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Review

Fibroblasts as architects of cancer pathogenesis ☆

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ABSTRACT

Studies of epithelial cancers (i.e., carcinomas) traditionally focused on transformation of the epithelium (i.e., the cancer cells) and how aberrant signaling within the cancer cells modulates the surrounding tissue of origin. In more recent decades, the normal cells, blood vessels, molecules, and extracellular components that surround the tumor cells, collectively known as the "tumor microenvironment" or "stroma", have received increasing attention and are now thought to be key regulators of tumor initiation and progression. Of particular relevance to the work reviewed herein are the fibroblasts, which make up the major cell type within the microenvironment of most carcinomas. Due to their inherent heterogeneity, plasticity, and function, it is perhaps not surprising that fibroblasts are ideal modulators of normal and cancerous epithelium; however, these aspects also present challenges if we are to interrupt their tumor-supportive functions. Here, we review the current body of knowledge and the many questions that still remain about the special entity known as the cancer-associated fibroblast. This article is part of a Special Issue entitled: Fibrosis: Translation of basic research to human disease.

1. Introduction

1.1. Form and function — normal, activated, and cancer-associated fibroblasts

Fibroblasts are derived from the primitive mesenchyme, have an elongated, spindle-like morphology, and are metabolically active (the suffix "blast" typically denotes an active metabolism). As the most abundant cells of the connective tissue in animals, fibroblasts both synthesize and degrade extracellular matrix (ECM) components by expressing collagens, fibronectins, laminins, elastins, proteoglycans, integrins, matrix metalloproteinases (MMPs), tissue inhibitors of metalloproteinases (TIMPs), and a host of other ECM proteins that are expressed in a tissue-specific manner (reviewed in [1,2]). Consequently, fibroblasts are responsible for providing structural integrity to most tissues. Fibroblasts also produce the tissue-specific basement membrane that provides a protective barrier around the specialized epithelium, thereby contributing to specificity, polarity, and functionality of the epithelium [2]. There is also evidence indicating that fibroblasts communicate through the basement membrane to alter epithelial homeostasis, proliferation, and differentiation [3].

Fibroblast activity is crucial during processes of wound healing and inflammation. When tissue damage occurs, resident fibroblast populations proliferate and invade the injured area in response to platelet clotting. Platelets adhere to exposed subendothelium at sites of vessel injury and release their bioactive cargo (e.g., TGFβ1, PDGF, IL1-B, MMPs, TIMPs), predominantly from α-granules that degrade the basement membrane, induce cell proliferation and migration, and recruit inflammatory cells and fibroblasts (reviewed in [4]). Under such conditions, fibroblasts are generally considered to be "activated". In particular, as the healing process progresses, fibroblasts turn on expression of a filamentous actin, alpa-smooth muscle actin (αSMA), which enables them to exert contractile forces to close the wound. Local tissue contractility is mediated by focal adhesions between the activated fibroblasts – at this point called myofibroblasts – and the ECM. Moreover, contractile fibroblasts are known to regulate interstitial fluid volume and pressure via cytoskeletal infrastructure [5]. After wound closure, the balance of MMPs and TIMPs secreted by fibroblasts is changed to favor ECM degradation (as opposed to synthesis) which leads to massive apoptosis of the myofibroblast population. Consequently, only quiescent, non-contractile fibroblasts are left at the resolved wound site, and as such, myofibroblasts are only observed under pathological conditions.

The wound healing process seems to be co-opted by tumors; indeed, tumors have been likened to "wounds that never heal" [6]. However, unlike wound resolution in which fibroblasts "de-activate", the myofibroblast population persists during fibrosis or tumorigenesis for reasons that are not clear. It seems that normal fibroblasts have a bimodal effect on cancerous cells in that early in tumorigenesis,

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fibroblasts work against malignant progression, yet as the malignancy advances, fibroblasts are subverted to promote tumor growth — these tumor-supportive fibroblasts are referred to as cancer-associated fibroblasts (CAFs). In some cases, normal fibroblasts suppress malignant conversion of immortalized prostate epithelium [7], whereas in the breast, normal fibroblasts can induce the transition of already transformed ductal carcinoma in situ to invasive carcinoma [8]. The oncogenic transformation of the epithelium may subvert normal fibroblasts and potentiate their ability to promote tumor growth. Concordantly, one study has shown that suppression of the retinoblastoma (Rb) protein in pancreatic epithelium induces a selection pressure for fibroblasts that lack p53 and subsequently results in p53-inactivated epithelium [9]. Although the reasons why CAFs remain perpetually activated remain to be elucidated, it is very clear that fibroblasts participate in an elaborate, reciprocal crosstalk with the cancerous epithelium.

2. Cancer-associated fibroblasts — heterogeneity or a spectrum of phenotypes?

It is widely accepted that CAFs are a heterogeneous cell type and that this diversity may arise from their cell (s) of origin, the tissue in which they develop, or their activation state at any given time. This heterogeneity has presented challenges to precisely and exclusively identifying CAFs and to distinguishing them from other cell types that express similar markers upon histopathological analysis of tumors and tissues (Fig. 1). Instead, CAFs are more readily distinguished from their normal counterparts by their phenotype, proliferation rate, and differential expression of ECM constituents [10].

