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Cancer associated fibroblasts (CAFs) in tumor microenvironment

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Abstract

Cancer associated fibroblasts (CAFs) is one of the most crucial components of the tumor microenvironment which promotes the growth and invasion of cancer cells by various mechanisms. CAFs demonstrate a high degree of heterogeneity due to their various origins; however, many distinct morphological features and physiological functions of CAFs have been identified. It is becoming clear that the crosstalk between the cancer cells and the CAFs plays a key role in the progression of cancer, and understanding this mutual relationship would eventually enable us to treat cancer patients by targeting CAFs. In this review, we will discuss the latest findings on the role of CAFs in tumorigenesis and metastasis as well as potential therapeutic implication of CAFs.

Keywords

CAFs; Cancer; Microenvironment; Review

2. INTRODUCTION

The progression of cancer is no longer being recognized as an independent event which only relates to the genetic mutation and uncontrollable growth of cancer cells. Different types of growth factors and cytokines secreted by the surrounding stromal cells and signal pathways induced by cell-cell interactions are thought to play key roles in the tumorigenesis and metastasis. A specialized group of fibroblasts called cancer associated fibroblasts, CAFs, is believed to actively participate in the growth and invasion of the tumor cells by providing a unique tumor microenvironment (1). Because of the close relationship between the cancer cells and CAFs, it is increasingly clear that the development of cancer cannot be dissociated from its local microenvironment. However, neither the origin of CAFs nor the criteria to distinguish CAFs from normal fibroblasts has been well established. In this review, we will discuss these issues by comparing different biological functions of both normal fibroblasts and CAFs in tumorigenesis, metastasis, and signaling pathways.

Normal stroma consists of various connective tissues that act like a supportive framework for tissues and organs. Among all the stromal components, fibroblasts are essential to synthesize and deposit the extracellular matrix (ECM) by producing a variety of collagens and fibronectin (2). In addition, they are indispensable for the formation of the basement membrane which separates the epithelium from the stroma by secreting laminin and type IV

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collagen (3). They are also an ample source of various soluble paracrine and autocrine growth factors that regulate the growth of the surrounding cells as well as themselves (4). Interestingly, fibroblasts not only maintain the integrity of the ECM and basement membrane, in some cases, they also contribute to the ECM remolding by secreting proteases such as matrix metalloproteinase(MMPs) which effectively degrade ECM (5). Another important role of fibroblasts is wound healing. In this process, they 'invade' the lesions and generate ECM which acts like a scaffold for tissue regeneration. During wound healing, fibroblasts are activated and become a specialized type of fibroblast, myofibroblast, which is endowed with a higher capability of ECM synthesis (6). However, the mechanism of how these myofibroblasts restore their original phenotype is still unclear. In addition to fibroblasts, other types of cells such as inflammatory cells and endothelium also contribute to the integrity and homeostasis of the stroma (7).

The formation of cancer to some extent depends on the ability of cancer cells to recruit a variety of stromal cells and to take advantage of them. Among the components of the stromal cells, an increasing number of CAFs which share a similar morphology with myofibroblasts observed in wound healing is often found in the cancer regions, while an enhancement of fibrin deposition is also observed (8). During the progression of cancer, tumor cells are able to alter the characteristics of the adjacent stroma to create a supportive microenvironment. This notion is strongly supported by the recent evidence that over 80% of the fibroblasts demonstrate an activated phenotype in breast cancer (9). Compared to normal fibroblasts and the myofibroblasts in wound healing, CAFs are perpetually activated, neither reverting back to a normal phenotype nor undergoing apoptosis (10). CAFs found in different cancers are highly heterogeneous, and they are possibly derived from resident fibroblasts, epithelia cells, endothelia cells or mesenchymal cells. The results of several *in vitro* and *in vivo* experiments indicate that CAFs promote cancer progression in both proliferation and invasion through multiple growth factors and signaling pathways. As the most abundant cell type in the tumor stroma and their tumor-promoting abilities, there is an increasing interest to study CAFs as drug targets for anticancer therapies.

3. CAFs CAN BE DISTINGUISHED FROM NORMAL FIBROBLASTS BY THEIR UNIQUE CHARACTERISTICS

CAFs found in the tumor stroma are large spindle shaped mesenchymal cells with stress fibers and well developed fibronexus (11). Based on immunohistochemical data, several CAFs markers were identified including alpha smooth muscle actin (alpha SMA), fibroblast activation protein (FAP), Thy-1, desmin, and S100A4 protein (12). Alpha SMA has been known to play a pivotal role in the embryonic stem cell-derived cardiomyocyte differentiation (13). On the other hand, expression of alpha SMA in the stroma blocks the migration of fibroblasts and contributes to alterations in cytoskeletal organization, which increases their contractile ability (14). Figure 1 shows an example of a high level of alpha SMA expression in invasive breast cancer; notably, in alpha SMA also expressed in myoepithelial cells of normal gland. FAP is a 95 kDa type II integral membrane glycoprotein belonging to the serine protease family (15). It has been shown to have both collagenase and dipeptidyl peptidase (DPP) activities which help to degrade the ECM (16). Chesa *et al.* performed a series of immunohistochemical experiments, and showed that FAP is excessively expressed by CAFs in over 90% of human epithelial carcinomas including breast, lung, and ovarian cancers (12). Thy-1 belongs to the glycoprotein family whose expression level determines the different profiles of cytokines of the fibroblasts, and only Thy-1+ orbital fibroblasts were able to differentiate to CAFs after treatment with transforming growth factor- beta (TGF-beta) (17, 18).

In most cases, CAFs are negative for epithelial or endothelial markers such as cytokeratin and CD31. It was reported that in breast invasive ductal carcinoma (IDC), alpha SMA $^{+}$ myofibroblasts are increased in the cancer regions and $CD34^{+}$ fibrocytes gradually disappear (19). Additionally, the expression level of laminin, whose function is to maintain the integrity of basement membrane is significantly reduced in the CAFs (20). Therefore, CAFs can be identified by their unique cell surface markers and morphological features which clearly distinguish them from the normal fibroblasts.

