HHS Public Access

Author manuscript

Cancer Metastasis Rev. Author manuscript; available in PMC 2015 May 15.

Published in final edited form as:

Cancer Metastasis Rev. 2013 June ; 32(0): 303–315. doi:10.1007/s10555-012-9415-3.

Stroma Cells in Tumor Microenvironment and Breast Cancer

Yan Mao[1], **Evan T. Keller**[2], **David H. Garfield**[1,3], **Kunwei Shen**[1,*], and **Jianhua Wang**[4,*]

[1]Shanghai Ruijin Hospital, Comprehensive Breast Health Center, Shanghai, 200025, China.

[2]Department of Urology, University of Michigan, Ann Arbor, Michigan, 48109, USA.

[3] University of Colorado Comprehensive Cancer Center, Aurora, Colorado, 80045, USA.

[4] Department of Biochemistry and Molecular & Cell Biology, Shanghai Jiao Tong University School of Medicine, Shanghai, 200025, China.

Abstract

Cancer is a systemic disease, encompassing multiple components of both tumor cells themselves and host stromal cells. It is now clear that stromal cells in the tumor microenvironment play an important role in cancer development. Molecular events through which reactive stromal cells affect cancer cells can be defined so that biomarkers and therapeutic targets can be identified. Cancer-associated fibroblasts (CAFs) make up the bulk of cancer stroma and affect the tumor microenvironment such that they promote cancer initiation, angiogenesis, invasion and metastasis. In breast cancer, CAFs not only promote tumor progression, but also induce therapeutic resistances. Accordingly, targeting CAFs provides a novel way to control tumors with therapeutic resistances. This review summarizes the current understanding of tumor stroma in breast cancer with a particular emphasis on the role of CAFs and the therapeutic implications of CAFs. The effects of other stromal components such as endothelial cells, macrophages and adipocytes in breast cancer are also discussed. Finally, we describe the biologic markers to sort patients into a specific and confirmed subtype for personalized treatment.

Keywords

Stroma cells; Tumor microenvironment; Cancer-associated fibroblasts (CAFs); Breast cancer

1. Introduction

Breast cancer is one of the most common causes of cancer-related death in women over the world. Approximately 230,000 new cases of invasive breast cancer are expected to be diagnosed in the United States in 2012, and almost 40,000 woman will die from this disease [1]. Although therapeutic approaches, such as surgery, chemotherapy, radiation, endocrine therapy and targeted therapy, have reduced cancer-specific mortality, there still are many therapeutic failures which result in cancer recurrence metastasis and death.

[*]Corresponding author: Jianhua Wang, Ph.D, Shanghai Jiao Tong University School of Medicine, Shanghai, 200025, China. Phone: 021-54660871; Fax: 021-63842157; jianhuaw2007@gmail.com Or Kunwei Shen, M.D, Shanghai Ruijin Hospital, Comprehensive Breast Health Center, Shanghai, 200025, China. kwshen@medmail.com.cn.

Conflict of interest statement: The author declares that there are no conflicts of interest.

The “seed and soil” hypothesis postulates that an appropriate host microenvironment (the soil) is needed for optimal growth of tumor cells (the seed) [2]. In the past four decades, many researchers have focused primarily on the tumor cells. However, emerging evidence indicates that tumors are composed of tumor parenchyma and stroma two discrete but interactive parts that crosstalk to promote tumor growth. Recently, many investigations support the notion that tumor stromal cells play important roles in tumor initiation, progression and metastasis. Cancer-associated fibroblasts (CAFs) are the most frequent component of tumor stroma, especially in breast and pancreatic cancer [3, 4]. Increasing data indicates that the depletion of fibroblast activation protein (FAP)-expressing tumor stromal cells led to stunted tumor growth and improved response to tumor vaccination providing evidence that the tumor microenvironment is fertile ground for development of novel therapies with the potential of augmenting existing treatment and prevention options [4, 5]. Actually, some new related therapeutic targets have been developed and are under pre-clinical evaluation and clinical trials as shown in **Table 1**. Herein we review the current understandings of tumor stroma interacting with breast cancer cells, with special focus on CAFs. In addition, we also review new emerging fields in breast cancer therapy associated with other tumor stromal cells.

2. Cancer-associated fibroblasts (CAFs)

2.1 Origin and markers of CAFs

Fibroblasts are the most abundant cells in connective tissues and form framework of tissues by secreting extracellular matrix (ECM) components [6]. In the past years, fibroblasts were found to be activated in wound healing and fibrosis with increasing expression of alpha smooth muscle actin (α-SMA) and the ED-A splice of fibronectin [6]. Currently, in agreement with the concept that tumors are similar to a chronic non-healing wound, fibroblasts have been found to be activated in cancer. These activated fibroblasts, termed cancer-associated fibroblasts (CAFs) [7], share many similarities with activated fibroblasts found in wounds and inflammatory sites. Currently, there is no precise definition of CAFs because of different cellular origins and expression markers. As shown in **Figure 1**, some evidence suggests that the origins of CAFs are 1) from activated resident fibroblasts; 2) from bone-marrow-derived mesenchymal stem cells (MSCs); 3) from cancer cells that undergo epithelial-mesenchymal transition (EMT); and 4) other mechanisms. For the first origin, there is evidence suggesting that the activation of resident fibroblasts is induced by many cancer-secreted factors, such as TGF-β and CXCL12/SDF-1[8] or by losing suppressor genes, such as PTEN, CAV-1, p53 and p21 [[9-15]. These hypotheses are also consistent with breast cancer xenograft models [8]. For the second source of CAFs, one study shows that *in vivo*, labeled MSCs have been found localized within tumor mass and differentiated into CAFs and pericytes with high expression of α-SMA, FAP, tenacin-C, etc [16], and moreover, TGF-β1 from the conditioned medium (CM) of MCF7 and MDA-MB-231 promote differentiation of human adipose tissue-derived stem cells (hASCs) into a CAF-like myofibroblastic phenotype (e.g., expression of α-SMA and tenascin-C) via Smad3 [17], which suggest that CAFs also origin from other kinds of stem cells. The third source of CAFs is from malignant tumor cells that undergo EMT changes [3, 16]. Malignant epithelial cancer cells can obtain high invasive and metastatic characteristics by exposure to many

factors (i.e. PDGF, TGF-β, EGF, etc). Moreover, CAFs may arise from endothelial cells by endothelial to mesenchymal transition (EndMT) with CD31 loss and α-SMA, fibroblast specific protein (FSP)-1 high expression [18]. At present, no evidence suggests which origin of CAFs is dominant, and it is the same situation in the markers of CAFs. The acceptable markers of CAFs consist of high expression of α-SMA, FSP-1, FAP, platelet-derived growth factor-α receptor (PDGFR-α), platelet-derived growth factor-β receptor (PDGFR-β), vimentin or loss of CAV-1, PTEN, p21 or TP53 mutation [9-15]. Furthermore, CAFS of different tissue origin may express different markers. In breast cancer, some groups use FAP as an important marker [5, 19], while other groups suggest that the combination of PDGFR-α and α-SMA is a distinguishing marker [20]. However, some findings confirm CAFs marker is mainly dependent on the tissue origin [21]. Recently, one study used the 4T1 breast cancer model and Rip tag2 pancreatic cancer model to find whether these markers are overlap in tumor stroma. The results indicated that α-SMA, PDGFR-β and NG2 (chondroitin sulfate proteoglycan) significantly overlap with each other in identifying a mixed population of fibroblasts (CAFs, myofibroblasts, pericytes and vascular smooth muscle cells), while α-SMA or vimentin alone is not a suitable marker for CAFs, but FSP1 alone can identify a unique group of CAFs without other marker expression [22]. The evidence above indicates that CAFs are also heterogeneous, like tumor cells, within the same type of cancer. Since breast cancer has been divided into five subtypes (luminal A, luminal B, HER2 positive, basal-like and normal-like) according to different gene expression [23], among different subtypes or in the same subtype, patients have different prognosis and different length of survival [24, 25], which may contribute to the heterogeneous stroma as suggested by our recent experiments (Unpublished data). Therefore, it may be difficult to use only one or two markers to identify these heterogeneous CAFs. The combination of some markers shown above may be a better choice for CAF identification [26], but the correct combination warrants investigation based on tumor phenotypes.

