dependent perivascular cell plasticity mediates pre-metastatic niche formation and metastasis. *Nat. Med.* **23**, 1176–1190 (2017).
13. Friedman, G. et al. Cancer-associated fibroblast compositions change with breast cancer progression linking the ratio of S100A4$^+$ and PDPN$^+$ CAFs to clinical outcome. *Nat. Cancer* **1**, 692–708 (2020).
14. Gascard, P. & Tlsty, T. D. Carcinoma-associated fibroblasts: orchestrating the composition of malignancy. *Genes Dev.* **30**, 1002–1019 (2016).
15. Levi-Galibov, O. et al. Heat shock factor 1-dependent extracellular matrix remodeling mediates the transition from chronic intestinal inflammation to colon cancer. *Nat. Commun.* **11**, 6245 (2020).
16. Alexander, J. & Cukierman, E. Stromal dynamic reciprocity in cancer: intricacies of fibroblastic–ECM interactions. *Curr. Opin. Cell Biol.* **42**, 80–93 (2016).
17. Monteran, L. & Erez, N. The dark side of fibroblasts: cancer-associated fibroblasts as mediators of immunosuppression in the tumor microenvironment. *Front. Immunol.* **10**, 1835 (2019).
18. Ozdemir, B. C. et al. Depletion of carcinoma-associated fibroblasts and fibrosis induces immunosuppression and accelerates pancreas cancer with reduced survival. *Cancer Cell* **25**, 719–734 (2014).
19. Rhim, A. D. et al. Stromal elements act to restrain, rather than support, pancreatic ductal adenocarcinoma. *Cancer Cell* **25**, 735–747 (2014).
20. Sugimoto, H., Mundel, T. M., Kieran, M. W. & Kalluri, R. Identification of fibroblast heterogeneity in the tumor microenvironment. *Cancer Biol. Ther.* **5**, 1640–1646 (2006).
21. Cox, T. R. The matrix in cancer. *Nat. Rev. Cancer* **21**, 217–238 (2021).
22. Neuzillet, C. et al. Inter- and intra-tumoural heterogeneity in cancer-associated fibroblasts of human pancreatic ductal adenocarcinoma. *J. Pathol.* **248**, 51–65 (2019).
23. Waghray, M. et al. GM-CSF mediates mesenchymal–epithelial cross-talk in pancreatic cancer. *Cancer Discov.* **6**, 886–899 (2016).
24. Bonneau, C. et al. A subset of activated fibroblasts is associated with distant relapse in early luminal breast cancer. *Breast Cancer Res.* **22**, 76 (2020).
25. Ohlund, D. et al. Distinct populations of inflammatory fibroblasts and myofibroblasts in pancreatic cancer. *J. Exp. Med.* **214**, 579–596 (2017).
26. Costa, A. et al. Fibroblast heterogeneity and immunosuppressive environment in human breast cancer. *Cancer Cell* **33**, 463–479 (2018).
27. Pelon, F. et al. Cancer-associated fibroblast heterogeneity in axillary lymph nodes drives metastases in breast cancer through complementary mechanisms. *Nat. Commun.* **11**, 404 (2020).

28. Su, S. et al. CD10+GPR77+ cancer-associated fibroblasts promote cancer formation and chemoresistance by sustaining cancer stemness. *Cell* **172**, 841–856 (2018).
29. Tang, F. et al. mRNA-seq whole-transcriptome analysis of a single cell. *Nat. Methods* **6**, 377–382 (2009).
30. Bartoschek, M. et al. Spatially and functionally distinct subclasses of breast cancer-associated fibroblasts revealed by single cell RNA sequencing. *Nat. Commun.* **9**, 5150 (2018).
31. Hwang, B., Lee, J. H. & Bang, D. Single-cell RNA sequencing technologies and bioinformatics pipelines. *Exp. Mol. Med.* **50**, 1–14 (2018).
32. Moncada, R. et al. Integrating microarray-based spatial transcriptomics and single-cell RNA-seq reveals tissue architecture in pancreatic ductal adenocarcinomas. *Nat. Biotechnol.* **38**, 333–342 (2020).
33. Waise, S. et al. An optimised tissue disaggregation and data processing pipeline for characterising fibroblast phenotypes using single-cell RNA sequencing. *Sci. Rep.* **9**, 9580 (2019).
34. Deasy, S. K. & Erez, N. A glitch in the matrix: organ-specific matrisomes in metastatic niches. *Trends Cell Biol.* **32**, 110–123 (2021).
35. Darby, I. A., Zakuan, N., Billet, F. & Desmouliere, A. The myofibroblast, a key cell in normal and pathological tissue repair. *Cell. Mol. Life Sci.* **73**, 1145–1157 (2016).