4. CAFs ARE DERIVED FROM VARIOUS ORIGINS

4.1. Normal fibroblasts can generate CAFs through genetic alteration

Cancer has been recognized as a disease due to its genetic alterations. It has been shown that CAFs are not only responsive to the extracellular molecules such as growth factors and cytokines, but also undergo frequent genetic alternations (21). Littlepage *et al.* found that even without exposure to cancer cells, the tumor promoting characteristics of CAFs can be stably maintained. These observations indicate that genetic or epigenetic changes may have already existed in the cancer stroma independent of the original tumor (22). Previous studies have reported a high frequency of genetic alternations such as point mutations, loss of heterozygosity (LOH), and gene copy number changes in oncogenes and tumor suppressors in CAFs that were isolated from various human cancers. Somatic mutations of P53 and PTEN are frequently observed in the epithelium of breast carcinoma. Both genes are indispensible to cell growth arrest whose malfunction directly leads to cancer progression (23). Interestingly, inactivation of these two genes are often detected in the CAFs around the cancer regions (24). However, it was also found that P53 expression level in the CAFs could be induced by the cancer cells through a paracrine mechanism which creates a selective pressure that promotes the expansion of the P53-negative CAFs (25). By analyzing the LOH in cancer, Kurose and colleagues found that LOH in the stromal compartment ranged from 17% to 61% in invasive breast cancer. They suggested that the genetic changes in the surrounding stromal cells are consequences of mutations in the epithelial compartment due to the higher LOH frequency in these cells (26). Furthermore, a significant correlation between the tumor grade and LOH signature of CAFs has been reported in breast cancer (27). Previous studies also suggest that genetic or phenotypic changes in stroma may be induced by adjacent carcinoma cells. For example, transplanting the human prostate cancer cell line C4-2 into athymic male nude mice indeed induced sarcomas of murine origin (28). On the contrary, in another study using single-nucleotide polymorphism (SNP) microarray and immunohistochemical approaches, no evidence was found to prove that CAFs undergo somatic copy number changes or p53 mutations in pancreatic cancer (29). Similarly, no clonally selected somatic or genetic alterations were found in CAFs from breast cancer biopsies by using comparative genomic hybridization (CGH) array (30). Due to the heterogeneity of the tumor samples and different analysis methods, the evidence for genetic alterations as a factor to generate the CAFs is still controversial and conflicting.

4.2. CAFs may derive from epithelial cells through epithelial- mesenchymal transition (EMT)

Epithelial-mesenchymal transition (EMT) is a term that refers to the event in which cells undergo a switch from an epithelial phenotype with tight junctions, to mesenchymal cells with a loose cell-cell adhesion (31). The importance of this process was initially thought as an early step in embryogenesis, but it was recently recognized as a potential mechanism of epithelial cancer metastasis (32). Because the molecular mechanisms of EMT has been well established, cancers characterized by alterations in stromal elements and fibrosis are also being considered as examples of EMT (33). Transdifferentiation of myofibroblasts from epithelial cells is a special case of EMT, which only generates CAFs instead of malignant

cancer cells (34). By providing the proper conditions, breast cancer cells may transdifferentiate to myoepithelial cells and finally become myofibroblasts, the ancestors of CAFs (35). Another example is that mouse squamous skin carcinoma cells acquire mesenchymal morphology with loss of adhesion marker, E-cadherin, by activating Ras and TGF- beta signaling (36). In most cases, CAFs generated through EMT may not be as malignant as cancer cells, but they are able to promote the cancer growth and metastasis significantly. It has been shown that kidney tubular epithelial cells which express β - galactosidase were observed in up to 30% of activated fibroblasts in the kidney, which indicates a potential source of CAFs (37). Recent genetic analysis showed that CAFs isolated from human breast cancer biopsies were indeed derived from the epithelial tumor cells (38).

Interestingly, genetic alterations present in both cancer cells and the CAFs are rarely identical, suggesting that only a small portion of the cancer cells and stromal cells may share a common origin (24). It is likely that CAFs are a population of early developmental precursors in normal mammary gland which respond to signals from the cancer cells (22). Although cancer cells fail to generate the majority of CAFs through EMT, surrounding normal epithelial cells may be an additional source of CAFs by undergoing EMT in response to stimuli from the microenvironment (37,39,40)

4.3. Mesenchymal cells are a potential source of CAFs

Bone-marrow (BM) derived circulating cells which belong to mesenchymal cells, have been demonstrated to be able to localize and proliferate in tumor stroma area (39). There is evidence that bone marrow contributes to the myofibroblasts and fibroblasts of the desmoplastic response as well as to tumor angiogenesis (40). The results of recent studies suggest that CAFs may develop from existing interstitial fibroblasts; however, animal studies by using transplanted bone marrow precursors have demonstrated that cells with features of CAFs can also be derived from myeloid precursor cells (41). Direkze and colleagues used transgenic mice with large T antigen (RIPTag) which was driven by rat insulin promoter as a model to study the origin of the CAFs. They transplanted alpha SMA^+ mesenchymal cells, which were labeled with green fluorescent protein (GFP), from a male donor to a female recipient and found that almost 25% of the total CAFs populations in the tumor area were GFP positive, which provides strong evidence to show that mesenchymal cells are a potential source of CAFs (39). Recent evidence also suggests that mesenchymal stem cells (MSC) selectively proliferate in tumor areas and contribute to the formation of CAFs. Mishra *et al.* found that when human bone marrow–derived mesenchymal stem cells (hMSCs) were treated with tumor-conditioned medium (TCM) derived from MDA-MB231 human breast cancer cell line in a prolonged period of time, cells exhibited functional properties of CAFs with sustained expression of SDF-1 (42).

Myofibroblast is known as an important cell type which is involved in wound healing by contracting the stroma and bringing the epithelial borders together (11). Besides wound healing, myofibroblasts are essential for tissue morphogenesis, stem cell niches formation and mucosal immunity (43). Myofibroblasts also appear in the cancer regions and are being recognized as a major source of the CAFs (44). They are large spindle-shaped mesenchymal cells with indented nuclei and well developed fibronexus(45). By using immunohistochemical approach, several myofibroblast markers were discovered; positive for vimentin and alpha SMA, and negative in smooth muscle myosin, desmin and cytokeratin (8). The matrix around cancer cells is profoundly influenced by myofibroblasts which are actively participating in ECM remodeling including increased production of fibronectin, proteoglycans and glycosaminoglycans (46, 47). The specialized adhesion structures formed by myofibroblasts and ECM are called focal adhesions (FAs) *in vitro* or fibronexus *in vivo* (48,49). Myofibroblasts observed at the invasion front in colon cancer

highly express pro-invasive factors such as scatter factor/hepatocyte growth factor (SF/HGF) and glycoprotein tenascin-C (TNC). Furthermore, laminin, an essential factor for the integrity of normal tissue was found to be down-regulated in the myofibroblasts which appear in the cancer lesion, providing another evidence that CAFs may be directly differentiated from myofibroblasts (50).

4.4. Endothelial cells may become CAFs through endothelial to mesenchymal transition (EndMT)

Endothelial cells exhibit different phenotypes according to the local microenvironment. It is reported that TGF-beta was able to induce proliferating endothelial cells to undergo a phenotypic conversion into fibroblast-like cells (51). Such endothelial to mesenchymal transition (EndMT) is associated with the emergence of mesenchymal marker fibroblast-specific protein-1 (FSP1) and down-regulation of CD31/PECAM (52). Almost 40% of the CAFs which express FSP1 share the same endothelial marker CD31 (53). CD31 was detected in 11% of the alpha SMA^+ CAFs. During EndMT, resident endothelial cells delaminate from an organized cell layer and invade the underlying tissue due to the loss of the adhesion molecular such as E-cadherin (52). Previous study of the EndMT mainly focused on its role in the embryonic development of the heart; however, recent evidence suggests that EndMT frequently occurs in a variety of cancers. Zeisberg *et al.* observed frequent EndMT in tumors in both B16F10 melanoma model and the Rip-Tag2 spontaneous pancreatic carcinoma model (53). They tagged endothelial cells by cross breeding Tie2-Cre mice with R26Rosa-lox-Stop-lox-LacZ animal, and provided evidence for EndMT as a source of CAFs at the invasive front of the tumors in these transgenic mice. Their results showed that EndMT is a unique mechanism for the accumulation of CAFs which facilitate cancer progression. Furthermore, mural cells such as vascular smooth muscle cells share a similar morphology and markers with myofibroblasts, providing another possibility that CAFs may be derived from the vascular origin (54).