2.2 Activation of CAFs in breast cancer

There is increasing evidence that suggests CAFs play prominent roles in cancer development and progression, however, the mechanisms for activation of CAFs are elusive. To date, TGF-β and CXCL12/SDF-1 are regarded as the major tumor cell-derived factors affecting CAF activation [8, 27] through a TGF-β and CXCL12/SDF-1 autocrine-signaling loop [8]. Nevertheless, other profibrotic factors released by cancer cells can also act on resident fibroblasts and induce their activation, including PDGF-α/β [28, 29], basic fibroblast growth factor (b-FGF) [30] or interleukin (IL)-6 [31, 32]. Another important mechanism in activation of CAFs is downregulation of tumor suppressor genes, such as p53, p21, PTEN and CAV-1, which are also implicated in repressing the procarcinogenic effects of breast stromal fibroblasts both *in vitro* and *in vivo* [9-15]. Interestingly, the findings identified caveolin-1 (Cav-1) as a mediator of CAF activation, and Cav-1 is a well-known marker of oncogenic transformation in fibroblasts [33]. However, transformation of NIH 3T3 fibroblastic cells by various oncogenes (v-abl, bcr-abl and crkl) leads to reduction of caveolins (Cav-1,2,3) which correlates very well with the bigger size of colonies formed by these transformed cells [33]. As compared with non-cancer-associated fibroblasts (NAFs), CAFs have lower level of Cav-1 protein in breast cancer, and CAFs also grow faster than NAFs, which confirm that loss of Cav-1 means the activation of CAFs [21, 26]. However,

the reason that Cav-1 expression is lost in CAFs still remains a puzzle. Currently, one of potential possibility of Cav-1 downregulation in CAFs may be due to lysosomal degradation [26] and autophagy [34]. More recently, another tumor suppressor gene, $p16^{INK4A}$, is found downregulated in breast cancer CAFs compared with NAFs isolated from the same patient [35], which also play critical roles in inhibition of cell cycle progression [36] and the induction of senescence [37]. Importantly, $p16^{INK4A}$ reduction in CAFs induces high level of CXCL12/SDF-1 and MMP-2 and tumors formed in the presence of $p16^{INK4A}$ -defective fibroblasts exhibits higher levels of active Akt, Cox-2, MMP-2 and MMP-9. Furthermore, the migration and invasion of breast cancer cells are also enhanced in an SDF-1-dependent manner which is mediated by EMT changes [35]. Moreover, the reduction in $p16^{INK4A}$ level is due to a decrease in the stability of the CDKN2A mRNA in CAFs, which results from the increase in the expression of RNA destabilizing protein AUF1 [35, 38]. Increasing $p16^{INK4A}$ level through ectopic expression or AUF1 downregulation, reduces the levels of SDF-1 and MMP-2 and suppresses the pro-carcinogenic effects of CAFs [35]. In this regard, understanding of the molecular events by which reactive stromal fibroblasts affect cancer cell is helpful to offer the better therapeutic effect in breast cancer treatment.

2.3 Role of CAFs in breast cancer progression

CAFs promote tumor onset and progression in different ways [39-42],such as affecting Estradiol (E2) levels, secreting many kinds of factors (HGF,TGF-β,SDF-1,VEGF, IL-6, etc) and matrix metalloproteinases (MMPs), inducing stemness, epigenetic changes, EMT, etc. Interestingly, some research has shown that CAFs promote pre-cancerous breast epithelial cells MCF10A and EIII8 growth and inhibit their differentiation by aromatase-mediated synthesis of estrogen in a three- dimensional cell-cell interaction model [43]. However, another study shows that both NAFs and CAFs have the ability to inhibit the growth of MCF10A [44]. In addition, NAFs have greater inhibitory capacity, and only NAFs significantly inhibit proliferation of the more transformed MCF10AT cells, suggesting that the ability of fibroblasts to inhibit epithelial cell proliferation is lost during breast cancer development [44] . Furthermore, the conditioned medium from NAFs also inhibits the growth of MCF-7 cells, while in contrast, conditioned medium from CAFs significantly enhances the growth of MCF-7 cells which due to increasing 17 beta-estradiol dehydrogenase (E2DH) activity in the reductive direction (estrone (E1)----estradiol (E2)) 2-3 fold in CAFs [45]. The result means CAFs promote pre-cancerous and cancerous breast epithelial cells growth by increasing E2 levels, which provides an explanation of faster tumor growth in estrogen receptor (ER) positive breast cancer.

Besides affecting the E2 level, increasing growth factors and losing suppressor genes in CAFs also contribute to breast cancer progression. In a mouse xenograft model of breast cancer, transient CAFs interactions increase tumor cell malignancy through a TGF-β-mediated mechanism [46]. IL-6 has been found 100-fold increase in CAFs compared with NAFs, and also promotes migration in MDA-MB-231 cells and induces EMT in ER positive cell lines (MCF7 or T47D) [32], suggesting that IL-6 secreted from CAFs potentiates the invasive phenotype in breast cancer. In another mouse model, co-inoculation of CAFs ^{Sip21} with MCF7 cells can promote breast cancer development compared with MCF7 cells inoculated alone, and the same results are also observed using MDA-MB-231 cell lines [12].

Moreover, when PTEN is overexpressed into CAFs, it can partly inhibit CAFs' role on tumor initiation [13], suggesting that inactivation of tumor suppressor genes in CAFs also promoted breast cancer onset and invasion.

2.4 CAFs and invasion and metastasis of breast cancer

CAFs not only induce mammary carcinogenesis, but also promote invasion and metastasis in breast cancer [39, 40, 43, 46, 47]. The transition from ductal carcinoma in situ (DCIS) to invasive ductal carcinoma (IDC) is a good example to understand the process of tumor invasion. It was found that CAFs induced the invasive ability of DCIS epithelial cell both *in vitro and in vivo* [47, 48] CAFs achieved this induction of invasion through increasing MMP14 expression and MMP9 activity. Cancer metastasis is a complicated process that requires multiple events including epithelial to mesenchymal transition (EMT) of the epithelial cancer cells, induction of angiogenesis, intravasation and extravasation of cancer cells, the EMT cells regaining epithelial traits (mesenchymal to epithelial transition (MET)), and finally forming a new colony in the appropriate distant microenvironment. In this process, not only CAFs, but other stromal cells work together to complete the organ-specific metastasis. It has been shown that CAFs induced EMT changes in breast epithelial cells [32], and also secrete CXCL12/SDF-1 to promote angiogenesis in the primary site by recruiting endothelial progenitor cells (EPCs) [40]. Then, the cancer cells secrete growth factors and chemokines, such as CCL2, not only to activate CAFs, but also to recruit the macrophages and promote their intravasation, [49]. Furthermore, this study demonstrated that,, CCL2 secreted from CAFs also increased breast cancer stem cells (CSCs) which promotes metastasis. In another study it was shown that when breast cancer cells arrived in the lung CCR-2 positive macrophages promoted their extravasation, and the cancer cellsunderwent MET and colonized to form lung metastases [50]. In addition, $CD4^+FOXP3^+$ Treg cells, recruited by CCL5 secreted from CAFs, also promoted lung metastasis by secreting receptor activator of nuclear factor-κB (RANK) ligand (RANKL) [51]. Interestingly, when breast cancer cells homed to the bone marrow through CXCL12/CXCR4 interaction caused by stem cells and circulating leukocytes [52], osteoclastic activation was induced by parathyroid hormone-related protein (PTHrP) and other soluble mediators released from the metastatic cells [53], at the same time, bone derived TGF-β also enhanced this process and tumor growth in a TGF-β-RANKL- PTHrP manner [54]. Besides, CCL18 from tumor associated macrophages (TAMs) also promote metastasis in breast cancer via PITPNM3 [55]. Interestingly, in addition to CAFs, NAFs also promote metastasis in breast cancer. One study found that the NAFs promoted the metastasis of prometastatic cancer cells (MCF10CA1a) *in vitro and in vivo* by TGF-β1 secreted by fibroblasts [46]. The evidence above shows that both in the primary and metastatic site, CAFs and other stromal cells may simultaneously contribute to tumor growth, invasion, metastasis and metastatic progression

2.5 CAFs and epigenetic modification

Research focused on the origins of cancer has identified that genetic mutations or epigenetic modification within tumor cells are critical in tumorigenesis and progression. However, there is less genetic evidence supporting a role for genetic changes in breast cancer stroma as contributing to cancer progression [56, 57]. Serial analyses demonstrated that epigenetic

changes in breast cancer cells can foster tumor malignancy, however, there are also dramatic and consistent modifications in gene expression within the fibroblasts from primary human breast tumors [58]. These changes include histone modifications, and alterations in expression of DNA methyltransferases, chromatin modifying factors, and microRNAs [57, 59, 60].

2.5.1 DNA methylation and histone modifications in CAFs—CAFs in breast cancer gain different DNA methylation patterns when compared to NAFs [60]. This has also been found in CAFs isolated from human gastric carcinomas [61], pancreatic cancers [62] and pulmonary fibrosis [63]. The CXorf12 gene has been found hypomethylated in breast CAFs [57], but its role in breast cancer progression still remains unclear. CYP19, encoding Cytochrome aromatase p450, is another gene that has been found hypomethylated in breast adipose fibroblasts (BAFs), which induce an increase aromatase levels in the breast [64]. Histone H3K27 also is hypomethylated in breast CAFs, resulting in high level of ADAM metallopeptidase with thrombospondin type 1 motif, (ADAMTS1) in CAFs which correlate to more invasive phenotype [65]. Moreover, loss of histone deacetylase 1(HDAC1) expression induces increased osteopontin (OPN) expression within the stromal compartment of invasive breast cancers, which then activate CAFs to promote tumor growth *in vivo.* These results suggest that histone modulations are presented in CAFs. All evidence above indicates that DNA methylation and histone modifications in CAFs also induce cancer progression and provide an enhanced understanding of cancer-stromal interactions in cancer evolution.

2.5.2 Role of microRNA in CAFs—MicroRNAs are a class of short noncoding regulatory RNAs that are involved in stem cell maintenance, developmental programming and cell fate specification, as well as various disease pathogeneses [66-69]. MicroRNA altered gene expression (both in tumor stroma as well as tumor cells) has been implicated in cancer promotion in several types of cancers, including breast cancer [70-75]. However, the contribution of specific microRNAs to CAFs remains largely unknown. miR21 has been found overexpressed in both tumor cells and breast tumor stroma [71], which significantly correlates with dual overexpression of TGF-β and poor patient outcome in breast cancer [76] . miR148-a is downregulated in endometrial CAFs compared to its counterpart NAFs, and then promote migration by WNT10B [77]. One study shows that in endometrial cancer, there are 11 differential expression microRNAs in CAFs and NAFs, and miR-31 is the most downregulated microRNA in CAFs, which overexpression of miR-31significantly impaired the ability of CAFs to stimulate tumor cell migration and invasion without affecting tumor cell proliferation [78]. In 23 prostate cancer cases, downregulation of miR-15 and miR-16 in CAFs promoted tumor growth and progression through the reduced post-transcriptional repression of Fgf-2 and its receptor Fgfr1, which affect both stromal and tumor cells and enhance cancer cell survival, proliferation and migration. Moreover, reconstitution of miR-15 and miR-16 impaired the tumor-supportive capability of stromal cells *in vitro* and *in vivo* [79]. Currently, while there is not much evidence describing microRNA changes in CAFs in breast cancer, these interesting findings from other tumors may offer some clues that the role of microRNA changes in CAFs and their potential importance in breast cancer progression.