CAFs are most often denoted by expression of αSMA. Several additional markers are used to identify CAFs, including: vimentin, platelet-derived growth factor receptor alpha (PDGFR-α), platelet-derived growth factor receptor beta (PDGFR-β), fibroblast specific protein (FSP-1), and fibroblast activation protein (FAP) [11–14]. Nevertheless, no one marker specifically labels all CAFs or clearly distinguishes CAFs from normal fibroblasts or other closely-related cell types. These other cell types include pericytes (cells that line blood vessels, also known as mural cells), smooth muscle cells, epithelial cells that have undergone an epithelial-to-mesenchymal transition (EMT), myoepithelial cells (specifically in the breast), and some adipocytes (Fig. 1). Most often, in order to generally classify these various cell types, a combination of markers must be used. For example, αSMA-positive CAFs can be distinguished from pericytes, which stain positively for neuron glial antigen 2 (NG2) and regulator of G-protein signaling 5 (RGS5). RGS5 has been shown to be overexpressed in abnormal tumor vasculature and colocalizes predominantly with PECAM-1/CD31 and less so with PDGFR-α and αSMA [15]. Although some carcinoma cells express FSP-1, FSP-1-positive fibroblast sub-populations present in the tumor microenvironment have been shown to facilitate malignant progression. For example, in a syngeneic mouse model of melanoma, PDGF-CC signaling recruited fibroblasts with differential expression of FSP-1, PDGFR-α and αSMA [11]. Additionally, vimentin is expressed in most mesenchymal cell types as well as epithelial cells that have undergone an epithelial-to-mesenchymal transition (EMT). Due to the apparent heterogeneity of fibroblasts and their diverse origins, it has therefore been difficult to distinguish true fibroblasts from fibroblast-like cells. Moreover, identifying markers to label fibroblast sub-populations that exclusively contribute to cancer progression in various organs has presented challenges (Fig. 1).

Molecular profiling studies have also revealed the heterogeneity of fibroblast and CAF populations, yet have also suggested that core signatures, at least among sup-populations of fibroblasts, might predict tumor-supportive function. For example, gene expression analysis of fibroblasts isolated from breast cancer patient tumors yielded subtype-specific molecular signatures, especially with respect to expression of genes encoding cytoskeletal and integrin signaling proteins [16]. On the other hand, a study in which fibroblasts were isolated from ten different anatomical regions and exposed to serum (mimicking a wound response), revealed a common transcriptional signature, termed the fibroblast core serum response (CSR), that was also identified in CAFs isolated from various carcinomas and predicted metastatic progression in patients with breast, lung, and gastric cancers [16]. Similarly, differences in tumor-promoting ability were found between normal tissue fibroblasts and CAFs when examined for their prostaglandin (PGE2) secretory phenotype, which is elevated in tumors [17]. Two recent studies defined very similar CAF expression profiles that represented pro-inflammatory signatures also found in CAFs derived from cancer patients. In one study using a K14-HPV16 mouse model of multistep squamous skin carcinogenesis, this signature included: Cox2, IL-1β, OPN, IL-6, CXCL1/2 [18]. In the other study using a xenograft model of breast cancer progression, enhanced expression of many of these same proteins were found in CAFs relative to normal mammary fibroblasts [19]. Importantly, this second study also identified the molecular modulator that caused fibroblasts to adopt this pro-tumorigenic CAF signature — the secreted growth factor, granulin (GRN) [19]. Hence, common biological responses of fibroblasts to their microenvironmental cues (e.g., serum exposure) might reveal how fibroblasts acquire their CAF phenotypes. However, these responses seem restricted to different subpopulations of fibroblasts. Given this diversity of biological functions and their obvious heterogeneity, markers and methods to identify different CAF populations for therapeutic purposes, while challenging, would seem of utmost importance.

3. Fibroblasts in cancer pathophysiology

It has long been thought that fibroblast behavior is dictated by the epithelium, but recently more attention has been paid to the possibility that fibroblasts actively drive tumorigenesis and cancer progression [8–11,20,21]. There is now evidence to suggest that fibroblasts play important roles during the entire course of tumor development, from the pre-neoplastic state until the terminal stage of cancer progression — metastasis.

3.1. Cancer initiation — do fibroblasts direct tumorigenesis?

Tumor initiation is typically conceptualized as the accumulation of genetic and epigenetic mutations in the epithelium that results in recruitment of a reactive stroma. While the role of fibroblasts in de novo transformation or induction of carcinoma from epithelium lacking oncogenic mutation is currently debated, some studies have shown that fibroblasts facilitate carcinoma formation from epithelium that is cancer-prone.

Studies of prostate cancer have demonstrated that isolated CAFs, but not normal fibroblasts, can induce the transformation of immortalized epithelial cells [20,22]. Transgenic mouse models have provided some insights into CAF-derived factors that are responsible for tumor initiation. For example, Wnt1 overexpression in fibroblasts transforms mammary epithelial cells from C57BL/6 mice [23]. Additionally, overexpression of HGF and/or TGFβ in fibroblasts was demonstrated to be sufficient for inducing ductal carcinoma in situ (DCIS), adenocarcinoma, and poorly differentiated tumors in the breast [24]. Knockout models and depletion experiments have also demonstrated the importance of fibroblast activation in tumorigenesis. One study using FSP-1-deficient mice showed reduced tumor growth and attenuated metastatic potential of an otherwise highly metastatic murine mammary carcinoma cell-line, whereas injection of wild type fibroblasts partially rescued this effect [25]. Furthermore, knockout of TGFβRII in FSP-1-positive cells promoted prostate neoplasia and forestomach squamous cell carcinoma [10].