As mentioned in previous paragraphs, the potential origins of the CAFs are summarized in Figure 2. Normal fibroblasts, cancer cells, mensenchymal cells and even endothelial cells could become CAFs under different tumor microenvironment.

5. CAF CONTRIBUTE TO TUMORIGENESIS AND METASTASIS BY ALTERING THE TUMOR MICROENVIRONMENT

5.1. CAFs Support primary tumor growth

Tumor cell proliferation is an essential step for the following invasion. In order to maintain continuous growth and propagation at metastatic sites, tumor cells have to recruit supportive stromal cells. Abundant growth-promoting factors were found in the conditioned medium from CAFs which were isolated from metastatic colon cancer patients as compared to the conditioned medium taken from normal skin fibroblasts (44). CAFs secrete various growth factors and cytokines into adjacent cancer cells such as TGF-beta and hepatocyte growth factor (HGF). Overexpression of these two growth factors in mouse fibroblasts is able to induce the initiation of breast cancer by co-injection with normal epithelium (55). Fibroblast secreted protein-1 (FSP1) produced by CAFs is another important factor in promoting the cancer cell growth. metastatic cancer cells transplanted into FSP1 knockout mice are less likely to form tumors, and co-injection of fibroblasts which overexpress FSP1 with the same tumor cells can restore the tumor formation (56). The results of this experiment indicate that FSP1 secreted by CAFs is capable of altering the cancer microenvironment which is favorable for cancer progression. Previous studies also demonstrated that stromal derived factor1 (SDF-1) derived from CAFs was able to recruit endothelial cell precursors (EPCs), thereby promoting angiogenesis (57). SDF-1α also known as CXCL12, is the ligand of

CXCR4 which is highly expressed on the cancer cells, and the activated CXCR4 can directly stimulate cancer cell proliferation (58). In melanoma, the fibroblasts which express N-cadherin serve as a positive stimulator for proliferation and migration of cancer cells. Because melanoma cells are unable to produce IGF-1 by themselves, they have to rely on the IGF-1 secreted by the surrounding fibroblasts to stimulate their growth (59). It should be noted that in lung and prostate cancers, CAFs can respond to androgens to produce growth factors that induce epithelial proliferation (60).

In addition to their abilities of secreting growth factors to enhance the cancer cell growth, CAFs can act like a mutagen which increases the tumorigenic ability of cancer cells. For example, reactive oxygen species (ROS) which are generated by CAFs under low pH and hypoxia environment could directly act as a mutagen to the surrounding cells (61). Tumorigenicity of normal mammary epithelial cells was indeed shown to be significantly enhanced by the irradiated fibroblasts *in vivo* (62). Such enhancement is due to the overexpression of TGF-beta from the irradiated stroma (63). Furthermore, a high level of TGF-beta expression has been detected in many malignant tumors including colon (64) and breast cancers (65). TGF-beta is the only known growth factor that is able to transdifferentiate fibroblasts into CAFs (66). On the other hand, TGF-beta has been identified as a potent chemoattractant for human dermal fibroblasts, which is able to recruit fibroblast outside of the tumor region (67). As an abundant cell type in tumor area, CAFs can also affect cancer cell growth by mediating their metabolic pathways. For example, during the proliferation of cancer cells, they prefer to turn to anaerobic glycolysis even in the presence of oxygen (68). Furthermore, CAFs are able to turn on some complementary metabolic pathways to buffer and recycle products of anaerobic metabolism in order to maintain the growth of cancer cells (68).

5.2. CAFs support cancer cell invasion and metastasis

CAFs found around the cancer regions are not only able to promote cancer cell growth but also to increase the invasiveness of the cancer cells through cell-cell interactions and various pro-invasive molecules, including cytokines, chemokines and various inflammatory mediators (69). CAFs induce invasive growth by transient heterotypic cell-cell contacts or by paracrine diffusible factors. On the other hand, proliferation of CAFs is mediated by the local growth factors including fibroblast growth factor (FGF), TGF-beta and other connective tissue growth factor (45). Of note, SPARC (secreted protein acidic and rich in cysteine) expressed by CAFs predicts a poor prognosis for patients with resectable pancreatic cancer (70). Signal cross-talk between the cancer cells and CAFs may direct both types of cells to modify the adjacent ECM and basement membrane. It has been widely recognized that breaking the basement membrane is the first step for the cancer cells to intravasate into the circulation system. On the other hand, the remodeled ECM proteins can alter the expression level of some specific genes that are essential for the structural scaffolding and cytoskeletal organization (71). In fact, CAFs are potentially invasive, and the results of *in vivo* experiments also showed that CAFs can invade into tumor areas (72). By using GFP transgenic mice under the control of the VEGF promoter, Fukumura *et al.* found that GFP positive CAFs were invading into tumor regions at different time points. However, in some cases, it is difficult to judge which one contributes more to the invasion, epithelial cells or CAFs. It has been shown that CAFs may serve as guidance structure that direct the migration of cancer cells as well. The result of co-culture experiment suggests that the leading invasive cells are of stromal origin, and they facilitate following cancer cells metastasis by degrading ECM and basement membrane (73). In order to make way for the invading cancer cells, CAFs will initiate both proteolytic and structural modification of the ECM to create the path for the following cancer cells. In another case, Cornil *et al* found that

CAFs support metastasis of melanoma cells by creating a niche which enhances the growth of cancer cells at distant sites (74).

The remodeling of the extracellular matrix by MMPs is one of the most crucial steps for cancer progression as well as for the formation of cancer microenvironment. Under normal physiological conditions, the balance between metalloproteinases and their inhibitors keeps ECM in a well organized shape. It has been shown that fibroblasts play major roles in tumor invasion by secreting various matrix-degrading proteases as well as their activators such as uPA (75). uPA can cleave MMPs to activate these proteins, and up-regulation of MMPs activity results in significant ECM degradation, which contribute to angiogenesis and metastasis (76). The tissue inhibitors of metalloproteinases (TIMPs) have been shown to down-regulate MMPs activity. TIMPs can also regulate other growth factors independent of the inhibition of the MMPs which indicates the TIMPs may also be involved in some important oncogenic signal pathways (77).