2.6 CAFs and therapeutic resistances

Therapeutic resistances are the major reason for breast cancer treatment failure. More importantly, tumor stroma also participates in therapeutic resistances which contributes to breast cancer progression and poor prognosis. Recently, increasing evidence shows that CAFs can induce endocrine/chemotherapy and target therapeutic resistances in breast cancer treatment [5, 80, 81]. Therefore ,targeting stroma as opposed to just targeting tumor cells, provide a novel notion and potentially more effective treatment strategy for breast cancer [82].

2.6.1 CAFs and chemotherapy resistance—Collagen type I secreted by CAFs contributes to decreasing chemotherapeutic drug uptake in tumors and plays a significant role in regulating tumor sensitivity to a variety of chemotherapies [5]. Furthermore, using construct an oral DNA vaccine targeting fibroblast activation protein (FAP) can greatly suppress primary tumor cell growth and metastasis of multidrug-resistant murine breast carcinoma [5]. The results suggest that targeting relatively stable fibroblasts maybe an emerging new effective therapy for breast cancer prevention and treatment. In addition, chemotherapy and radiation induced DNA damage in fibroblasts promote secretion of WNT16B and consequently result in breast cancer cell proliferation, invasion and induce mitoxantrone (MIT) resistance by NF-κB pathway activation. Moreover, the β-catenin inhibitor XAV939 and NF-KB mutation can reverse the sensitivity to MIT [81], which is also observed in prostate cancer and ovary cancer [81]. The findings suggest that between treatment time periods, cancer cells have chance to recover through the Wnt signaling pathway; however adding a Wnt pathway inhibitor, may allow for the cancer to restore sensitivity to the original chemotherapy.

2.6.2 CAFs and endocrine resistance—In addition to induction of chemo-resistance, CAFs can also induce endocrine resistance. Tamoxifen is a classic endocrine therapeutic drug for ER positive breast cancer patients and greatly improve disease-free survival and overall survival in more than 15 years follow up, but about 33% patients still have recurrence and metastasis [83]. Recently, many results indicate that CAFs play critical roles in tamoxifen resistance. One study showed that when co-cultured with CAFs from ER-α +/PgR+ or ER-α-/PgR-breast tumors, estrogen receptor (ER)-α tamoxifen-sensitive premalignant (EIII8) cell line underwent epithelial morphogenesis; while EIII8 cells co-cultured with only ER-α-/PgR- tumor-derived CAFs exhibited decreased tamoxifen sensitivity compared with cells co-cultured with ER-α+/PgR+ tumor-derived CAFs. The results also indicated that CAF induced tamoxifen resistance was accompanied by mitogen-activated protein kinase (MAPK) and Akt hyperactivation, reduced sensitivity to U0126 or LY294002, and ER-α hyperphosphorylation in the activation function-1 domain, but not mediated by epidermal growth factor receptor or insulin-like growth factor (IGF)-1R axes. Another study found that CAF-induced tamoxifen and fulvestrant resistance with 4.4 and 2.5-fold reductions in MCF7 by chaning mitochondrial functions in cancer cells, and mitochondrial“poisons” (metformin and arsenic trioxide (ATO)) are able to re-sensitize these cancer cells to tamoxifen [80]. The findings suggest that CAF-induced mitochondrial dysfunction in breast cancer cells can change their sensitivity to tamoxifen. Notably, the conditioned media of CAFs induce tamoxifen resistance also through activation of EGFR

and PI3K/AKT, with the involvement of β1 integrin [84]. Indeed, our recent results further confirmed that inflammatory cytokines from the conditioned media of CAFs result in tamoxifen resistance through induction of EMTs (Unpublished data).Therefore, tamoxifen resistance modulated by CAFs in breast cancer treatment may provide an alternative explanation for why some patients become refractory to hormone-therapy.

2.6.3 CAFs and target resistance—Emerging evidence also indicates that CAFs also induced target resistance in breast cancer and other types of cancers [85-87]. The results showed that HGF secreted by CAFs activated Met and lead to EGFR/Met crosstalk and resistance to EGFR TKIs gefitinib in triple-negative breast cancer (TNBC) [85], which indicates that targeting EGFR and Met in combination may be an effective therapeutic strategy for TNBC. Interestingly, one study suggested that CAFs can also sensitize some cancers to targeted therapy. Specifically, it was shown that mesenchymal stem cells (MSCs) and CAFs increased the cytotoxic effect of RAF inhibitor RAF265 on MDA-MB-231 cells by downregulating ERK1/2 phosphorylation and sensitized MCF7 cells to the mTOR inhibitor RAD001 [87]. Moreover, the data indicated that both MSCs and CAFs have no effects on the response to PDGFR/FGFR/VEGFR inhibitor TKI258 in breast cancer cell lines [87]. This observation showed that CAFs may not contribute to all mechanisms of drug-resistance; however, the potential reason may be ascribed to heterogeneity of CAFs in drug response. Based on these findings, many new drugs and new combinations have been emerging to improve breast cancer patients treatment by targeting CAFs in therapeutic resistance, such as XAV939 [81], metformin [77, 80], and PD0332991 [88], as shown in **Table 1**.

2.7 CAFs and breast cancer prognosis

As described above, breast cancers are divided into five molecular subtypes with different prognosis and treatment. With a deeper understanding of the role of tumor microenvironment, it is interesting to explore whether breast cancer is likely to be classified into subtypes based on its different stromal phenotypes. Recently, one group found that 22K oligonucleotide Agilent microarrays can be used to divided breast cancers into four main groups (ECM1–4) according to 278 ECM-related genes [89]. The ECM1 signature (MARCO, PUNC, and SPARC, whose expression levels were associated with breast cancer survival and risk of recurrence) had a poorer prognosis with high expression of integrins and metallopeptidases, and low expression of several laminin chains [89]. ECM2 tumors were characterized by a more heterogeneous expression of ECM-related genes. ECM3 tumors showed mainly up-regulation of genes encoding macromolecules involved in the maintenance of connective tissue; in particular, collagens, laminins, fibrillins, and the matrix-associated proteins [89]. However, the ECM4 group had a favorable outcome and with overexpression of a set of protease inhibitors belonging to the serpin family. These findings supporting the hypothesis that clinical outcome is strongly related to stromal characteristics. According to differential gene expression patterns in breast tumor stroma, Finak et al have developed a 26-gene predictor (stroma-derived prognostic predictor, SDPP) that predicts disease outcome with greater accuracy than predictors or signatures derived from whole tissue [90]. Tumor stroma samples from the good-outcome cluster overexpress a distinct set of immune-related genes, including T cell and NK cell markers indicative of a

TH1–type immune response (GZMA, CD52, CD247, CD8A) [90].Therefore, individuals with this gene expression pattern may provide benefits from treatments targeting tumor cells via the immune response, such as vaccine therapies in the adjuvant setting. More recently, Sloan et al found high levels of caveolin-1 in the stromal tissue surrounding the tumor, rather than within tumor cells, associated strongly with reduced metastasis and improved survival ($p < 0.0001$) [91]. The similar results were also observed by another group [92], which shows a loss of stromal Cav-1 in human breast cancers is associated with tumor recurrence, metastasis, and poor clinical outcome. Moreover, Farmer et al reported that a 50-gene signature that predicts poor response to anthracycline-based neoadjuvant chemotherapy (5-fluorouracil, epirubicin and cyclophosphamide (FEC) in subjects in the EORTC 10994/BIG 00-01 trial), but unable to predict survival in subjects who did not receive chemotherapy, which suggests that the stromal metagene is predictive rather than prognostic [93]. Interestingly, one study divided tumor stroma into 3 groups: collagen dominant (C), fibroblast dominant (F), or lymphocyte dominant (L), and found that dominant stroma type as an independent predictor of disease-free survival, especially in patients with high-grade tumors. The L type predicted longest disease free interval(DFI), followed by F and C types [94]. The results above supports a previous study showing that lymphocytic infiltration is associated with favorable prognosis [95]. Notably, in human breast tumors, infiltrating tumor associated macrophages(TAMs) correlate with poor prognostic features [96, 97], higher tumor grade [98], and decreased disease-free survival [99, 100], which will be discussed below.

3. Other tumor stroma cells and breast cancer

Cancer is a systemic disease within which it may keep an ecosystem, encompassing multiple components of tumor and stroma cells that are a prerequisite for tumor cell invasion and metastasis. As shown in **Figure 2**, in addition to CAFs, there are also other types of stroma which play central roles in breast cancer, such as macrophage, endothelial cells, adipocytes and leukocytes, et al.

3.1 Tumor-associated macrophages (TAMs) and breast cancer

Macrophages are derived from $CD34^+$ bone marrow progenitors that continually proliferate and shed for their progeny into the bloodstream as pro-monocytes. They then develop onto monocytes and extravasate into tissues where they differentiate into a specific type of “resident” tissue macrophage. Macrophages are also prominent in the stoma compartment of virtually all types of malignancy [101].