A recent study using mice containing conditional alleles of *Pten* and an *Fsp-cre* transgene, showed that inactivation of PTEN specifically in mammary fibroblasts significantly increased the incidence and rate of progression to adenocarcinoma of MMTV-ErbB2/Neu-driven tumors [21]. Upon examination of the pre-neoplastic mammary glands of the mice in this study, significant increases in ECM

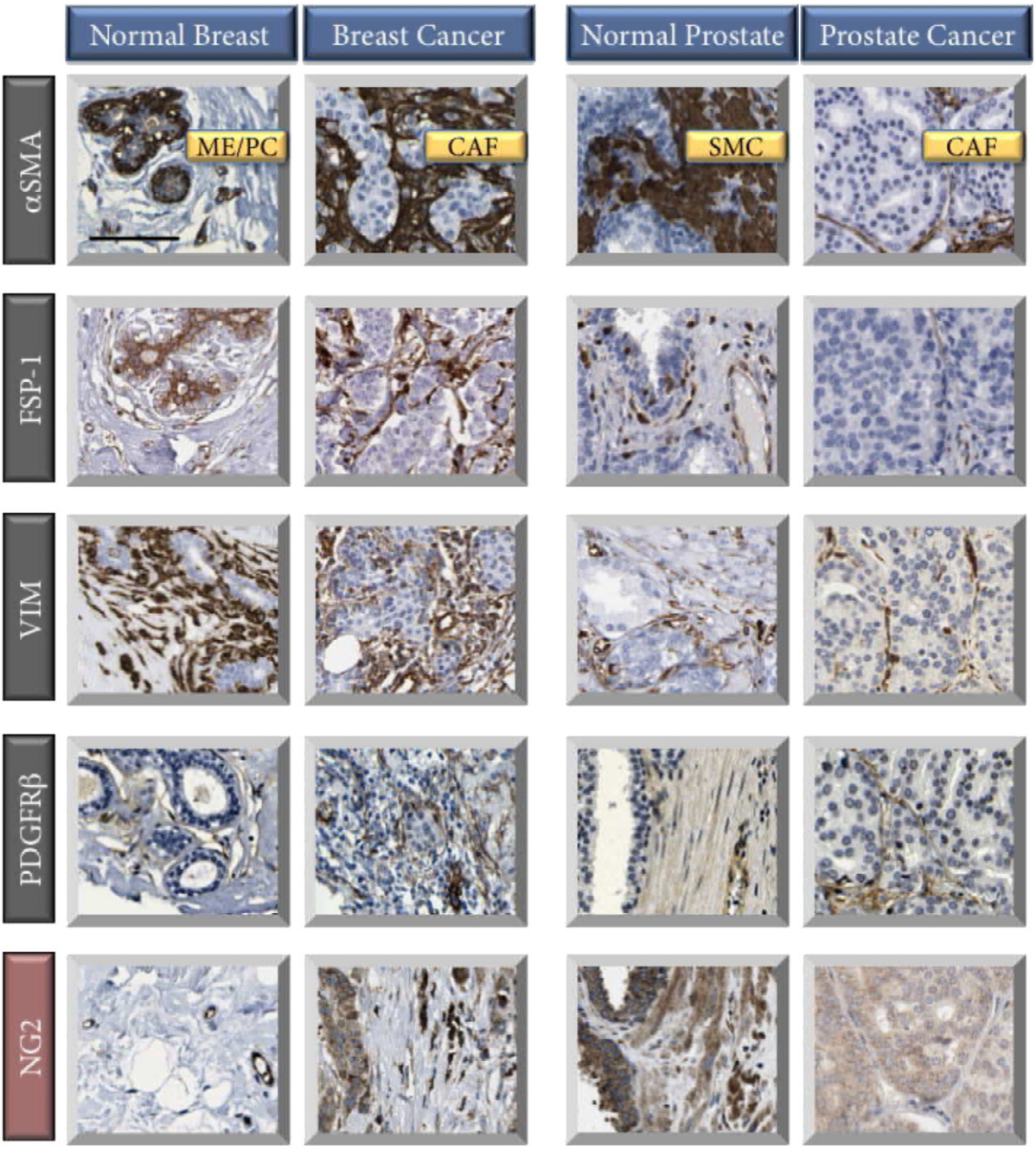

Fig. 1. Histological images of sections from human tissue surgical specimens that were immunohistochemically stained with some markers commonly used to identify cancer-associated fibroblasts: alpha-smooth muscle actin (αSMA), fibroblast-specific protein-1 (FSP-1), vimentin (VIM), platelet-derived growth factor receptor beta (PDGFR-β); and pericytes: neuron glial antigen 2 (NG2). As discussed herein, no one marker specifically labels all CAFs or clearly distinguishes CAFs from normal fibroblasts or other closely related cell types. For example, αSMA not only denotes cancer-associated fibroblasts (CAFs), but also myoepithelial cells (ME), pericytes (PC), and smooth muscle cells (SMC) in both normal and cancer tissues. Shown is normal breast tissue is from a female, age 23 (with the exception of the vimentin stain, which is from a 45 year old woman); normal prostate tissue is from a 51 year old man; cancerous breast and prostate tumor tissues are from a 40 year old woman with ductal carcinoma and a 64 year old man with high grade adenocarcinoma, respectively. Positive staining for indicated proteins appears brown. Tissue sections were counterstained with hematoxylin (blue) to indicate cell nuclei and extracellular material. Scale bar = 100 μm. Images were adapted and used with permission from The Human Protein Atlas (www.proteinatlas.org) and only protein stainings with annotated expression ranked as high reliability are displayed.