There are many MMPs that are known to play pivotal roles in cancer metastasis. Overexpression of MMP3 by the CAFs stimulates epithelial hyperplasia and abnormal branching in the mammary gland (78). A high level of MMP2 production in stromal cells is required to support tumor growth and pathological neoangiogenesis of gliomas (79). The interstitial collagenase MMP1 was observed at increased amounts in the peritumoral fibroblasts isolated from a primary melanoma (80). Up-regulation of MMP1 has been recognized as a putative breast cancer predictive marker (81), and it can stimulate growth and invasion pathways by cleaving protease-activated receptor-1 (PAR1) on the cancer cell surface. On the other hand, knock-down of PAR1 can block the invasiveness of metastatic cancer cells in a nude mice model (82). The reasons which cause the activation of the MMPs are somewhat complicated. Fibroblasts are not the only source of the MMPs, and high activity of MMPs and uPA are also observed in various cancer cells. Tumor and stromal cells interaction is a mutual event and the soluble factors secreted by cancer cells also affect the expression of MMPs. For example, the expression of MMP2 in the stroma of malignant tumors is increased by the paracrine stimulation factors; however, the expression level of MMP9 in tumor derived fibroblasts requires direct contact with malignant tumor epithelium which indicates a different mechanism of MMPs activation (83).

Results of *in vivo* experiments also indicate the importance of MMPs in tumorigenesis. A mammary-specific transgenic mouse of stromelysin-1, one of the members of MMPs family, was constructed using the MMTV promoter. The overexpression of stromelysin-1 significantly increased the incidence of tumor formation by vigorously degrading the basement membrane (84). Interestingly the tumorigenic ability is reduced in mice bearing both TIMP-1 and stromelysin-1 transgenes. Collectively, CAFs affect cancer cell invasion through both cell-cell contact and pro-invasive factors secretion, they are also one of the most significant contributors to produce MMPs which play a major role in cancer metastasis. Hence, it is promising to develop anti-cancer treatments targeting MMPs which are secreted from CAFs.

5.3. CAFs induce inflammation in cancer regions

The initial assumption of chronic inflammation contributing to tumorigenesis was claimed by Virchow who observed that cell proliferation was enhanced by inflammation caused by irritants (85). Many clinical and experimental data also support the notion that fibroblasts play crucial roles in immune responses through production of cytokines and chemokines (86). In the thymus and lymph nodes, they help to identify distinct anatomical compartments (87). Furthermore, fibroblasts not only mediate the quality but also the quantity of the immune response (88). In normal physiology, fibroblasts can terminate immune responses

by withdrawing survival signals and normalize the chemokine gradients which accelerate the apoptosis or withdraw the tissue through the lymphatic vessels (89).

In clinical settings, chronic inflammation and cancer are closely related, and cancer is referred to as “wounds that never heal” (90). Pro-inflammatory cytokines are secreted by cancer cells and CAFs attract excessive immune cells to the cancer region. Macrophages, neutrophils, and lymphocytes could be recruited to the tumor stroma by secreting factors from the CAFs. Macrophages are actively attracted into tumor regions along defined chemotactic gradients, and release a number of factors that influence endothelial cell behavior including VEGF, HGF, MMP2, IL-8. Once macrophages reach the tumor, they start to differentiate into tumor-associated macrophages (TAMs) which further enhance the growth and metastasis of cancer cells (91). Stromal cells are instrumental in creating the unique environment of chronic inflammation and immune tolerance, allowing cancer cells to be exposed to growth factors while avoiding immune surveillance by secreting various cytokines, chemokines, and other factors (92).

In order for the tumor to survive, any immune response directed toward the tumor cells needs to be suppressed as a net result. Stromal cells are the main source of thrombospondin-1(TSP-1) which has both positive and negative effects on angiogenesis and interaction with immune cells (10). As mentioned previously, CAFs excessively secrete MMPs which degrade basement membrane, and cleaved products of MMPs such as fibronectin and collagen are chemotactic for leukocytes, meanwhile, they also modulate the proliferation of the immune cells (93). Cyclooxygenase-2 (COX2) is an enzyme which specifically catalyzes the production of prostaglandins which trigger inflammation (94). By using microarray expression analysis on CAFs from metastatic colon cancer and normal skin fibroblasts, approximately 170 genes were found to be up-regulated in CAFs as compared to skin fibroblasts (95). Among the up-regulated genes, COX2 expression level in CAFs was six times higher than that in normal skin fibroblasts (95). IL-8 belongs to CXC chemokine family which is involved in recruitment of leukocytes to the site of inflammation (96). Normally, cells without external stimulation secrete a very low amount of IL-8; however, certain pro-inflammatory cytokines such as TNFα, IL-1b and IL-6 can elevate the IL-8 expression in both transcriptional and post transcriptional levels (97). Mueller and colleagues investigated IL-8 induction in CAFs which were isolated from metastatic colon cancer patients (98). They found that TNFα mediates IL-8 expression through NF-κB signal and promotes angiogenesis and tumor cell invasion, and such induction can be abrogated by using NF-κB inhibitor parthenolide (98). Therefore, various pro-inflammatory factors produced by CAFs keep cancer region in a chronic inflammatory state which facilitates cancer cell metastasis.

6. CANCER STEM CELLS (CSCs) NICHE AND CAFs

Several lines of evidence indicate that aberrant regulation of adult stem cells leads to tumor formation. Many solid tumors have been shown to contain certain number of stem cells, and they appear to be highly tumorigenic and metastatic (99). Cancer stem cells were first found in acute myeloid leukemia (100), and it was also detected in breast and brain tumors later (101). It is believed that the stromal niche is able to regulate the differentiation and proliferation of the stem cells by providing a unique microenvironment (102). For example, quiescent prostatic epithelial tissues which contain adult stem cells were capable of modulating the differentiation in response to the mesenchymal cells (103).

Similar to the normal stem cells, cancer stem cells also need a favorable environment to support their self-renewal or differentiation. In basal cell carcinomas and some solid tumors, the tumor niche comprised by CAFs is indeed molecularly distinct from those found in

normal stroma. Sneddon and colleagues claimed that tumor niche secretes several factors (e.g., BMP antagonists) that are also produced by the normal stem cell niche to maintain self-renewal. On the other hand, unlike their normal counterparts, skin CAFs express high levels of the BMP antagonist, Gremlin 1 (104). In contrast, BMP2 and BMP4 are highly expressed in basal carcinoma cells. Gremlin 1 protein is capable of preventing the basal cell carcinoma from undergoing differentiation, which suggest that expression of secreted BMP antagonists by CAFs may promote self-renewal of CSCs (104). It has been reported that brain CSCs reside in a vascular niche which benefits their self-renewal (105). Disrupting this niche blocks self-renewal of the brain cancer stem cells and hence significantly inhibits tumor growth. The microenvironment created by bone marrow endothelial cells appears to be required for the homing of both normal hematopoietic stem cells (HSCs) and leukemic cells (106). Moreover, ECM components and growth factors in the HSC microenvironment can promote cell survival of acute myeloid leukemia (AML) as well as chemo-resistance (107). In order to examine whether tumor stroma can provide a distinct niche for stem cells, Risbridger *et al.* combined human ES cells with CAFs and grafted into the kidney capsule of host mice (108). Interestingly, they found that the grafted ES cells alone rapidly formed large teratomas, and when combined with CAFs, the teratoma formation was significantly inhibited, indicating that CAFs-derived niche is able to direct the differentiation of the ES cells (108).