Tumor-associated macrophages (TAMs) are mostly regarded as the M2 phenotype, which secrete growth factors that promote angiogenesis [100-105], growth [101], invasion, migration [106], metastatic spread [107] and immunosuppression. In breast cancer, infiltrating TAMs correlate with poor prognostic features [96, 108], higher tumor grade [98], high vascular grade, increased necrosis [100] and decreased disease-free survival [99, 100] and overall survival [100]. Recently, one study found that chemokine (C-C motif) ligand 18 (CCL18) were highly expressed in TAMs and promoted the invasion and metastasis of cancer cells by triggering integrin clustering and enhancing their adherence to extracellular matrix [55]. Importantly, the results indicated that the functional receptor of CCL18,

PITPNM3, is able to promote breast cancer progression through interaction of CCL18 and PITPNM3. Epigenetic changes also impact TAMs. For example, macrophage infiltration associated with miR92a expression in breast cancer tissue which links to tumor stage and disease-free survival [109]. Another study found that macrophages activated by IL-4 also regulate the invasiveness of breast cancer cells through exosome-mediated delivery of oncogenic miR-223 via the Mef2c-β-catenin pathway [110]. Considering that macrophages are derived from the same cell lineage as osteoclasts, the major target of bisphosphonates (BPs), which also increase apoptosis and decrease proliferation, migration and invasion in breast cancer cell lines and mice models. Therefore, targeting TAMs by BPs is a potential choice and it also has been used to good effect *in vitro* and mouse models [105, 111-118]. Given that BPs has been FDA approved for breast cancer patients who have bone metastases, it may be the first effective drug which targets tumor stroma, and warrants additional research in clinical trials.

3.2 Other leukocytes and breast cancer

Notably, not only macrophages but also other kinds of infiltrating leukocytes promote breast cancer progression. One study showed that more infiltrating leukocytes were found in DCIS with focal myoepithelial cell layer disruptions [119], which indicated that leukocytes may promote breast cancer invasive progress. In a spontaneous mouse model of breast cancer, $CD4^+$ Treg lymphocytes were found increasingly infiltrated in tumor and depletion of these T cells by interleukin-2 (IL-2) immunotoxin fusion protein can inhibit tumor growth [120]. Another study showed that the metastatic spread of Erbb2-transformed carcinoma cells required $CD4^+CD25^+$ T cells, who secrete receptor activator of nuclear factor-κB (RANK) ligand (RANKL) and implicate into metastatic process [51]. Moreover, the cells which secrete RANKL also with high expression of forkhead box P3 (FOXP3), a transcription factor produced by regulatory T cells, so the $CD4^+CD25^+FOXP3^+$ Treg cells can stimulate the metastatic progression by RANKL in the RANK-expressing breast/mammary carcinoma cells. This indicates that anti-RANKL-RANK maybe an effective strategy to prevent breast cancer metastasis. Interestingly, recent findings also suggest that infiltrating number of $CD8^+$ T lymphocytes positively correlate with patient survival [121] and high CD8 and low FOXP3 cell infiltrating after neoadjuvant chemotherapy was significantly relate to improved recurrence free survival (RFS) and overall survival (OS). Based on these findings, targeting immune cells may be an emerging strategy for cancer treatment. Indeed, blockade of macrophage recruitment with colony stimulating factor 1 receptor (CSF1R)-signaling antagonists is an good example [96]. Cytotoxic agents induced cancer cells to produce CSF1 and interleukin-34 and recruited monocytes/macrophages infiltrating by CSF1R–dependent manner, and in a mammary tumor–bearing mice model, CSF1R antagonist and paclitaxel in combination improved survival by slowing primary tumor development and reducing pulmonary metastasis in $CD8^+$ T-cell–dependent manner [96]. Recently, another study showed different components of leukocytes play different roles in breast cancer [122]. They found that activated T lymphocytes predominate in tumor tissue, whereas myeloid lineage cells in “normal” breast tissue [122]. Importantly, compared with tissue from patients treated primarily by surgery alone, the tissue from patients who received neoadjuvant chemotherapy contained increased percentages of infiltrating myeloid cells, accompanied by an increased CD8/CD4 T-cell ratio and higher numbers of granzyme B-expressing cells. This study

indicates that chemotherapy may affect the tumor immune environment and a deeper understanding of this interaction should be pursued.

3.3 Endothelial cells and breast cancer

Endothelial cells also play important roles in cancer growth and invasion. Human umbilical endothelial cells (HUVECs) induced higher proliferation of preneoplastic MCF10AT1-EIII8 (referred as EIII8) in the EIII8-fibroblasts-HUVEC tricultures than EIII8-fibroblast co-cultures [43]. This finding suggests that endothelial cells can help breast cancer initiation. Moreover, TNF-α production by endothelial and other stromal cells induced by chemotherapeutic agents increases the CXCL1/2 expression in cancer cells via NF-κB, and then CXCL1/2 attract $CD11b^+$ $Gr1^+$ myeloid cells into the tumor, which produce chemokines including S100A8/9 that enhance cancer cell survival, thus amplifying the CXCL1/2-S100A8/9 loop and causing chemo-resistance. This network of endothelial-carcinoma-myeloid signaling interactions provides a mechanism linking chemo-resistance and metastasis, with opportunities for intervention by a CXCR2 blocker [123]. This network also highlights that tumor stroma components have interactions in promoting malignant in cancer cells.

3.4 Adipose tissue and breast cancer

Adipose tissue, consisting of mainly mature adipocytes and progenitors (preadipocytes and adipose-derived stem cells (ADSCs), is the most abundant component surrounding breast cancer cells. There is cumulative evidence supporting that cancer-associated adipose (CAA) tissue is a key component of breast cancer progression and carcinogenesis. It has been shown that collagen VI (COLVI) is abundantly expressed in CAAs and involved in mammary tumor progression *in vivo* [124, 125]. Moreover, IL-6 plays a role in CAA-cancer cell interaction and promotes an aggressive phenotype in prostate cancer [126]. There is also evidence that ADSCs promote growth and survival of breast cancer cells as well as their migratory and invasive capacities *in vitro* and *in vivo* by secreting cytokines(IL-6, IL8, CCL-5 and CXCL12/SDF-1), the expansion of cancer stem cells and induce EMT in the cancer cells in a PDGF dependent manner [127-132]. Like CAFs, CAAs also contribute to radioresistance in breast cancer [133]. The role of CAAs in breast cancer progression may explain that obesity is an independent negative prognosis factor for breast cancer independently of menopause status [134, 135].

4. Conclusions

The tumor microenvironment has been demonstrated to promote breast cancer initiation, growth, migration, metastasis and therapeutic resistances. CAFs, TAMs, EC, CAAs, and leukocytes, et al, are critical components of tumor stroma which compromise the tumor microenvironment and take part in induction of malignancy in breast cancer through various mechanisms (**Figure 2**). Research to date provides a greater understanding of cancer evolution, potential targets to reverse refractory tumors into a sensitive phenotype, and improve DFS (disease free survival) and OS (overall survival). Moreover, with the novel concept that best therapy is personalized treatment in breast cancer patients, it is important to explore more biologic markers to sort patients into a specific and confirmed subtype, and

use effective markers to predict therapeutic response. The interaction between cancer cells and stromal cells in the tumor microenvironment may be useful to screen potential candidate markers and provide a great impact in cancer therapy in the future.

Acknowledgements

We apologize to the many authors whose excellent work we could not cite owing to space limitation. Research in the authors' laboratory is supported by National Natural funding of China (81272404, 81071747), National key program (973) for Basic Research of China (2011CB510106, 2011CB504300), Shanghai Education Committee Key Discipline and Specialties Foundation (J50208), the Program for Professor of Special Appointment (Eastern Scholar to J, Wang) at Shanghai Institutions of Higher Learning, and Shanghai Pujiang Program (10PJ1406400), Shanghai Committee of Science and Technology (11DZ2260200) and Program of Shanghai Municipal Health Bureau Subject Chief Scientist (XBR20110052); National Institutes of Health Grant P01 CA093900.