remodeling and immune cell infiltration were observed. Furthermore, the PTEN-specific gene signature of the pre-neoplastic fibroblasts from these mice was remarkably similar to stromal signatures found in patients with breast cancer. However, as mentioned earlier, FSP-1 marks both normal and activated fibroblasts, as well as some epithelial cells, in the mammary gland [26], so it is not clear that the accelerated growth was due exclusively to the PTEN-null fibroblasts.

Finally, a number of studies have implicated reactive stroma, including activated fibroblasts, in accelerating the appearance of carcinoma (reviewed in [27]). Many of these studies incorporate the use of irradiated fibroblasts with pre-transformed epithelium that bear mutations in tumor suppressor genes. The most recent example comes from a study in which Trp53-deficient mammary epithelilum was transplanted into irradiated hosts (whole body irradiation) that had previously undergone mammary fat pad clearance [28]. In this study, estrogen receptor-negative tumors developed with increased incidence and at an accelerated pace above controls. Another type of reactive fibroblast, the senescent fibroblast, has also been implicated in

driving cancer progression from pre-malignant epithelium. A recent study using a model of skin carcinogenesis elegantly showed that senescent fibroblasts drove progression of pre-neoplastic epithelium in an osteopontin-dependent manner [29].

3.2. Early stage cancer — fibroblasts pave the way for cancer

There is clear evidence that CAFs play an important role during the early stages of tumorigenesis. As tumors progress, the architecture of the host tissue becomes highly distorted with aberrant accumulation of ECM components. One hallmark of this process within the tumor microenvironment is collagen cross-linking. Hints about the molecular basis of this come from experimental models showing that CAFs are a major source of lysyl-oxidase (LOX), which cross-links collagen, and that inhibiting LOX delays the onset of breast cancer [30]. In fact, LOX activity has been observed in many types of aggressively growing cancers [30]. Likewise, in breast and colon cancer models, CAFs express uPA and uPAR, which modulate ECM degradation, which, in turn, increases the bioavailability of growth factors that are typically sequestered by the ECM [31].

In addition to modulating ECM components, CAFs are known to regulate proliferation of the epithelium. In some cases, enhanced proliferation is the result of direct paracrine interactions between tumor cells and CAFs, and in others, it appears to be an indirect effect of inflammatory processes in which CAFs serve as mediators (discussed in more detail later). Under various contexts, CAFs produce a repertoire of growth factors and cytokines that influence the behavior of the epithelium, including HGF, EGF, IGFs, IGFBPs, b-FGF, and TGFβ, to name a few (reviewed in [20]). In other contexts, inflammatory processes that impinge upon fibroblasts endow them with the ability to directly affect tumor cell behavior. For example, generation of reactive oxygen species (ROS) in the inflammatory microenvironment has been shown to modulate CAF transdifferentiation, thereby enhancing their tumor-promoting functions [32,33]. Interestingly, it has also been shown that induction of autophagy in the CAF compartment helps to promote tumor cell survival via processes involving downregulation of caveolin-1 and the subsequent stabilization of hypoxia-inducible factor-1 alpha (HIF1-α) [34].

3.3. CAFs in cancer progression and metastasis

Metastasis, which is the leading cause of death in patients with solid tumors, is a highly inefficient process, and it is estimated that less than 1% of disseminated tumor cells are able to form viable metastases. Not surprisingly, successful metastatic progression also involves fibroblasts. Within the primary tumor site, it has been shown that factors secreted by CAFs, including TGFβ-1, induce tumor cells to undergo EMT, thereby promoting tumor cell motility, invasion, and metastasis [35,36]. Likewise, knockout of one allele of TGFβRII in FSP-1-positive cells yielded more metastases in the MMTV-PyMT mammary tumor model [37]. Underscoring these results, transgenic mice deficient in FSP-1 cells did not incur metastases when engrafted with a highly metastatic murine mammary carcinoma cells [25]. As primary modulators of the ECM, as mentioned earlier, CAFs certainly help tumor cells to invade the surrounding tissue by forming the invasive front [38]. Indeed, it was demonstrated that CAF expression of caveolin-1 leads to matrix remodeling, invasiveness, and increased metastases in mice injected with breast adenocarcinoma cells [39].

Fibroblasts likely play a role at metastatic sites as well, as activated fibroblasts have been observed at metastatic sites where they promote tumor cell proliferation [40] . Fibroblast production of periostin at the site of a micrometastasis was shown to be crucial for metastatic colonization and maintenance of cancer stem cell-like properties, which are thought to be necessary for initiation of a new tumor [41]. Other studies have shown that systemic signaling cascades that operate as a consequence to the body's response to cancer, aid construction of the tumor-supportive microenvironment, including CAFs, at metastatic sites [19,42]. Such studies have raised important questions that have yet to be fully elucidated, such as: what is the source of CAFs found in metastatic sites? Do disseminated tumor cells have to "start all over again" to build their specialized CAF compartment? One early study suggested the interesting possibility that tumor cells take their stroma, including CAFs, with them [43]. The stromal requirements of micrometastases is a rate limiting step in the colonization of secondary organs and fully elucidating the mechanisms by which normal stromal compartments of the secondary organ prevent or promote metastatic growth is crucial to developing therapies against this life threatening condition.