How the niche of CSC is generated is an important question and several models have been proposed to explain the relationship between CSCs and their niches. First possibility is that CSCs do not need a particular niche for self-renewal and they share the same niche with normal stem cells. It is also likely that CSCs may be capable of transmitting signals that turn a quiescent niche to become activated, and take advantage of this niche. Another possibility is that CSCs may rely on the niche which is already created by CAFs or ECM components for colonial expansion (109). Clarifying the exact origin of CSC niche and understanding the roles of CAFs in the environment are critical questions for developing effective anti-cancer drugs.

7. CLINICAL SIGNIFICANCE AND FUTURE DIRECTION

The degree of the cancer progression is greatly affected by its microenvironment, and understanding the underlying mechanisms likely leads to an identification of novel targets for anti-cancer therapy. In order to target the CAFs, several obstacles should be overcome. The fibroblasts, ECs, and inflammatory cells are not malignant, therefore, successful therapy needs to precisely target the cancer components and avoid attacking the surrounding normal cells. Additionally, delivery of agents to the stroma can be problematic because of insufficient and defective vascular structures, hypoxia, and pH alterations. Therefore, successful approaches will require identification of appropriate targets and designing efficient delivery methods. Examples of effective anti-stromal therapy include targeting cancer-associated inflammation through the use of non-steroidal anti-inflammatory drugs (NSAIDs) (110). Cancer cell often develops resistance to various types of therapies, in large part due to their inherent genomic instability. An alternative approach is to focus on targeting various non-neoplastic cells which are associated with the tumor microenvironment, such as fibroblasts and endothelial cells. Although genetic alternations have been found in CAFs, CAFs are more genetically stable compare to tumor cells, which makes them an optional target of immunotherapy (111). There are several potential therapeutic targets of CAFs (Table 1). VEGF is the most important signal which mediates the growth of the blood vessels, and several VEGF inhibitors are in Phase I or II trials for colon and lung carcinomas. These drugs specifically target the endothelial cells, one of the potential sources of the CAFs. Tenascin-C is abundantly expressed in CAFs, and it has been known to be able to promote colon cancer metastasis in response to TGF-beta signaling

(112). Anti-tenascin monoclonal antibody, 81C6, is currently under Phase II trial for malignant brain tumor patients. The median survival rate of recurrent malignant glioma patients who were treated with 81C6 followed by chemotherapy was significantly higher than that of a control group treated with surgery plus iodine-125 therapy (113). Another potential target, FAP, is highly expressed in CAFs compared to normal fibroblasts in cancer patients, which make it an ideal target for anti-cancer therapy. A monoclonal antibody called Sibrotuumab against the FAP is indeed under a phase I trail for colorectal cancer patients (114). Connective tissue growth factor (CTGF) is another promising therapeutic target which is excessively expressed in CAFs. Recent data showed that CTGF secreted by CAFs isolated from a differential reactive stroma (DRS) xenograft model promoted tumorigenesis and angiogenesis of prostate cancer (115). Aikawa *et al.* developed a fully humanized CTGF-specific monoclonal antibody (FG-3019) as a novel therapeutic approach for pancreatic ductal adenocarcinoma (120). Inhibition of CTGF by FG-3019 indeed resulted in significant decrease in the volume of intra-pancreatic tumor and attenuated the metastatic potential of the tumor. As discussed above, various types of MMPs are also expressed by CAFs and clinical trials of a series of MMPs inhibitors were performed to test the efficacy on a wide range of tumor types (116). However, results from these trials have been disappointing. However, considering the diverse functions of various MMPs, inhibiting only a fraction of MMPs among over thirty members may not significantly affect tumor invasion (117). The preclinical tests also suggest that the most efficacious treatment time is the early stage of cancer. As the net outcome of global MMPs inhibition depends on multiple factors including the tumor stage and tissue specificity, the current goal is more focused on developing highly selective MMPs inhibitors based on the types and stages of the cancer (118).

In summary, the roles of CAFs in tumor progression are now clearly recognized and their underlying mechanisms are gradually revealed. However, many key questions regarding the origins and functions of CAFs still remains to be answered. Mutual interactions between CAFs and tumor cells and their resulting signaling are also of paramount interest for identifying potential therapeutic targets. Although there are several drugs that partially block the function of CAFs and some of them are even in clinical trials, their specificities need to be improved. Further understanding of detailed functional mechanisms and their pathological roles of CAFs in tumor microenvironment is expected to lead to development of novel approaches for cancer therapy.

REFERENCES

1. Liotta LA, Kohn EC. The microenvironment of the tumour-host interface. Nature. 2001; 411:375–379. [PubMed: 11357145]
2. Rodemann HP, Muller GA. Characterization of human renal fibroblasts in health and disease: II. *In vitro* growth, differentiation, and collagen synthesis of fibroblasts from kidneys with interstitial fibrosis. Am J Kidney Dis. 1991; 17:684–686. [PubMed: 2042650]
3. Chang HY, Chi JT, Dudoit S, Bondre C, van de Rijn M, Botstein D, Brown PO. Diversity, topographic differentiation, and positional memory in human fibroblasts. Proc Natl Acad Sci U S A. 2002; 99:12877–12882. [PubMed: 12297622]
4. Nakamura T, Matsumoto K, Kiritoshi A, Tano Y, Nakamura T. Induction of hepatocyte growth factor in fibroblasts by tumor-derived factors affects invasive growth of tumor cells: *in vitro* analysis of tumor-stromal interactions. Cancer Res. 1997; 57:3305–3313. [PubMed: 9242465]
5. Simian M, Hirai Y, Navre M, Werb Z, Lochter A, Bissell MJ. The interplay of matrix metalloproteinases, morphogens and growth factors is necessary for branching of mammary epithelial cells. Development. 2001; 128:3117–3131. [PubMed: 11688561]