References

1. Siegel R, et al. Cancer statistics, 2012. CA Cancer J Clin. 2012; 62(1):10–29. [PubMed: 22237781]
2. Paget S. The distribution of secondary growths in cancer of the breast. 1889. Cancer Metastasis Rev. 1989; 8(2):98–101. [PubMed: 2673568]
3. Kalluri R, et al. Fibroblasts in cancer. Nat Rev Cancer. 2006; 6(5):392–401. [PubMed: 16572188]
4. Ostman A, et al. Cancer-associated fibroblasts and tumor growth--bystanders turning into key players. Curr Opin Genet Dev. 2009; 19(1):67–73. [PubMed: 19211240]
5. Loeffler M, et al. Targeting tumor-associated fibroblasts improves cancer chemotherapy by increasing intratumoral drug uptake. J Clin Invest. 2006; 116(7):1955–1962. [PubMed: 16794736]
6. Gabbiani G, et al. Presence of modified fibroblasts in granulation tissue and their possible role in wound contraction. Experientia. 1971; 27(5):549–550. [PubMed: 5132594]
7. Dvorak HF. Tumors: wounds that do not heal. Similarities between tumor stroma generation and wound healing. N Engl J Med. 1986; 315(26):1650–1659. [PubMed: 3537791]
8. Kojima Y, et al. Autocrine TGF-beta and stromal cell-derived factor-1 (SDF-1) signaling drives the evolution of tumor-promoting mammary stromal myofibroblasts. Proc Natl Acad Sci U S A. 2010; 107(46):20009–20014. [PubMed: 21041659]
9. Moskovits N, et al. p53 Attenuates cancer cell migration and invasion through repression of SDF-1/CXCL12 expression in stromal fibroblasts. Cancer Res. 2006; 66(22):10671–10676. [PubMed: 17108103]
10. Aboussekhra A. Role of cancer-associated fibroblasts in breast cancer development and prognosis. Int J Dev Biol. 2011; 55(7-9):841–849. [PubMed: 22161840]
11. Kiaris H, et al. Evidence for nonautonomous effect of p53 tumor suppressor in carcinogenesis. Cancer Res. 2005; 65(5):1627–1630. [PubMed: 15753354]
12. Trimis G, et al. Expression of p21waf1/Cip1 in stromal fibroblasts of primary breast tumors. Hum Mol Genet. 2008; 17(22):3596–3600. [PubMed: 18713757]
13. Trimboli AJ, et al. Pten in stromal fibroblasts suppresses mammary epithelial tumours. Nature. 2009; 461(7267):1084–1091. [PubMed: 19847259]
14. Trimmer C, et al. Caveolin-1 and mitochondrial SOD2 (MnSOD) function as tumor suppressors in the stromal microenvironment: a new genetically tractable model for human cancer associated fibroblasts. Cancer Biol Ther. 2011; 11(4):383–394. [PubMed: 21150282]
15. Hill R, et al. Selective evolution of stromal mesenchyme with p53 loss in response to epithelial tumorigenesis. Cell. 2005; 123(6):1001–1011. [PubMed: 16360031]
16. Spaeth EL, et al. Mesenchymal stem cell transition to tumor-associated fibroblasts contributes to fibrovascular network expansion and tumor progression. PLoS One. 2009; 4(4):e4992. [PubMed: 19352430]
17. Jotzu C, et al. Adipose tissue-derived stem cells differentiate into carcinoma-associated fibroblast-like cells under the influence of tumor-derived factors. Anal Cell Pathol (Amst). 2010; 33(2):61–79. [PubMed: 20978328]

18. Zeisberg EM, et al. Discovery of endothelial to mesenchymal transition as a source for carcinoma-associated fibroblasts. Cancer Res. 2007; 67(21):10123–10128. [PubMed: 17974953]
19. Garin-Chesa P, et al. Cell surface glycoprotein of reactive stromal fibroblasts as a potential antibody target in human epithelial cancers. Proc Natl Acad Sci U S A. 1990; 87(18):7235–7239. [PubMed: 2402505]
20. Erez N, et al. Cancer-Associated Fibroblasts Are Activated in Incipient Neoplasia to Orchestrate Tumor-Promoting Inflammation in an NF-kappaB-Dependent Manner. Cancer Cell. 2010; 17(2): 135–147. [PubMed: 20138012]
21. Mercier I, et al. Human breast cancer-associated fibroblasts (CAFs) show caveolin-1 downregulation and RB tumor suppressor functional inactivation: Implications for the response to hormonal therapy. Cancer Biol Ther. 2008; 7(8):1212–1225. [PubMed: 18458534]
22. Sugimoto H, et al. Identification of fibroblast heterogeneity in the tumor microenvironment. Cancer Biol Ther. 2006; 5(12):1640–1646. [PubMed: 17106243]
23. Tafani M, et al. Up-regulation of pro-inflammatory genes as adaptation to hypoxia in MCF-7 cells and in human mammary invasive carcinoma microenvironment. Cancer Sci. 2010; 101(4):1014–1023. [PubMed: 20151982]
24. Haque R, et al. PS1-05: Disparities in Breast Cancer Survival by Molecular Subtype and Race/Ethnicity. Clin Med Res. 2012; 10(3):145.
25. Onitilo AA, et al. Breast cancer subtypes based on ER/PR and Her2 expression: comparison of clinicopathologic features and survival. Clin Med Res. 2009; 7(1-2):4–13. [PubMed: 19574486]
26. Martinez-Outschoorn UE, et al. Tumor cells induce the cancer associated fibroblast phenotype via caveolin-1 degradation: implications for breast cancer and DCIS therapy with autophagy inhibitors. Cell Cycle. 2010; 9(12):2423–2433. [PubMed: 20562526]
27. Lohr M, et al. Transforming growth factor-beta1 induces desmoplasia in an experimental model of human pancreatic carcinoma. Cancer Res. 2001; 61(2):550–555. [PubMed: 11212248]
28. Bronzert DA, et al. Synthesis and secretion of platelet-derived growth factor by human breast cancer cell lines. Proc Natl Acad Sci U S A. 1987; 84(16):5763–5767. [PubMed: 3039506]
29. Shao ZM, et al. Human breast carcinoma desmoplasia is PDGF initiated. Oncogene. 2000; 19(38): 4337–4345. [PubMed: 10980609]
30. Strutz F, et al. Basic fibroblast growth factor expression is increased in human renal fibrogenesis and may mediate autocrine fibroblast proliferation. Kidney Int. 2000; 57(4):1521–1538. [PubMed: 10760088]
31. Giannoni E, et al. Reciprocal activation of prostate cancer cells and cancer-associated fibroblasts stimulates epithelial-mesenchymal transition and cancer stemness. Cancer Res. 2010; 70(17): 6945–6956. [PubMed: 20699369]
32. Hugo HJ, et al. Contribution of Fibroblast and Mast Cell (Afferent) and Tumor (Efferent) IL-6 Effects within the Tumor Microenvironment. Cancer Microenviron. 2012
33. Koleske AJ, et al. Reduction of caveolin and caveolae in oncogenically transformed cells. Proc Natl Acad Sci U S A. 1995; 92(5):1381–1385. [PubMed: 7877987]
34. Martinez-Outschoorn UE, et al. Oxidative stress in cancer associated fibroblasts drives tumor-stroma co-evolution: A new paradigm for understanding tumor metabolism, the field effect and genomic instability in cancer cells. Cell Cycle. 2010; 9(16):3256–3276. [PubMed: 20814239]
35. Al-Ansari MM, et al. p16(INK4A) Represses the paracrine tumor-promoting effects of breast stromal fibroblasts. Oncogene. 2012
36. Serrano M, et al. A new regulatory motif in cell-cycle control causing specific inhibition of cyclin D/CDK4. Nature. 1993; 366(6456):704–707. [PubMed: 8259215]
37. Ohtani N, et al. The p16INK4a-RB pathway: molecular link between cellular senescence and tumor suppression. J Med Invest. 2004; 51(3-4):146–153. [PubMed: 15460900]
38. Matros E, et al. BRCA1 promoter methylation in sporadic breast tumors: relationship to gene expression profiles. Breast Cancer Res Treat. 2005; 91(2):179–186. [PubMed: 15868446]
39. Hasebe T, et al. Highly proliferative fibroblasts forming fibrotic focus govern metastasis of invasive ductal carcinoma of the breast. Mod Pathol. 2001; 14(4):325–337. [PubMed: 11301349]

40. Orimo A, et al. Stromal fibroblasts present in invasive human breast carcinomas promote tumor growth and angiogenesis through elevated SDF-1/CXCL12 secretion. Cell. 2005; 121(3):335–348. [PubMed: 15882617]
41. Saito RA, et al. Forkhead box F1 regulates tumor-promoting properties of cancer-associated fibroblasts in lung cancer. Cancer Res. 2010; 70(7):2644–2654. [PubMed: 20233876]
42. Zhang C, et al. Fibroblast growth factor receptor 2-positive fibroblasts provide a suitable microenvironment for tumor development and progression in esophageal carcinoma. Clin Cancer Res. 2009; 15(12):4017–4027. [PubMed: 19509166]
43. Shekhar MP, et al. Breast stroma plays a dominant regulatory role in breast epithelial growth and differentiation: implications for tumor development and progression. Cancer Res. 2001; 61(4): 1320–1326. [PubMed: 11245428]
44. Sadlonova A, et al. Breast fibroblasts modulate epithelial cell proliferation in three-dimensional in vitro co-culture. Breast Cancer Res. 2005; 7(1):R46–59. [PubMed: 15642169]
45. Adams EF, et al. Effects of human breast fibroblasts on growth and 17 beta-estradiol dehydrogenase activity of MCF-7 cells in culture. Breast Cancer Res Treat. 1988; 11(2):165–172. [PubMed: 3042052]
46. Stuelten CH, et al. Transient tumor-fibroblast interactions increase tumor cell malignancy by a TGF-Beta mediated mechanism in a mouse xenograft model of breast cancer. PLoS One. 2010; 5(3):e9832. [PubMed: 20352126]
47. Hu M, et al. Role of COX-2 in epithelial-stromal cell interactions and progression of ductal carcinoma in situ of the breast. Proc Natl Acad Sci U S A. 2009; 106(9):3372–3377. [PubMed: 19218449]
48. Hu M, et al. Regulation of in situ to invasive breast carcinoma transition. Cancer Cell. 2008; 13(5): 394–406. [PubMed: 18455123]
49. Tsuyada A, et al. CCL2 mediates cross-talk between cancer cells and stromal fibroblasts that regulates breast cancer stem cells. Cancer Res. 2012; 72(11):2768–2779. [PubMed: 22472119]
50. Qian BZ, et al. CCL2 recruits inflammatory monocytes to facilitate breast-tumour metastasis. Nature. 2011; 475(7355):222–225. [PubMed: 21654748]
51. Tan W, et al. Tumour-infiltrating regulatory T cells stimulate mammary cancer metastasis through RANKL-RANK signalling. Nature. 2011; 470(7335):548–553. [PubMed: 21326202]
52. Rossi D, et al. The biology of chemokines and their receptors. Annu Rev Immunol. 2000; 18:217–242. [PubMed: 10837058]
53. Roodman GD. Mechanisms of bone metastasis. N Engl J Med. 2004; 350(16):1655–1664. [PubMed: 15084698]
54. Azim HA, et al. Bone metastasis in breast cancer: The story of RANK-Ligand. J Egypt Natl Canc Inst. 2012; 24(3):107–114. [PubMed: 22929916]
55. Chen J, et al. CCL18 from tumor-associated macrophages promotes breast cancer metastasis via PITPNM3. Cancer Cell. 2011; 19(4):541–555. [PubMed: 21481794]
56. Campbell I, et al. Clonal mutations in the cancer-associated fibroblasts: the case against genetic coevolution. Cancer Res. 2009; 69(17):6765–6768. discussion 6769. [PubMed: 19706773]
57. Hu M, et al. Distinct epigenetic changes in the stromal cells of breast cancers. Nat Genet. 2005; 37(8):899–905. [PubMed: 16007089]
58. Allinen M, et al. Molecular characterization of the tumor microenvironment in breast cancer. Cancer Cell. 2004; 6(1):17–32. [PubMed: 15261139]
59. Enkelmann A, et al. Specific protein and miRNA patterns characterise tumour-associated fibroblasts in bladder cancer. J Cancer Res Clin Oncol. 2011; 137(5):751–759. [PubMed: 20607552]
60. Fiegl H, et al. Breast cancer DNA methylation profiles in cancer cells and tumor stroma: association with HER-2/neu status in primary breast cancer. Cancer Res. 2006; 66(1):29–33. [PubMed: 16397211]
61. Jiang L, et al. Global hypomethylation of genomic DNA in cancer-associated myofibroblasts. Cancer Res. 2008; 68(23):9900–9908. [PubMed: 19047171]