4. Tracing the origins of CAFs

Perhaps one of the most asked questions in the study of tumor microenvironment is: what is the source of cancer-associated fibroblasts? It is likely that CAFs are derived from a variety of sources. The origin of myofibroblasts in fibrosis has been extensively studied and has provided insight, both technically and conceptually, into the source of CAFs in cancer. Currently, there are three prevailing hypotheses regarding the source of activated fibroblasts in tumors. The first, and most commonly studied, is that CAFs derive from resident cells that are recruited from local sources into the tumor microenvironment by the malignant epithelium. These resident cells might include normal tissue fibroblasts (as discussed in detail above), and tissue mesenchymal stem cells (MSCs). Second, circulating fibrocyte progenitors and bone marrow-derived cells have been demonstrated to extravasate into the tissue where the tumor resides and differentiate into cells with fibroblast-like phenotypes. A third and final scenario is that other cell types, such as pericytes, myoepithelial cells, and endothelial cells transdifferentiate to give rise to the tumor fibroblast-like population.

4.1. Calling upon resident fibroblasts

As discussed at length earlier, several lines of evidence suggest that activation of resident fibroblast populations give rise to CAFs. Studies of the origin of fibroblasts during wound healing and organ fibrosis have helped shed light on how this process occurs in cancer. As stated earlier, the tumor and fibrotic microenvironment is a key regulator of pathological progression. Resident fibroblasts surrounding the malignancy are commonly thought to be the first responders to the site of insult that the tumor provides.

Although not well understood, resident mesenchymal stem/progenitor cells (MSCs) have been shown to contribute to tumor growth in various model systems [44], suggesting that these cells, which share a lineage relationship with fibroblasts, might provide a source of CAFs. MSCs have been shown to differentiate into myofibroblast-like cells (marked by αSMA and collagen 1) [45] and to secrete tumor-promoting factors, including VEGF, IL-8, HGF and IGF-1 [46]. It is not clear whether resident MSCs differentiate into CAFs for several reasons. First, techniques to isolate MSCs that can differentiate into the various lineages from resident tissue is challenging and it is not clear which cell-surface markers would clearly and exclusively distinguish MSCs, if possible. Second, once MSCs are brought into cell culture, they are highly susceptible to differentiation and selection pressures.

4.2. Bone marrow and circulating cells as CAF reservoirs

As vasculature in tumors is highly permeable, the influx of circulating cells increases and the relative contribution of bone marrow-derived cells to the tumor stroma also increases. The contribution of bone marrow-derived cells to the CAF population is debated and appears to be context-dependent. In a study of inflammation-induced gastric cancer, up to 20% of the αSMA+ in the tumor stroma were shown to be derived from bone marrow mesenchymal progenitor cells [47]. Yet studies of pancreatic and other cancers demonstrated that bone marrow-derived cells contribute a small percentage of the

CAF population [48,49]. A study of breast cancer, in which tumors formed with a highly reactive, myofibroblast-rich stroma, demonstrated that none of the αSMA+ population was of bone marrow origin [19]. However, interesting studies in patients who had previously received a bone marrow transplant from a donor of the opposite sex indicated that some tissue fibroblasts were of donor origin [50,51].

Bone marrow-derived MSCs have been of interest due to their ability to home to sites of inflammation, tissue repair, and neoplasia in experimental models [52–54]. Other studies have identified some of the growth factors and cytokines that attract MSCs to tumor sites (e.g., VEGF, EGF, HGF, b-FGF, PDGF, CCL2) [55–57]. In studies using an ovarian cancer cell-line, Skov3, admixed with human MSCs, the resulting tumor microenvironment harbored abundant cells with both CAF and pericyte-like phenotypes [57]; however, this study did not determine whether the CAFs arose from the implanted MSCs or from recruitment of other host cells that gave rise to the CAF population. Bone marrow-derived MSCs have also been shown to play an important role in tumor progression [58]. Nevertheless, whether or not MSCs should be equated with CAFs is not yet clear.

Circulating fibrocytes have also been considered as a source of CAFs, as they have been shown to be recruited to injured tissues [59,60] and to contribute to the αSMA myofibroblasts observed in a mouse model of allergic asthma [61]. Furthermore, in a model of bleomycin-induced lung fibrosis, GFP bone marrow chimera mice have elevated levels of GFP+, collagen 1 producing fibroblasts in the lungs [62]. Fibrocytes are thought to be of bone marrow origin due to their expression of leukocyte marker CD45, bone marrow stem cell antigen CD34, CD11b, and fibroblastic markers such as vimentin, collagen I/III and fibronection (reviewed in [63]). As fibrocytes can lose CD45 and CD34 markers when in circulation, it is likely that the cumulative effects of fibrocytes in tissue fibrosis and cancer are unrecognized.