36. Kim, N. et al. Single-cell RNA sequencing demonstrates the molecular and cellular reprogramming of metastatic lung adenocarcinoma. *Nat. Commun.* **11**, 2285 (2020).
37. Lambrechts, D. et al. Phenotype molding of stromal cells in the lung tumor microenvironment. *Nat. Med.* **24**, 1277–1289 (2018).
38. Davidson, S. et al. Single-cell RNA sequencing reveals a dynamic stromal niche that supports tumor growth. *Cell Rep.* **31**, 107628 (2020).
39. Chen, S. et al. Single-cell analysis reveals transcriptomic remodellings in distinct cell types that contribute to human prostate cancer progression. *Nat. Cell Biol.* **23**, 87–98 (2021).
40. Zhou, Y. et al. Author Correction: Single-cell RNA landscape of intratumoral heterogeneity and immunosuppressive microenvironment in advanced osteosarcoma. *Nat. Commun.* **12**, 2567 (2021).
41. Li, H. et al. Reference component analysis of single-cell transcriptomes elucidates cellular heterogeneity in human colorectal tumors. *Nat. Genet.* **49**, 708–718 (2017).
42. Guerrero-Juarez, C. F. et al. Single-cell analysis reveals fibroblast heterogeneity and myeloid-derived adipocyte progenitors in murine skin wounds. *Nat. Commun.* **10**, 650 (2019).
43. Cannon, A. et al. Desmoplasia in pancreatic ductal adenocarcinoma: insight into pathological function and therapeutic potential. *Genes Cancer* **9**, 78–86 (2018).
44. Elyada, E. et al. Cross-species single-cell analysis of pancreatic ductal adenocarcinoma reveals antigen-presenting cancer-associated fibroblasts. *Cancer Discov.* **9**, 1102–1123 (2019).
45. Miyazaki, Y. et al. Adipose-derived mesenchymal stem cells differentiate into heterogeneous cancer-associated fibroblasts in a stroma-rich xenograft model. *Sci. Rep.* **11**, 4690 (2021).
46. Wang, Y. et al. Single-cell analysis of pancreatic ductal adenocarcinoma identifies a novel fibroblast subtype associated with poor prognosis but better immunotherapy response. *Cell Discov.* **7**, 36 (2021).
47. Peng, J. et al. Single-cell RNA-seq highlights intra-tumoral heterogeneity and malignant progression in pancreatic ductal adenocarcinoma. *Cell Res.* **29**, 725–738 (2019).
48. Bernard, V. et al. Single-cell transcriptomics of pancreatic cancer precursors demonstrates epithelial and microenvironmental heterogeneity as an early event in neoplastic progression. *Clin. Cancer Res.* **25**, 2194–2205 (2019).
49. Hosein, A. N. et al. Cellular heterogeneity during mouse pancreatic ductal adenocarcinoma progression at single-cell resolution. *JCI Insight* **5**, e129212 (2019).
50. Dominguez, C. X. et al. Single-cell RNA sequencing reveals stromal evolution into LRRC15+ myofibroblasts as a determinant of patient response to cancer immunotherapy. *Cancer Discov.* **10**, 232–253 (2020).
51. Sebastian, A. et al. Single-cell transcriptomic analysis of tumor-derived fibroblasts and normal tissue-resident fibroblasts reveals fibroblast heterogeneity in breast cancer. *Cancers* **12**, 1307 (2020).
52. Peng, S., Hebert, L. L., Eschbacher, J. M. & Kim, S. Single-cell RNA sequencing of a postmenopausal normal breast tissue identifies multiple cell types that contribute to breast cancer. *Cancers* **12**, 3639 (2020).
53. Lu, T. et al. Single-cell transcriptome atlas of lung adenocarcinoma featured with ground glass nodules. *Cell Discov.* **6**, 69 (2020).
54. Zhang, M. et al. Single-cell transcriptomic architecture and intercellular crosstalk of human intrahepatic cholangiocarcinoma. *J. Hepatol.* **73**, 1118–1130 (2020).
55. Bhattacharjee, S. et al. Tumor restriction by type I collagen opposes tumor-promoting effects of cancer-associated fibroblasts. *J. Clin. Invest.* **131**, e146987 (2021).
56. Hornburg, M. et al. Single-cell dissection of cellular components and interactions shaping the tumor immune phenotypes in ovarian cancer. *Cancer Cell* **39**, 928–944 (2021).
57. Shih, A. J. et al. Identification of grade and origin specific cell populations in serous epithelial ovarian cancer by single cell RNA-seq. *PLoS ONE* **13**, e0206785 (2018).
58. Chen, Z. et al. Single-cell RNA sequencing highlights the role of inflammatory cancer-associated fibroblasts in bladder urothelial carcinoma. *Nat. Commun.* **11**, 5077 (2020).