62. Yu J, et al. Unlike pancreatic cancer cells pancreatic cancer associated fibroblasts display minimal gene induction after 5-aza-2′-deoxycytidine. PLoS One. 2012; 7(9):e43456. [PubMed: 22984426]
63. Robinson CM, et al. Hypoxia-induced dna hypermethylation in human pulmonary fibroblasts is ssociated with thy-1 promoter methylation and the development of a pro-fibrotic Phenotype. Respir Res. 2012; 13(1):74. [PubMed: 22938014]
64. Knower KC, et al. Epigenetic mechanisms regulating CYP19 transcription in human breast adipose fibroblasts. Mol Cell Endocrinol. 2010; 321(2):123–130. [PubMed: 20211687]
65. Tyan SW, et al. Breast cancer cells induce stromal fibroblasts to secrete ADAMTS1 for cancer invasion through an epigenetic change. PLoS One. 2012; 7(4):e35128. [PubMed: 22514714]
66. Gangaraju VK, et al. MicroRNAs: key regulators of stem cells. Nat Rev Mol Cell Biol. 2009; 10(2):116–125. [PubMed: 19165214]
67. Fazi F, et al. MicroRNA: basic mechanisms and transcriptional regulatory networks for cell fate determination. Cardiovasc Res. 2008; 79(4):553–561. [PubMed: 18539629]
68. Leung AK, et al. Function and localization of microRNAs in mammalian cells. Cold Spring Harb Symp Quant Biol. 2006; 71:29–38. [PubMed: 17381277]
69. Tiscornia G, et al. MicroRNAs in embryonic stem cell function and fate. Genes Dev. 2010; 24(24): 2732–2741. [PubMed: 21159814]
70. Davis-Dusenbery BN, et al. MicroRNA in Cancer: The Involvement of Aberrant MicroRNA Biogenesis Regulatory Pathways. Genes Cancer. 2010; 1(11):1100–1114. [PubMed: 21533017]
71. Farazi TA, et al. MicroRNA sequence and expression analysis in breast tumors by deep sequencing. Cancer Res. 2011; 71(13):4443–4453. [PubMed: 21586611]
72. Iliopoulos D, et al. An epigenetic switch involving NF-kappaB, Lin28, Let-7 MicroRNA, and IL6 links inflammation to cell transformation. Cell. 2009; 139(4):693–706. [PubMed: 19878981]
73. Iliopoulos D, et al. STAT3 activation of miR-21 and miR-181b-1 via PTEN and CYLD are part of the epigenetic switch linking inflammation to cancer. Mol Cell. 2010; 39(4):493–506. [PubMed: 20797623]
74. Iorio MV, et al. MicroRNA gene expression deregulation in human breast cancer. Cancer Res. 2005; 65(16):7065–7070. [PubMed: 16103053]
75. Jazbutyte V, et al. MicroRNA-21: from cancer to cardiovascular disease. Curr Drug Targets. 2010; 11(8):926–935. [PubMed: 20415649]
76. Qian B, et al. High miR-21 expression in breast cancer associated with poor disease-free survival in early stage disease and high TGF-beta1. Breast Cancer Res Treat. 2009; 117(1):131–140. [PubMed: 18932017]
77. Aprelikova O, et al. Silencing of miR-148a in cancer-associated fibroblasts results in WNT10B-mediated stimulation of tumor cell motility. Oncogene. 2012
78. Aprelikova O, et al. The role of miR-31 and its target gene SATB2 in cancer-associated fibroblasts. Cell Cycle. 2010; 9(21):4387–4398. [PubMed: 20980827]
79. Musumeci M, et al. Control of tumor and microenvironment crosstalk by miR-15a and miR-16 in prostate cancer. Oncogene. 2010; 30(41):4231–4242. [PubMed: 21532615]
80. Martinez-Outschoorn UE, et al. Anti-estrogen resistance in breast cancer is induced by the tumor microenvironment and can be overcome by inhibiting mitochondrial function in epithelial cancer cells. Cancer Biol Ther. 2011; 12(10):924–938. [PubMed: 22041887]
81. Sun Y, et al. Treatment-induced damage to the tumor microenvironment promotes prostate cancer therapy resistance through WNT16B. Nat Med. 2012
82. Tchou J, et al. Targeting the tumor stroma as a novel treatment strategy for breast cancer: shifting from the neoplastic cell-centric to a stromacentric paradigm. Adv Pharmacol. 2012; 65:45–61. [PubMed: 22959023]
83. Davies C, et al. Relevance of breast cancer hormone receptors and other factors to the efficacy of adjuvant tamoxifen: patient-level meta-analysis of randomised trials. Lancet. 2011; 378(9793): 771–784. [PubMed: 21802721]
84. Pontiggia O, et al. The tumor microenvironment modulates tamoxifen resistance in breast cancer: a role for soluble stromal factors and fibronectin through beta1 integrin. Breast Cancer Res Treat. 2012; 133(2):459–471. [PubMed: 21935603]

85. Mueller KL, et al. Fibroblast-secreted hepatocyte growth factor mediates epidermal growth factor receptor tyrosine kinase inhibitor resistance in triple-negative breast cancers through paracrine activation of Met. Breast Cancer Res. 2012; 14(4):R104. [PubMed: 22788954]

86. Johansson AC, et al. Cancer-associated fibroblasts induce matrix metalloproteinase-mediated cetuximab resistance in head and neck squamous cell carcinoma cells. Mol Cancer Res. 2012; 10(9):1158–1168. [PubMed: 22809838]

87. Dittmer A, et al. Mesenchymal stem cells and carcinoma-associated fibroblasts sensitize breast cancer cells in 3D cultures to kinase inhibitors. Int J Oncol. 2011; 39(3):689–696. [PubMed: 21667024]

88. Capparelli C, et al. CDK inhibitors (p16/p19/p21) induce senescence and autophagy in cancer-associated fibroblasts, “fueling” tumor growth via paracrine interactions, without an increase in neo-angiogenesis. Cell Cycle. 2012; 11(19)

89. Bergamaschi A, et al. Extracellular matrix signature identifies breast cancer subgroups with different clinical outcome. J Pathol. 2008; 214(3):357–367. [PubMed: 18044827]

90. Finak G, et al. Stromal gene expression predicts clinical outcome in breast cancer. Nat Med. 2008; 14(5):518–527. [PubMed: 18438415]

91. Sloan EK, et al. Stromal cell expression of caveolin-1 predicts outcome in breast cancer. Am J Pathol. 2009; 174(6):2035–2043. [PubMed: 19411449]

92. Pavlides S, et al. The reverse Warburg effect: aerobic glycolysis in cancer associated fibroblasts and the tumor stroma. Cell Cycle. 2009; 8(23):3984–4001. [PubMed: 19923890]

93. Farmer P, et al. A stroma-related gene signature predicts resistance to neoadjuvant chemotherapy in breast cancer. Nat Med. 2009; 15(1):68–74. [PubMed: 19122658]

94. Ahn S, et al. The prognostic significance of tumor-associated stroma in invasive breast carcinoma. Tumour Biol. 2012

95. Martinet L, et al. Human solid tumors contain high endothelial venules: association with T- and B-lymphocyte infiltration and favorable prognosis in breast cancer. Cancer Res. 2011; 71(17):5678–5687. [PubMed: 21846823]

96. DeNardo DG, et al. Leukocyte complexity predicts breast cancer survival and functionally regulates response to chemotherapy. Cancer Discov. 2011; 1(1):54–67. [PubMed: 22039576]

97. Laoui D, et al. Tumor-associated macrophages in breast cancer: distinct subsets, distinct functions. Int J Dev Biol. 2011; 55(7-9):861–867. [PubMed: 22161841]

98. Lee AH, et al. Angiogenesis and inflammation in invasive carcinoma of the breast. J Clin Pathol. 1997; 50(8):669–673. [PubMed: 9301551]