4.3. Transdifferentiation of resident tissue cells

The plasticity of various epithelial and mesenchymal cell types during pathological processes has become a topic of intense investigation. A number of cell types found in the tumor microenvironment or in proximal tissue have been proposed as candidates for the CAF compartment. These cells include: pericytes, adipocytes, endothelial cells, and even the epithelial cancer cells.

Pericytes, which inherently express αSMA, have been of particular interest as a speculated source of fibroblasts in recent studies, mainly due to their reported functions in other pathological conditions. One study utilized lineage tracing of pericytes/perivascular cells to demonstrate their transdifferentiaton into interstitial, proliferative myofibroblast-like cells in kidney fibrosis [64]. Additionally, ADAM12+ perivascular cells in fibrotic regions have been shown to undergo a progressive differentiation program into myofibroblasts [65]. Furthermore, pericytes have been shown to contribute to scar formation in spinal cord injury [66]. Pericytes are known to detach from tumor vasculature, thereby contributing to the inherent "leakiness" of tumor vasculature [67] [68], leading to the idea that these pericytes, should they remain viable in the tumor microenvironment, might give rise to cells with a CAF-like phenotype. Nevertheless, whether or not pericytes contribute in a major way to the CAF population remains to be determined conclusively.

Given their mesenchymal lineage relationship with fibroblasts, tissue adipocytes have been proposed as a likely source of CAFs. For example, in a breast cancer model, local adipose tissue contributed ~29% of the αSMA-positive population and comprised ~27% of the NG2-positive cells [69]. Moreover, adipose precursor cells have been shown to induce expression of fibronectin, αSMA, and vimentin in 4T1 murine mammary carcinoma cells, consistent with tumor-associated fibroblastic cells [70]. Finally, one study demonstrated that breast tumor-derived TNF-α and IL-11 prevented differentiation of adipocyte precursors, causing them to expand as a fibroblastic population to contribute to the desmoplastic stroma [71]. Nevertheless, direct proof that mature adipocytes de-differentiate within the tumor microenvironment (e.g., in the mammary gland) to give rise to the CAF population has not been provided as of yet.

One lineage tracing experiment in a model of kidney fibrosis demonstrated that endothelial cells acquire fibroblast markers during a processes coined "EndMT" — endothelial to mesenchymal transition [72]. In this context, endothelial cells underwent a partial transdifferentiation to acquire fibroblastic markers αSMA, FSP-1, vimentin, and N-cadherin, while retaining endothelial markers VE-Cadherin, CD31, Tie1, and Tie2 (reviewed in [73]). A related study showed that up to 40% of CAFs arise as a consequence of EndMT in two different murine cancer models [74].

Perhaps the most accepted transdifferentiation program occurs during embryonic development when epithelial cells transition into mesenchymal cells, in a process termed the epithelial-to-mesenchymal transition (EMT). The existence of a permanent and irreversible EMT in adult tissue is often debated. Recently, the EMT has been classified into three functionally different processes that occur in distinct biological settings [35]. Type 1 which occurs during implantation, embryogenesis, and organ development is well studied and will not be discussed here (reviewed in [35,75]). Type 2 occurs during tissue regeneration and fibrosis (*discussed by other authors in this special issue*). Type 3, discussed below, is associated with tumor progression and metastasis. As discussed earlier, in general, the cancer research community views EMT as a process by which cancerous epithelial cells undergo a partial, and possibly reversible, transition to a mesenchymal-like state for the puposes of invasion and metastasis. Epithelial cells that undergo EMT in vitro are marked by acquisition of mesenchymal markers (e.g., αSMA, FSP-1, vimentin, desmin, and N-cadherin) and loss of epithelial markers (e.g., E-cadherin), while for the most part, retaining expression of epithelial-specific cytokeratins (reviewed in [75]). It has been suggested that epithelial cells undergo an EMT after exposure to oxidative stress induced my MMPs to become myofibroblasts [76]. The EMT phenotype therefore supports functions that are not normal to terminally differentiated epithelial cells, such as anchorage-independent survival, loss of homotypic cell-cell contact, cell motility, invasion, and ability to breach the basement membrane (reviewed in [35,77,78]), several properties that are characteristics of fibroblast-like cells. Indeed, an epithelial cell-line derived from a breast cancer biopsy displayed fibroblast characteristics and had the ability to differentiate into myofibroblasts [79]. Cancer cells that have undergone EMT are typically found at the invasive front of tumors in various model systems, further supporting their role in the metastatic process. At this point, while it is generally thought that the EMT is important for cancer progression, it is still debated whether cells that have undergone an EMT fill the role of CAFs.

The underlying heterogeneity of CAFs and the promiscuity of markers that are used to identify them (Fig. 1) also present challenges to identifying their origins. Additional studies using transgenic models and in vivo lineage-tracing techniques that incorporate precise, well-accepted, cell-specific promoters might help us find more answers to the questions about the origins of CAFs.

5. Interactions in the tumor microenvironment

Crosstalk within the tumor microenvironment is not limited to paracrine signaling between CAFs and malignant cells, but also occurs between different resident and distant stromal cell types. CAFs actively engage these multiple other stromal cell types, including endothelial cells, bone marrow-derived cells (BMDCs) and inflammatory cells, in order to stimulate cancer growth, angiogenesis and metastatic spread.