6. Tomasek JJ, Gabbiani G, Hinz B, Chaponnier C, Brown RA. Myofibroblasts and mechano-regulation of connective tissue remodelling. Nat Rev Mol Cell Biol. 2002; 3:349–363. [PubMed: 11988769]
7. Hanahan D, Weinberg RA. The hallmarks of cancer. Cell. 2000; 100:57–70. [PubMed: 10647931]
8. Eyden B. The myofibroblast: phenotypic characterization as a prerequisite to understanding its functions in translational medicine. J Cell Mol Med. 2008; 12:22–37. [PubMed: 18182061]
9. Sappino AP, Skalli O, Jackson B, Schurch W, Gabbiani G. Smooth-muscle differentiation in stromal cells of malignant and non-malignant breast tissues. Int J Cancer. 1988; 41:707–712. [PubMed: 2835323]
10. Li H, Fan X, Houghton J. Tumor microenvironment: the role of the tumor stroma in cancer. J Cell Biochem. 2007; 101:805–815. [PubMed: 17226777]
11. De Wever O, Demetter P, Mareel M, Bracke M. Stromal myofibroblasts are drivers of invasive cancer growth. Int J Cancer. 2008; 123:2229–2238. [PubMed: 18777559]
12. Garin-Chesa P, Old LJ, Rettig WJ. Cell surface glycoprotein of reactive stromal fibroblasts as a potential antibody target in human epithelial cancers. Proc Natl Acad Sci U S A. 1990; 87:7235–7239. [PubMed: 2402505]
13. Clement S, Stouffs M, Bettiol E, Kampf S, Krause KH, Chaponnier C, Jaconi M. Expression and function of alpha-smooth muscle actin during embryonic-stem-cell-derived cardiomyocyte differentiation. J Cell Sci. 2007; 120:229–238. [PubMed: 17179203]
14. Ronnov-Jessen L, Petersen OW. A function for filamentous alpha-smooth muscle actin: retardation of motility in fibroblasts. J Cell Biol. 1996; 134:67–80. [PubMed: 8698823]
15. Cheng JD, Valianou M, Canutescu AA, Jaffe EK, Lee HO, Wang H, Lai JH, Bachovchin WW, Weiner LM. Abrogation of fibroblast activation protein enzymatic activity attenuates tumor growth. Mol Cancer Ther. 2005; 4:351–360. [PubMed: 15767544]
16. Scanlan MJ, Raj BK, Calvo B, Garin-Chesa P, Sanz-Moncasi MP, Healey JH, Old LJ, Rettig WJ. Molecular cloning of fibroblast activation protein alpha, a member of the serine protease family selectively expressed in stromal fibroblasts of epithelial cancers. Proc Natl Acad Sci U S A. 1994; 91:5657–5661. [PubMed: 7911242]
17. Hagood JS, Prabhakaran P, Kumbla P, Salazar L, MacEwen MW, Barker TH, Ortiz LA, Schoeb T, Siegal GP, Alexander CB, Pardo A, Selman M. Loss of fibroblast Thy-1 expression correlates with lung fibrogenesis. Am J Pathol. 2005; 167:365–379. [PubMed: 16049324]
18. Koumas L, Smith TJ, Feldon S, Blumberg N, Phipps RP. Thy-1 expression in human fibroblast subsets defines myofibroblastic or lipofibroblastic phenotypes. Am J Pathol. 2003; 163:1291–1300. [PubMed: 14507638]
19. Di Tommaso L, Pasquinelli G, Damiani S. Smooth muscle cell differentiation in mammary stromo-epithelial lesions with evidence of a dual origin: stromal myofibroblasts and myoepithelial cells. Histopathology. 2003; 42:448–456. [PubMed: 12713621]
20. Tlsty TD. Stromal cells can contribute oncogenic signals. Semin Cancer Biol. 2001; 11:97–104. [PubMed: 11322829]
21. Tsellou E, Kiaris H. Fibroblast independency in tumors: implications in cancer therapy. Future Oncol. 2008; 4:427–432. [PubMed: 18518767]
22. Littlepage LE, Egeblad M, Werb Z. Coevolution of cancer and stromal cellular responses. Cancer Cell. 2005; 7:499–500. [PubMed: 15950897]
23. Mayo LD, Dixon JE, Durden DL, Tonks NK, Donner DB. PTEN protects p53 from Mdm2 and sensitizes cancer cells to chemotherapy. J Biol Chem. 2002; 277:5484–5489. [PubMed: 11729185]
24. Kurose K, Gilley K, Matsumoto S, Watson PH, Zhou XP, Eng C. Frequent somatic mutations in PTEN and TP53 are mutually exclusive in the stroma of breast carcinomas. Nat Genet. 2002; 32:355–357. [PubMed: 12379854]
25. Hill R, Song Y, Cardiff RD, Van Dyke T. Selective evolution of stromal mesenchyme with p53 loss in response to epithelial tumorigenesis. Cell. 2005; 123:1001–1011. [PubMed: 16360031]
26. Kurose K, Hoshaw-Woodard S, Adeyinka A, Lemeshow S, Watson PH, Eng C. Genetic model of multi-step breast carcinogenesis involving the epithelium and stroma: clues to tumour-microenvironment interactions. Hum Mol Genet. 2001; 10:1907–1913. [PubMed: 11555627]

27. Fukino K, Shen L, Patocs A, Mutter GL, Eng C. Genomic instability within tumor stroma and clinicopathological characteristics of sporadic primary invasive breast carcinoma. Jama. 2007; 297:2103–2111. [PubMed: 17507346]
28. Pathak S, Nemeth MA, Multani AS, Thalmann GN, von Eschenbach AC, Chung LW. Can cancer cells transform normal host cells into malignant cells? Br J Cancer. 1997; 76:1134–1138. [PubMed: 9365160]
29. Walter K, Omura N, Hong SM, Griffith M, Goggins M. Pancreatic cancer associated fibroblasts display normal allelotypes. Cancer Biol Ther. 2008; 7:882–888. [PubMed: 18344687]
30. Allinen M, Beroukhim R, Cai L, Brennan C, Lahti-Domenici J, Huang H, Porter D, Hu M, Chin L, Richardson A, Schnitt S, Sellers WR, Polyak K. Molecular characterization of the tumor microenvironment in breast cancer. Cancer Cell. 2004; 6:17–32. [PubMed: 15261139]
31. Hay ED. An overview of epithelio-mesenchymal transformation. Acta Anat (Basel). 1995; 154:8–20. [PubMed: 8714286]
32. Thiery JP. Epithelial-mesenchymal transitions in tumour progression. Nat Rev Cancer. 2002; 2:442–454. [PubMed: 12189386]
33. Zvaifler NJ. Relevance of the stroma and epithelial-mesenchymal transition (EMT) for the rheumatic diseases. Arthritis Res Ther. 2006; 8:210. [PubMed: 16689999]
34. Forino M, Torregrossa R, Ceol M, Murer L, Della Vella M, Del Prete D, D'Angelo A, Anglani F. TGFbeta1 induces epithelial-mesenchymal transition, but not myofibroblast transdifferentiation of human kidney tubular epithelial cells in primary culture. Int J Exp Pathol. 2006; 87:197–208. [PubMed: 16709228]
35. Petersen OW, Lind Nielsen H, Gudjonsson T, Villadsen R, Ronnov-Jessen L, Bissell MJ. The plasticity of human breast carcinoma cells is more than epithelial to mesenchymal conversion. Breast Cancer Res. 2001; 3:213–217. [PubMed: 11434871]
36. Oft M, Akhurst RJ, Balmain A. Metastasis is driven by sequential elevation of H-ras and Smad2 levels. Nat Cell Biol. 2002; 4:487–494. [PubMed: 12105419]
37. Iwano M, Plieth D, Danoff TM, Xue C, Okada H, Neilson EG. Evidence that fibroblasts derive from epithelium during tissue fibrosis. J Clin Invest. 2002; 110:341–350. [PubMed: 12163453]
38. Petersen OW, Nielsen HL, Gudjonsson T, Villadsen R, Rank F, Niebuhr E, Bissell MJ, Ronnov-Jessen L. Epithelial to mesenchymal transition in human breast cancer can provide a nonmalignant stroma. Am J Pathol. 2003; 162:391–402. [PubMed: 12547698]
39. Direkze NC, Hodivala-Dilke K, Jeffery R, Hunt T, Poulsom R, Oukrif D, Alison MR, Wright NA. Bone marrow contribution to tumor-associated myofibroblasts and fibroblasts. Cancer Res. 2004; 64:8492–8495. [PubMed: 15574751]
40. Direkze NC, Alison MR. Bone marrow and tumour stroma: an intimate relationship. Hematol Oncol. 2006; 24:189–195. [PubMed: 16795113]
41. Russo FP, Alison MR, Bigger BW, Amofah E, Florou A, Amin F, Bou-Gharios G, Jeffery R, Iredale JP, Forbes SJ. The bone marrow functionally contributes to liver fibrosis. Gastroenterology. 2006; 130:1807–1821. [PubMed: 16697743]
42. Mishra PJ, Mishra PJ, Humeniuk R, Medina DJ, Alexe G, Mesirov JP, Ganesan S, Glod JW, Banerjee D. Carcinoma-associated fibroblast-like differentiation of human mesenchymal stem cells. Cancer Res. 2008; 68:4331–4339. [PubMed: 18519693]
43. Powell DW, Adegboyega PA, Di Mari JF, Mifflin RC. Epithelial cells and their neighbors I. Role of intestinal myofibroblasts in development, repair, and cancer. Am J Physiol Gastrointest Liver Physiol. 2005; 289:G2–G7. [PubMed: 15961883]
44. Schmitt-Graff A, Desmouliere A, Gabbiani G. Heterogeneity of myofibroblast phenotypic features: an example of fibroblastic cell plasticity. Virchows Arch. 1994; 425:3–24. [PubMed: 7921410]
45. De Wever O, Mareel M. Role of tissue stroma in cancer cell invasion. J Pathol. 2003; 200:429–447. [PubMed: 12845611]
46. Howlett AR, Bissell MJ. The influence of tissue microenvironment (stroma and extracellular matrix) on the development and function of mammary epithelium. Epithelial Cell Biol. 1993; 2:79–89. [PubMed: 8353596]