59. Kumar, V. et al. Single-cell atlas of lineage states, tumor microenvironment, and subtype-specific expression programs in gastric cancer. *Cancer Discov* **12**, 670–691 (2021).
60. Grunberg, N. et al. Cancer-associated fibroblasts promote aggressive gastric cancer phenotypes via heat shock factor 1-mediated secretion of extracellular vesicles. *Cancer Res.* **81**, 1639–1653 (2021).
61. Farbehi, N. et al. Single-cell expression profiling reveals dynamic flux of cardiac stromal, vascular and immune cells in health and injury. *eLife* **8**, e43882 (2019).
62. Phan, Q. M., Sinha, S., Biernaskie, J. & Driskell, R. R. Single-cell transcriptomic analysis of small and large wounds reveals the distinct spatial organization of regenerative fibroblasts. *Exp. Dermatol.* **30**, 92–101 (2021).
63. Deng, C.-C. et al. Single-cell RNA-seq reveals fibroblast heterogeneity and increased mesenchymal fibroblasts in human fibrotic skin diseases. *Nat. Commun.* **12**, 3709 (2021).
64. Xie, T. et al. Single-cell deconvolution of fibroblast heterogeneity in mouse pulmonary fibrosis. *Cell Rep.* **22**, 3625–3640 (2018).
65. Krenkel, O., Hundertmark, J., Ritz, T. P., Weiskirchen, R. & Tacke, F. Single cell RNA sequencing identifies subsets of hepatic stellate cells and myofibroblasts in liver fibrosis. *Cells* **8**, 503 (2019).
66. Smillie, C. S. et al. Intra- and inter-cellular rewiring of the human colon during ulcerative colitis. *Cell* **178**, 714–730 (2019).
67. Martin, J. C. et al. Single-cell analysis of Crohn's disease lesions identifies a pathogenic cellular module associated with resistance to anti-TNF therapy. *Cell* **178**, 1493–1508 (2019).
68. Calon, A. et al. Stromal gene expression defines poor-prognosis subtypes in colorectal cancer. *Nat. Genet.* **47**, 320–329 (2015).
69. Erez, N., Truitt, M., Olson, P., Arron, S. T. & Hanahan, D. Cancer-associated fibroblasts are activated in incipient neoplasia to orchestrate tumor-promoting inflammation in an NF-κB-dependent manner. *Cancer Cell* **17**, 135–147 (2010).
70. Biffi, G. & Tuveson, D. A. Diversity and biology of cancer-associated fibroblasts. *Physiol. Rev.* **101**, 147–176 (2021).
71. Schlesinger, Y. et al. Single-cell transcriptomes of pancreatic preinvasive lesions and cancer reveal acinar metaplastic cells' heterogeneity. *Nat. Commun.* **11**, 4516 (2020).
72. Lin, W. et al. Single-cell transcriptome analysis of tumor and stromal compartments of pancreatic ductal adenocarcinoma primary tumors and metastatic lesions. *Genome Med.* **12**, 80 (2020).
73. Chen, K. et al. Single-cell RNA-seq reveals dynamic change in tumor microenvironment during pancreatic ductal adenocarcinoma malignant progression. *EBioMedicine* **66**, 103315 (2021).
74. Afshar-Kharghan, V. The role of the complement system in cancer. *J. Clin. Invest.* **127**, 780–789 (2017).
75. Biffi, G. et al. IL1-induced JAK/STAT signaling is antagonized by TGFβ to shape CAF heterogeneity in pancreatic ductal adenocarcinoma. *Cancer Discov.* **9**, 282–301 (2019).
76. Steele, N. G. et al. Inhibition of Hedgehog signaling alters fibroblast composition in pancreatic cancer. *Clin. Cancer Res.* **27**, 2023–2037 (2021).
77. Hutton, C. et al. Single-cell analysis defines a pancreatic fibroblast lineage that supports anti-tumor immunity. *Cancer Cell* **39**, 1244 (2021).
78. Kieffer, Y. et al. Single-cell analysis reveals fibroblast clusters linked to immunotherapy resistance in cancer. *Cancer Discov.* **10**, 1330–1351 (2020).
79. Wu, S. Z. et al. Stromal cell diversity associated with immune evasion in human triple-negative breast cancer. *EMBO J.* **39**, e104063 (2020).
80. Valdes-Mora, F. et al. Single-cell transcriptomics reveals involution mimicry during the specification of the basal breast cancer subtype. *Cell Rep.* **35**, 108945 (2021).
81. Givel, A. M. et al. miR200-regulated CXCL12β promotes fibroblast heterogeneity and immunosuppression in ovarian cancers. *Nat. Commun.* **9**, 1056 (2018).