5.1. CAFs and endothelial cells

Cancer-associated fibroblasts participate in processes supporting tumor growth, angiogenesis and progression through multiple and principally different mechanisms involving crosstalk with other cellular

compartments within the tumor microenvironment. In particular, CAFs have been highlighted as providers of a multitude of pro-angiogenic cues (growth factors, proteases, extra-cellular matrix constituents, cellular recruitment) in various tumor types. The stimulation of angiogenesis afforded by stromal fibroblasts is in line with their primary localization at the leading edge of tumors, where there is a manifested demand for an expanded vascular supply [38,80]. CAFs secrete and deposit numerous growth factors in the tumor microenvironment that stimulate endothelial cell growth and angiogenesis. Studies using mice genetically engineered with a reporter for vascular endothelial growth factor (VEGF)-A have demonstrated dramatic induction of VEGF-A transcription in the stroma of both spontaneously arising, as well as implanted tumors [81]. Indeed, stromal cells in ovarian carcinomas provide most angiogenic growth factors in higher quantities than do the overt malignant cells [82]. Provision of angiopoietin-1 and -2 by CAFs acts to stabilize the neo-vasculature of ovarian carcinomas [83], indicating that fibroblasts in this context also stimulate vessel patency.

While neo-angiogenesis is typically initiated by the hypoxic tumor microenvironment, CAFs are also in many cases induced to secrete pro-angiogenic factors in response to paracrine signaling events. Production of various isoforms of the platelet-derived growth factor (PDGF) family by malignant cells serves to recruit and activate pro-angiogenic CAFs in various cancers [84]. Hence, paracrine PDGF stimulation of CAFs induces production of the prototypical pro-angiogenic inducer fibroblast growth factor (FGF)-2 in both cervical carcinomas and melanoma [11,85].

Interestingly, CAFs activated by PDGF-CC in melanomas also secrete the extracellular matrix protein osteopontin, the action of which is known to synergistically stimulate angiogenesis together with FGF-2 and promote autocrine VEGF-A signaling in endothelial cells [11,86–88]. Consistent with the role of PDGFs as essential upstream mediators of angiogenic cues provided by CAFs, malignant cells genetically deficient for VEGF-A make up for this lack by paracrine activation of PDGFR-α in CAFs, thus stimulating production of stromal VEGF-A [89,90]. Moreover, autocrine stimulation of CAFs by the cytokine CXCL14 in prostate cancers also results in induction of FGF-2 and subsequent neo-angiogenesis [91].

Finally, CAFs indirectly regulate the process of angiogenesis through abundant provision of extracellular matrix (ECM) products, such as collagen (s), osteopontin and tenascin-C, as well as by secreting matrix-remodeling proteases, including members of the matrix metalloproteinase (MMP) and the a disintegrin and metallo-proteinase (ADAM) families [92]. Paradoxically, excessive production of ECM and a prolonged fibrotic reaction is detrimental to the angiogenic process, most likely by physically restricting endothelial sprout formation and tip cell migration, and attenuation of the desmoplastic response in pancreatic adenocarincomas through inhibition of hedgehog signaling alleviates the angiogenic blockade and improves tumor perfusion [93,94]. Taken together, our current knowledge points towards a pivotal role for stromal fibroblasts in releasing tumors from dormancy through activation of the angiogenic switch, and identifies CAF-endothelial cell crosstalk as a hitherto unexploited target for cancer therapy.

5.2. CAFs and bone marrow-derived cells

In addition to supporting tumor growth through orchestration of paracrine signaling networks within the tumor parenchyma, CAFs mediate long distance effects through mobilization of various BMDCs. Strikingly, CAFs, but not normal fibroblasts, secrete stromal cell-derived factor (SDF)-1 in order to recruit $Sca1^{+}CD31^{+}$ BMDCs that act as endothelial progenitors and incorporate into the neo-vasculature [95]. In this study, SDF-1 was specifically expressed by αSMA-positive myofibroblasts.

In addition to CAFs affecting BMDCs, BMDCs can affect the CAF phenotype. Interestingly, recruitment of a distinct set of $Sca1^{+}c\text{-}Kit^{-}$ hematopoietic BMDCs induces myofibroblast differentiation and expression of αSMA through secretion of granulin (GRN) [19]. The mature myofibroblast component subsequently promotes multiple aspects of tumor growth, angiogenesis and progression systemically through production of an array of cytokines and growth factors, including CXCL1, CXCL2, IL1A and B, IL6 and IL8 [19]. Thus, reciprocal signaling between CAFs and BMDCs represents a conceptually novel and viable cancer drug target, e.g., through targeting of SDF-1 or GRN.

5.3. CAFs and immune modulation

It is increasingly appreciated that phenotypic modulation of the immune response is a feature of most, if not all, cancers [96]. CAFs manipulate the inflammatory microenvironment through two principally distinct mechanisms. Firstly, CAFs harbor a pro-inflammatory expression profile that serves to recruit macrophages, neutrophils and other stimulatory immune cells that promote various aspects of tumor progression [19,97,98]. Notably, CAF-derived S100A4 mediates tumor infiltration of T-cells, the action of which promotes lung metastatic formation in experimental breast cancers [99].

Secondly, immune editing by CAF-derived factors act to suppress tumor detection and rejection by the host immune system [100]. In this context, stromal fibroblasts from melanoma, but not from normal skin, impede NK cell cytotoxicity both through cell-to-cell contacts and through release of PGE_2 [101]. In addition, stromal cells expressing FAP orchestrate an immuno-suppressive tumor microenvironment in pancreatic adenocarcinomas [102]. In accordance with their role as modulators of the immune response in tumors, depletion of CAFs from experimental breast cancers – via DNA vaccination against FAP – results in suppression of metastatic spread through shifting the polarization of the immune microenvironment from Th2 to Th1 [103].