47. Mahfouz SM, Chevallier M, Grimaud JA. Distribution of the major connective matrix components of the stromal reaction in breast carcinoma. An immunohistochemical study. Cell Mol Biol. 1987; 33:453–467. [PubMed: 3311367]
48. Dugina V, Fontao L, Chaponnier C, Vasiliev J, Gabbiani G. Focal adhesion features during myofibroblastic differentiation are controlled by intracellular and extracellular factors. J Cell Sci. 2001; 114:3285–3296. [PubMed: 11591817]
49. Singer II, Kawka DW, Kazazis DM, Clark RA. *In vivo* co-distribution of fibronectin and actin fibers in granulation tissue: immunofluorescence and electron microscope studies of the fibronexus at the myofibroblast surface. J Cell Biol. 1984; 98:2091–2106. [PubMed: 6373789]
50. Tlsty TD, Hein PW. Know thy neighbor: stromal cells can contribute oncogenic signals. Curr Opin Genet Dev. 2001; 11:54–59. [PubMed: 11163151]
51. Zeisberg EM, Tarnavski O, Zeisberg M, Dorfman AL, McMullen JR, Gustafsson E, Chandraker A, Yuan X, Pu WT, Roberts AB, Neilson EG, Sayegh MH, Izumo S, Kalluri R. Endothelial-to-mesenchymal transition contributes to cardiac fibrosis. Nat Med. 2007; 13:952–961. [PubMed: 17660828]
52. Potenta S, Zeisberg E, Kalluri R. The role of endothelial-to-mesenchymal transition in cancer progression. Br J Cancer. 2008; 99:1375–1379. [PubMed: 18797460]
53. Zeisberg EM, Potenta S, Xie L, Zeisberg M, Kalluri R. Discovery of endothelial to mesenchymal transition as a source for carcinoma-associated fibroblasts. Cancer Res. 2007; 67:10123–10128. [PubMed: 17974953]
54. Skalli O, Pelte MF, Peclet MC, Gabbiani G, Gugliotta P, Bussolati G, Ravazzola M, Orci L. Alpha-smooth muscle actin, a differentiation marker of smooth muscle cells, is present in microfilamentous bundles of pericytes. J Histochem Cytochem. 1989; 37:315–321. [PubMed: 2918221]
55. Kuperwasser C, Chavarria T, Wu M, Magrane G, Gray JW, Carey L, Richardson A, Weinberg RA. Reconstruction of functionally normal and malignant human breast tissues in mice. Proc Natl Acad Sci U S A. 2004; 101:4966–4971. [PubMed: 15051869]
56. Grum-Schwensen B, Klingelhofer J, Berg CH, El-Naaman C, Grigorian M, Lukanidin E, Ambartsumian N. Suppression of tumor development and metastasis formation in mice lacking the S100A4(mts1) gene. Cancer Res. 2005; 65:3772–3780. [PubMed: 15867373]
57. Goh PP, Sze DM, Roufogalis BD. Molecular and cellular regulators of cancer angiogenesis. Curr Cancer Drug Targets. 2007; 7:743–758. [PubMed: 18220534]
58. Orimo A, Gupta PB, Sgroi DC, Arenzana-Seisdedos F, Delaunay T, Naeem R, Carey VJ, Richardson AL, Weinberg RA. Stromal fibroblasts present in invasive human breast carcinomas promote tumor growth and angiogenesis through elevated SDF-1/CXCL12 secretion. Cell. 2005; 121:335–348. [PubMed: 15882617]
59. Li G, Satyamoorthy K, Meier F, Berking C, Bogenrieder T, Herlyn M. Function and regulation of melanoma-stromal fibroblast interactions: when seeds meet soil. Oncogene. 2003; 22:3162–3171. [PubMed: 12789292]
60. Cunha GR, Hayward SW, Wang YZ. Role of stroma in carcinogenesis of the prostate. Differentiation. 2002; 70:473–485. [PubMed: 12492490]
61. Yuan J, Glazer PM. Mutagenesis induced by the tumor microenvironment. Mutat Res. 1998; 400:439–446. [PubMed: 9685702]
62. Barcellos-Hoff MH, Ravani SA. Irradiated mammary gland stroma promotes the expression of tumorigenic potential by unirradiated epithelial cells. Cancer Res. 2000; 60:1254–1260. [PubMed: 10728684]
63. Barcellos-Hoff. Radiation-induced transforming growth factor beta and subsequent extracellular matrix reorganization in murine mammary gland. Cancer Res. 1993; 53:3880–3886. [PubMed: 8358713]
64. Tsushima H, Kawata S, Tamura S, Ito N, Shirai Y, Kiso S, Imai Y, Shimomukai H, Nomura Y, Matsuda Y, Matsuzawa Y. High levels of transforming growth factor beta 1 in patients with colorectal cancer: association with disease progression. Gastroenterology. 1996; 110:375–382. [PubMed: 8566583]