82. Izar, B. et al. A single-cell landscape of high-grade serous ovarian cancer. *Nat. Med.* **26**, 1271–1279 (2020).
83. Bota-Rabassedas, N. et al. Contextual cues from cancer cells govern cancer-associated fibroblast heterogeneity. *Cell Rep.* **35**, 109009 (2021).
84. Xing, X. et al. Decoding the multicellular ecosystem of lung adenocarcinoma manifested as pulmonary subsolid nodules by single-cell RNA sequencing. *Sci. Adv.* **7**, eabd9738 (2021).

85. Kwon, O. J. et al. Functional heterogeneity of mouse prostate stromal cells revealed by single-cell RNA-seq. *iScience* **13**, 328–338 (2019).
86. Vickman, R. E. et al. Heterogeneity of human prostate carcinoma-associated fibroblasts implicates a role for subpopulations in myeloid cell recruitment. *Prostate* **80**, 173–185 (2020).
87. Banales, J. M. et al. Cholangiocarcinoma 2020: the next horizon in mechanisms and management. *Nat. Rev. Gastroenterol. Hepatol.* **17**, 557–588 (2020).
88. Massalha, H. et al. A single cell atlas of the human liver tumor microenvironment. *Mol. Syst. Biol.* **16**, e9682 (2020).
89. Affo, S. et al. Promotion of cholangiocarcinoma growth by diverse cancer-associated fibroblast subpopulations. *Cancer Cell* **39**, 866–882 (2021).
90. Kambayashi, T. & Laufer, T. M. Atypical MHC class II-expressing antigen-presenting cells: can anything replace a dendritic cell? *Nat. Rev. Immunol.* **14**, 719–730 (2014).
91. Kerdidani, D. et al. Lung tumor MHCII immunity depends on in situ antigen presentation by fibroblasts. *J. Exp. Med.* **219**, e20210815 (2022).
92. LeBleu, V. S. & Kalluri, R. A peek into cancer-associated fibroblasts: origins, functions and translational impact. *Dis. Model. Mech.* **11**, dmm029447 (2018).
93. Aoki, S. et al. Placental growth factor promotes tumour desmoplasia and treatment resistance in intrahepatic cholangiocarcinoma. *Gut* **71**, 185–193 (2021).
94. Gao, Y. et al. $CD63^{+}$ cancer-associated fibroblasts confer tamoxifen resistance to breast cancer cells through exosomal miR-22. *Adv. Sci.* **7**, 2002518 (2020).
95. Roulis, M. et al. Paracrine orchestration of intestinal tumorigenesis by a mesenchymal niche. *Nature* **580**, 524–529 (2020).
96. Brugger, M. D., Valenta, T., Fazilaty, H., Hausmann, G. & Basler, K. Distinct populations of crypt-associated fibroblasts act as signaling hubs to control colon homeostasis. *PLoS Biol.* **18**, e3001032 (2020).
97. Buechler, M. B. et al. Cross-tissue organization of the fibroblast lineage. *Nature* **593**, 575–579 (2021).
98. Purcell, J. W. et al. LRRC15 is a novel mesenchymal protein and stromal target for antibody–drug conjugates. *Cancer Res.* **78**, 4059–4072 (2018).
99. Lim, Y. W. et al. Single-cell transcriptomics reveals the effect of PD-L1/TGF-β blockade on the tumor microenvironment. *BMC Biol.* **19**, 107 (2021).
100. Helms, E. J. et al. Mesenchymal lineage heterogeneity underlies nonredundant functions of pancreatic cancer-associated fibroblasts. *Cancer Discov.* **12**, 484–501 (2022).
101. Karakasheva, T. A. et al. IL-6 mediates cross-talk between tumor cells and activated fibroblasts in the tumor microenvironment. *Cancer Res.* **78**, 4957–4970 (2018).
102. Wang, W. et al. Stromal induction of BRD4 phosphorylation results in chromatin remodeling and BET inhibitor resistance in colorectal cancer. *Nat. Commun.* **12**, 4441 (2021).
103. Herting, C. J., Karpovsky, I. & Lesinski, G. B. The tumor microenvironment in pancreatic ductal adenocarcinoma: current perspectives and future directions. *Cancer Metastasis Rev.* **40**, 675–689 (2021).
104. Gorchs, L. & Kaipe, H. Interactions between cancer-associated fibroblasts and T cells in the pancreatic tumor microenvironment and the role of chemokines. *Cancers* **13**, 2995 (2021).
105. Norton, J., Foster, D., Chinta, M., Titan, A. & Longaker, M. Pancreatic cancer associated fibroblasts (CAF): under-explored target for pancreatic cancer treatment. *Cancers* **12**, 1347 (2020).

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Competing interests

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