99. Campbell MJ, et al. Proliferating macrophages associated with high grade, hormone receptor negative breast cancer and poor clinical outcome. Breast Cancer Res Treat. 2011; 128(3):703–711. [PubMed: 20842526]

100. Leek RD, et al. Association of macrophage infiltration with angiogenesis and prognosis in invasive breast carcinoma. Cancer Res. 1996; 56(20):4625–4629. [PubMed: 8840975]

101. Lewis CE, et al. Distinct role of macrophages in different tumor microenvironments. Cancer Res. 2006; 66(2):605–612. [PubMed: 16423985]

102. Lewis JS, et al. Expression of vascular endothelial growth factor by macrophages is up-regulated in poorly vascularized areas of breast carcinomas. J Pathol. 2000; 192(2):150–158. [PubMed: 11004690]

103. Dirkx AE, et al. Monocyte/macrophage infiltration in tumors: modulators of angiogenesis. J Leukoc Biol. 2006; 80(6):1183–1196. [PubMed: 16997855]

104. Roland CL, et al. Inhibition of vascular endothelial growth factor reduces angiogenesis and modulates immune cell infiltration of orthotopic breast cancer xenografts. Mol Cancer Ther. 2009; 8(7):1761–1771. [PubMed: 19567820]

105. Melani C, et al. Amino-biphosphonate-mediated MMP-9 inhibition breaks the tumor-bone marrow axis responsible for myeloid-derived suppressor cell expansion and macrophage infiltration in tumor stroma. Cancer Res. 2007; 67(23):11438–11446. [PubMed: 18056472]

106. Lin EY, et al. Colony-stimulating factor 1 promotes progression of mammary tumors to malignancy. J Exp Med. 2001; 193(6):727–740. [PubMed: 11257139]

107. Oosterling SJ, et al. Macrophages direct tumour histology and clinical outcome in a colon cancer model. J Pathol. 2005; 207(2):147–155. [PubMed: 16104052]
108. Mukhtar RA, et al. Tumor-associated macrophages in breast cancer as potential biomarkers for new treatments and diagnostics. Expert Rev Mol Diagn. 2011; 11(1):91–100. [PubMed: 21171924]
109. Nilsson S, et al. Downregulation of miR-92a is associated with aggressive breast cancer features and increased tumour macrophage infiltration. PLoS One. 2012; 7(4):e36051. [PubMed: 22563438]
110. Yang M, et al. Microvesicles secreted by macrophages shuttle invasion-potentiating microRNAs into breast cancer cells. Mol Cancer. 2011; 10:117. [PubMed: 21939504]
111. Hiraoka K, et al. Inhibition of bone and muscle metastases of lung cancer cells by a decrease in the number of monocytes/macrophages. Cancer Sci. 2008; 99(8):1595–1602. [PubMed: 18754872]
112. Gazzaniga S, et al. Targeting tumor-associated macrophages and inhibition of MCP-1 reduce angiogenesis and tumor growth in a human melanoma xenograft. J Invest Dermatol. 2007; 127(8):2031–2041. [PubMed: 17460736]
113. Zeisberger SM, et al. Clodronate-liposome-mediated depletion of tumour-associated macrophages: a new and highly effective antiangiogenic therapy approach. Br J Cancer. 2006; 95(3):272–281. [PubMed: 16832418]
114. Zhang W, et al. Depletion of tumor-associated macrophages enhances the effect of sorafenib in metastatic liver cancer models by antimetastatic and antiangiogenic effects. Clin Cancer Res. 2010; 16(13):3420–3430. [PubMed: 20570927]
115. Veltman JD, et al. Zoledronic acid impairs myeloid differentiation to tumour-associated macrophages in mesothelioma. Br J Cancer. 2010; 103(5):629–641. [PubMed: 20664588]
116. Luckman SP, et al. Heterocycle-containing bisphosphonates cause apoptosis and inhibit bone resorption by preventing protein prenylation: evidence from structure-activity relationships in J774 macrophages. J Bone Miner Res. 1998; 13(11):1668–1678. [PubMed: 9797474]
117. Cecchini MG, et al. Effect of bisphosphonates on proliferation and viability of mouse bone marrow-derived macrophages. J Bone Miner Res. 1987; 2(2):135–142. [PubMed: 3455162]
118. Coscia M, et al. Zoledronic acid repolarizes tumour-associated macrophages and inhibits mammary carcinogenesis by targeting the mevalonate pathway. J Cell Mol Med. 2010; 14(12): 2803–2815. [PubMed: 19818098]
119. Man YG, et al. The significance of focal myoepithelial cell layer disruptions in human breast tumor invasion: a paradigm shift from the “protease-centered” hypothesis. Exp Cell Res. 2004; 301(2):103–118. [PubMed: 15530847]
120. Knutson KL, et al. IL-2 immunotoxin therapy modulates tumor-associated regulatory T cells and leads to lasting immune-mediated rejection of breast cancers in neu-transgenic mice. J Immunol. 2006; 177(1):84–91. [PubMed: 16785502]
121. Mahmoud SM, et al. Tumor-infiltrating CD8+ lymphocytes predict clinical outcome in breast cancer. J Clin Oncol. 2011; 29(15):1949–1955. [PubMed: 21483002]
122. Ruffell B, et al. Leukocyte composition of human breast cancer. Proc Natl Acad Sci U S A. 2012; 109(8):2796–2801. [PubMed: 21825174]
123. Acharyya S, et al. A CXCL1 paracrine network links cancer chemoresistance and metastasis. Cell. 2011; 150(1):165–178. [PubMed: 22770218]
124. Iyengar P, et al. Adipocyte-derived collagen VI affects early mammary tumor progression in vivo, demonstrating a critical interaction in the tumor/stroma microenvironment. J Clin Invest. 2005; 115(5):1163–1176. [PubMed: 15841211]
125. Iyengar P, et al. Adipocyte-secreted factors synergistically promote mammary tumorigenesis through induction of anti-apoptotic transcriptional programs and proto-oncogene stabilization. Oncogene. 2003; 22(41):6408–6423. [PubMed: 14508521]
126. Finley DS, et al. Periprostatic adipose tissue as a modulator of prostate cancer aggressiveness. J Urol. 2009; 182(4):1621–1627. [PubMed: 19683746]
127. Yu JM, et al. Mesenchymal stem cells derived from human adipose tissues favor tumor cell growth in vivo. Stem Cells Dev. 2008; 17(3):463–473. [PubMed: 18522494]

128. Walter M, et al. Interleukin 6 secreted from adipose stromal cells promotes migration and invasion of breast cancer cells. Oncogene. 2009; 28(30):2745–2755. [PubMed: 19483720]
129. Welte G, et al. Interleukin-8 derived from local tissue-resident stromal cells promotes tumor cell invasion. Mol Carcinog. 2011
130. Pinilla S, et al. Tissue resident stem cells produce CCL5 under the influence of cancer cells and thereby promote breast cancer cell invasion. Cancer Lett. 2009; 284(1):80–85. [PubMed: 19427114]
131. Devarajan E, et al. Epithelial-mesenchymal transition in breast cancer lines is mediated through PDGF-D released by tissue-resident stem cells. Int J Cancer. 2011; 131(5):1023–1031. [PubMed: 22038895]
132. Muehlberg FL, et al. Tissue-resident stem cells promote breast cancer growth and metastasis. Carcinogenesis. 2009; 30(4):589–597. [PubMed: 19181699]
133. Bochet L, et al. Cancer-associated adipocytes promotes breast tumor radioresistance. Biochem Biophys Res Commun. 2011; 411(1):102–106. [PubMed: 21712027]
134. Ligibel J. Obesity and breast cancer. Oncology (Williston Park). 2011; 25(11):994–1000. [PubMed: 22106549]
135. Dirat B, et al. Unraveling the obesity and breast cancer links: a role for cancer-associated adipocytes? Endocr Dev. 2010; 19:45–52. [PubMed: 20551667]
136. Harris RE. Cyclooxygenase-2 (cox-2) blockade in the chemoprevention of cancers of the colon, breast, prostate, and lung. Inflammopharmacology. 2009; 17(2):55–67. [PubMed: 19340409]
137. Dougall WC, et al. Molecular pathways: osteoclast-dependent and osteoclast-independent roles of the RANKL/RANK/OPG pathway in tumorigenesis and metastasis. Clin Cancer Res. 2012; 18(2):326–335. [PubMed: 22031096]