6. The CAF as a clinical entity

Given the importance of stromal support in carcinogenesis and tumor progression, CAFs are thought to provide an important target for therapeutic strategies to improve outcomes for cancer patients. In the normal breast, fibroblasts are quite abundant and, in the case of carcinogenesis, infiltration of CAFs and their ECM is the reason why breast cancer often presents as a palpable lump. Additionally, high mammographic density is associated with an increased risk of developing breast cancer [104]. Recent clinical and translational studies have made it clear that the extent of stromal desmoplasia is linked to prognosis and that molecular profiling of stromal markers is predictive of outcome [105,106]. In the clinic, a myofibroblast-rich, reactive stroma is almost always found in human adenocarcinomas and is associated with invasiveness and poor prognosis [20,107].

Understanding the heterogeneity within fibroblast populations will be important if we are to effectively target the tumor promoting functions of these cells. Indeed, fibroblasts express differential levels of key interleukins and chemokines in breast cancers of different subtypes (i.e., basal-like and luminal-like) [108]. An interesting recent study provided a plausible explanation for why triple negative breast cancer (ER−/PR−/HER2−; TNBC) is more prevalent among African American women than Caucasian women by showing that normal mammary fibroblasts from African American women support the growth of TNBC cells while those from Caucasian women did not [109]. As TNBC denotes poor prognosis and there are no targeted therapies against this kind of tumor, studies aiming to elucidate why certain fibroblasts promote one kind of disease or another are crucial.

Unfortunately, however, there are no current therapies deisgned to directly inhibit or eliminate tumor-promoting CAFs. Currently, an ongoing clinical trial is investigating the effect of irradiation of the area around non-invasive or early invasive breast cancer (http://www.clinicaltrials.gov). This trial is based on pre-clinical data that support the role of the microenvironment in progression and invasion of breast cancer; however, in some pre-clinical models irradiation of the mammary gland actually promotes transformation of immortalized

tumor cells [110]. A separate study on pancreatic tumors suggested that blocking HGF signaling in addition to irradiation of the stroma may help prevent invasion of cancerous epithelial cells [111].

Perhaps indirect targeting of other components in the tumor microenvironment will serve to inhibit CAF accumulation or function. For example, certain tyrosine kinase inhibitors that target signaling pathways (e.g., VEGFR, PDGFR) that are active in stromal cell populations (e.g. endothelial cells and fibroblasts) have been proposed. In the past decades, there has been much interest in the engineering of anti-angiogenesis molecules that prevent the angiogenic switch in early malignancies and thereby prevent tumor growth. Unfortunately, these efforts have been met with disappointment, as patients often acquire resistance to drugs like Avastin (VEGFA antibody), which has recently been removed as an FDA-approved therapy for breast cancer [112]. Studies show that cancer cells can evolve resistance to blockade of the VEGF pathway; however, concomitant inhibition of FGF and VEGF during this process results in tumor stasis and a reduction in tumor burden [113]. Conversely, results from pre-clinical models have led to suggestions that enhancing tumor vascularization (e.g., inhibiton of PDGF-B or hedgehog) might help improve delivery of chemotherapy, thereby attenuating tumor progression [94,114].

Targeting the structure and homeostasis of the abundant ECM found within tumors may one day prove useful in therapeutic approaches as well. For example, lysyl oxidase enzymes, responsible for collagen crosslinking, are elevated in many cancers [115]; however, the ECM turnover in these conditions is abnormal. Although strategies to inhibit MMP activity have been proposed [116], clinical trials using MMP inhibitors yielded disappointing results due to serious toxicity and lack of specificity [117].

Finally, targeting factors that promote and sustain the CAF phenotype, as demonstrated in pre-clinical models, might lead to novel therapeutic approaches. For example, targeting BMDC-derived granulin, which was shown to induce the tumor-promoting function of normal mammary fibroblasts, might provide a means to prevent CAF support of cancer progression [19]. Autocrine factors, such as TGFb and SDF-1, that drive and sustain CAF populations might provide a basis for exploring ways to inhibit the tumor support network [118]. Likewise, elucidating some of the currently unknown factors derived from cancer cells that promote CAF function [18,119] should also lead to identification of therapeutic candidates.

7. Perspectives

The aims of many pre-clinical cancer research efforts have been to elucidate mechanisms by which certain tumors progress toward life-threatening malignancy and acquire resistance to treatments. As such, many studies consider the variety of cell types that comprise a solid tumor. In that vein, if targeted therapies are to be successful, then one must take into account not only the molecular target of a given therapy, but also the patient population that is most likely to respond to that therapy. Also, delineating pathways that provide tumors with adaptive resistance to current therapies allows for the opportunity to attack tumors via multiple modalities. It is likely that understanding similarities between cancer pathogenesis, fibrosis, and wound healing will be a step in that direction. Defining CAFs, understanding their behavior, and elucidating how they arise are one part of this challenging task and hold the promise of designing ways to attack tumor-supportive CAFs, perhaps while sparing normal fibroblasts that promote homeostasis.

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