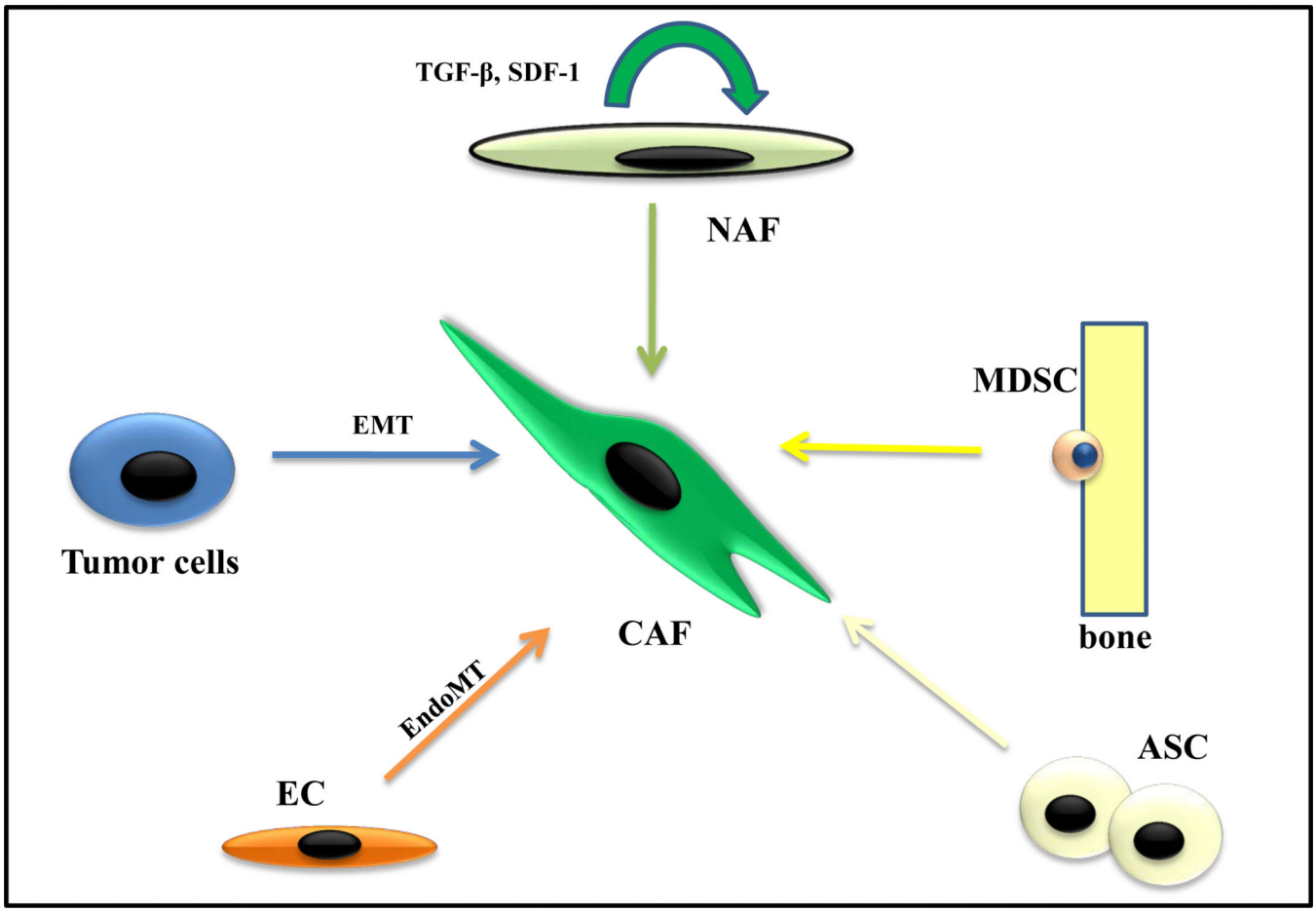

Figure 1. The origin of CAFs

Schematic of cells that may transit to (**arrows**) CAFs. **Abbreviations:** CAF, cancer-associated fibroblast; NAF, normal tissue derived fibroblast; MDSC, mesenchymal derived stem cell; EC, endothelial cell; EMT, epithelial mesenchymal transition; EndoMT, endocrine mesenchymal transition; ASC, adipose tissue-derived stem cells.

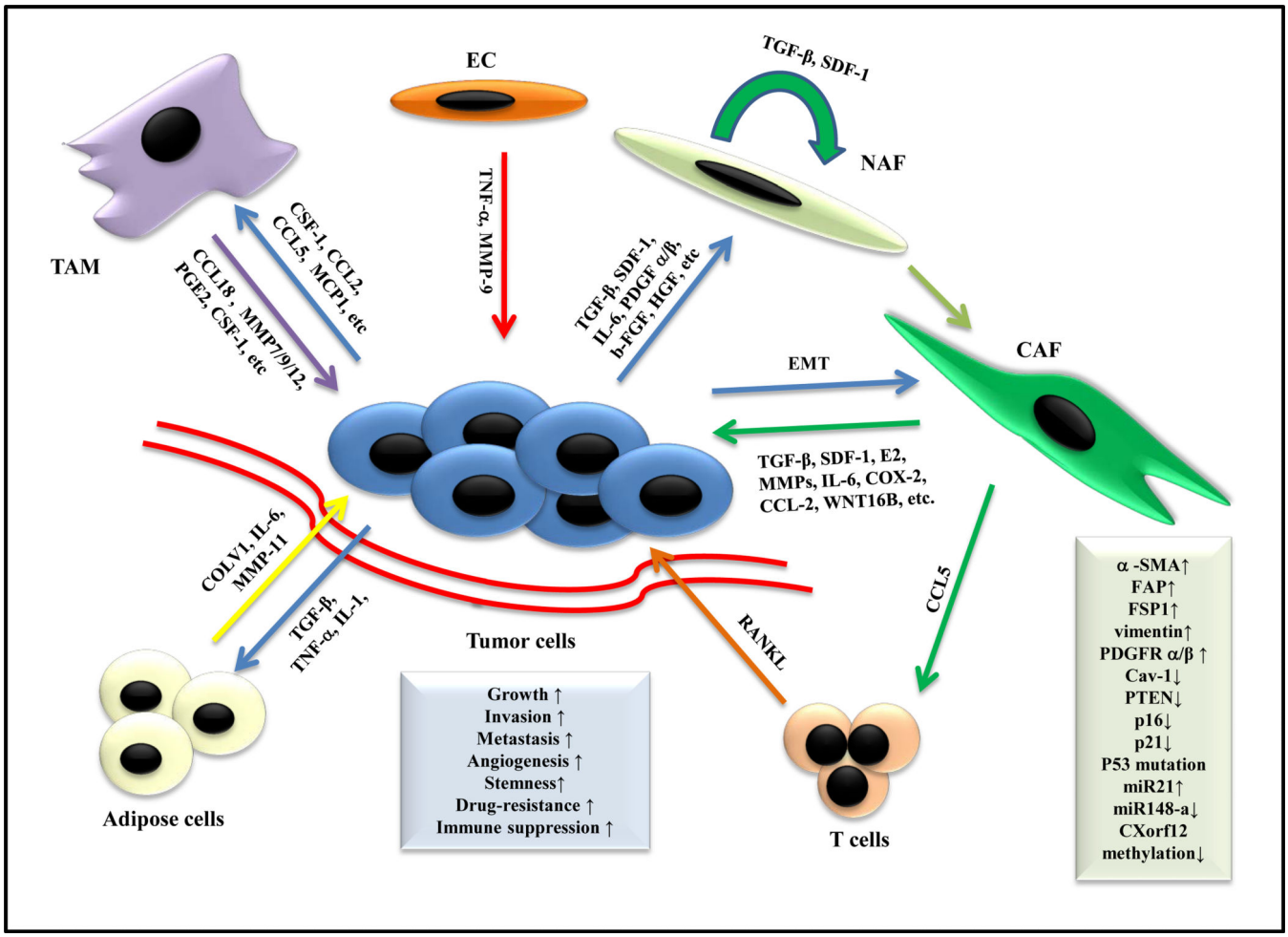

Figure 2. Schematic representation showing the role of stroma cells in microenvironment and breast cancer progression

The tumor microenvironment is a dynamic composite of cells broadly categorized as multiple components of no-stroma and stroma cells, where tumor cells thrive. Stroma cells promote tumor growth, invasion, and metastasis by secreting multiple cytokines, chemokines and other growth factors, et al. Moreover, tumor cells also affect the phenotype of stroma cells. Obviously, the tumor and stroma cell interactions are truly reciprocal; while stroma cells may support tumors, tumor cells in turn modulate the microenvironments within which they inside. **Abbreviations:** SDF-1, stroma-derived factor; TNF- α, tumor necrosis factor; TGF-β, transforming growth factor-β; NF-κB, nuclear factor κB; MMP-7,9,11 matrixmetalloproteinase-2,9; α-SMA, alpha smooth muscle actin; FAP , fibroblast activation protein; FSP-1, fibroblast specific protein; PDGFR-α/β, platelet-derived growth factor-α/β; FGF, fibroblast growth factor; Cav-1, caveolin-1; IL-1,4,6,10,13, interleukin -1,4,6,10,13; E2, estrone -E2; CCL2, 5, 18, chemokine ligand 2, 5, 18; RANKL , nuclear factor-κB (RANK) ligand; CSF-1, colony stimulating factor-1; COLVI , collagen VI; VEGF, vascular endothelial growth factor; HGF, hepatocyte growth factor.

Table 1

Drugs targeting different stroma cells in breast cancer

Drug	Target	Types of cancer	Clinical phase	Reference
Celecoxib Rofecoxib (anti-COX2)	CAFs	Colon, Breast, Prostate, Lung.	Phase II/III	[132]
PLX3397	CAFs	TNBC	phase I /II	[131]
chloroquine	CAFs	Breast	Phase I/II	[22]
metformin or ATO	CAFs	Breast	Pre-clinical	[71, 75]
PD0332991	CAFs	Breast ,Melanoma, Lymphoma	Phase II	[81]
SB431542	CAFs	Breast	Pre-clinical	[96]
XAV939	CAFs	Breast, Prostate, Ovary	Pre-clinical	[74],
Anti-Met	CAFs	TNBC	Pre-clinical	[78]
An anti-FAP vaccine	CAFs	Breast cancer	Pre-clinical	[18]
BPs	TAMs	Breast cancer,	Phase III	[98,105,112]
CSF-1R antagonist + paclitaxel	TAMs	Breast cancer	Pre-clinical	[89]
Denosumab (antu-RANKL)	Anti-Tregs	Breast cancer	Phase III	[133]