65. Serra R, Crowley MR. TGF-beta in mammary gland development and breast cancer. Breast Dis. 2003; 18:61–73. [PubMed: 15687689]
66. Ronnov-Jessen L, Petersen OW. Induction of alpha-smooth muscle actin by transforming growth factor-beta 1 in quiescent human breast gland fibroblasts. Implications for myofibroblast generation in breast neoplasia. Lab Invest. 1993; 68:696–707. [PubMed: 8515656]
67. Postlethwaite AE, Keski-Oja J, Moses HL, Kang AH. Stimulation of the chemotactic migration of human fibroblasts by transforming growth factor beta. J Exp Med. 1987; 165:251–256. [PubMed: 3491869]
68. Koukourakis MI, Giatromanolaki A, Harris AL, Sivridis E. Comparison of metabolic pathways between cancer cells and stromal cells in colorectal carcinomas: a metabolic survival role for tumor-associated stroma. Cancer Res. 2006; 66:632–637. [PubMed: 16423989]
69. Powell DW, Mifflin RC, Valentich JD, Crowe SE, Saada JI, West AB. Myofibroblasts. I. Paracrine cells important in health and disease. Am J Physiol. 1999; 277:C1–C9. [PubMed: 10409103]
70. Infante JR, Matsubayashi H, Sato N, Tonascia J, Klein AP, Riall TA, Yeo C, Iacobuzio-Donahue C, Goggins M. Peritumoral fibroblast SPARC expression and patient outcome with resectable pancreatic adenocarcinoma. J Clin Oncol. 2007; 25:319–325. [PubMed: 17235047]
71. Lukashev ME, Werb Z. ECM signalling: orchestrating cell behaviour and misbehaviour. Trends Cell Biol. 1998; 8:437–441. [PubMed: 9854310]
72. Fukumura D, Xavier R, Sugiura T, Chen Y, Park EC, Lu N, Selig M, Nielsen G, Taksir T, Jain RK, Seed B. Tumor induction of VEGF promoter activity in stromal cells. Cell. 1998; 94:715–725. [PubMed: 9753319]
73. Gaggioli C, Hooper S, Hidalgo-Carcedo C, Grosse R, Marshall JF, Harrington K, Sahai E. Fibroblast-led collective invasion of carcinoma cells with differing roles for RhoGTPases in leading and following cells. Nat Cell Biol. 2007; 9:1392–1400. [PubMed: 18037882]
74. Cornil I, Theodorescu D, Man S, Herlyn M, Jambrosic J, Kerbel RS. Fibroblast cell interactions with human melanoma cells affect tumor cell growth as a function of tumor progression. Proc Natl Acad Sci U S A. 1991; 88:6028–6032. [PubMed: 2068080]
75. Camps JL, Chang SM, Hsu TC, Freeman MR, Hong SJ, Zhau HE, von Eschenbach AC, Chung LW. Fibroblast-mediated acceleration of human epithelial tumor growth *in vivo* . Proc Natl Acad Sci U S A. 1990; 87:75–79. [PubMed: 2296606]
76. Imai K. Matrix metalloproteinases and cancer cell invasion and metastasis. Tanpakushitsu Kakusan Koso. 1997; 42:1694–1700. [PubMed: 9279101]
77. Flavell SJ, Hou TZ, Lax S, Filer AD, Salmon M, Buckley CD. Fibroblasts as novel therapeutic targets in chronic inflammation. Br J Pharmacol. 2008; 153(Suppl 1):S241–S246. [PubMed: 17965753]
78. Thomasset N, Lochter A, Sympson CJ, Lund LR, Williams DR, Behrendtsen O, Werb Z, Bissell MJ. Expression of autoactivated stromelysin-1 in mammary glands of transgenic mice leads to a reactive stroma during early development. Am J Pathol. 1998; 153:457–467. [PubMed: 9708806]
79. Takahashi M, Fukami S, Iwata N, Inoue K, Itohara S, Itoh H, Haraoka J, Saido T. *In vivo* glioma growth requires host-derived matrix metalloproteinase 2 for maintenance of angioarchitecture. Pharmacol Res. 2002; 46:155–163. [PubMed: 12220955]
80. Wandel E, Grasshoff A, Mittag M, Haustein UF, Saalbach A. Fibroblasts surrounding melanoma express elevated levels of matrix metalloproteinase-1 (MMP-1) and intercellular adhesion molecule-1 (ICAM-1). in vitro. Exp Dermatol. 2000; 9:34–41.
81. Poola I, DeWitty RL, Marshalleck JJ, Bhatnagar R, Abraham J, Leffall LD. Identification of MMP-1 as a putative breast cancer predictive marker by global gene expression analysis. Nat Med. 2005; 11:481–483. [PubMed: 15864312]
82. Sternlicht MD, Werb Z. How matrix metalloproteinases regulate cell behavior. Annu Rev Cell Dev Biol. 2001; 17:463–516. [PubMed: 11687497]
83. Singer CF, Kronsteiner N, Marton E, Kubista M, Cullen KJ, Hirtenlehner K, Seifert M, Kubista E. MMP-2 and MMP-9 expression in breast cancer-derived human fibroblasts is differentially regulated by stromal-epithelial interactions. Breast Cancer Res Treat. 2002; 72:69–77. [PubMed: 12000221]

84. Wilson CL, Heppner KJ, Labosky PA, Hogan BL, Matrisian LM. Intestinal tumorigenesis is suppressed in mice lacking the metalloproteinase matrilysin. Proc Natl Acad Sci U S A. 1997; 94:1402–1407. [PubMed: 9037065]

85. Balkwill F, Mantovani A. Inflammation and cancer: back to Virchow? Lancet. 2001; 357:539–545. [PubMed: 11229684]

86. Bucala R, Ritchlin C, Winchester R, Cerami A. Constitutive production of inflammatory and mitogenic cytokines by rheumatoid synovial fibroblasts. J Exp Med. 1991; 173:569–574. [PubMed: 1997647]

87. Anderson G, Jenkinson EJ. Lymphostromal interactions in thymic development and function. Nat Rev Immunol. 2001; 1:31–40. [PubMed: 11905812]

88. Parsonage G, Falciani F, Burman A, Filer A, Ross E, Bofill M, Martin S, Salmon M, Buckley CD. Global gene expression profiles in fibroblasts from synovial, skin and lymphoid tissue reveals distinct cytokine and chemokine expression patterns. Thromb Haemost. 2003; 90:688–697. [PubMed: 14515190]

89. Buckley CD, Pilling D, Lord JM, Akbar AN, Scheel-Toellner D, Salmon M. Fibroblasts regulate the switch from acute resolving to chronic persistent inflammation. Trends Immunol. 2001; 22:199–204. [PubMed: 11274925]

90. Dvorak HF. Tumors: wounds that do not heal. Similarities between tumor stroma generation and wound healing. N Engl J Med. 1986; 315:1650–1659. [PubMed: 3537791]

91. Leek RD, Harris AL. Tumor-associated macrophages in breast cancer. J Mammary Gland Biol Neoplasia. 2002; 7:177–189. [PubMed: 12463738]

92. Swann JB, Smyth MJ. Immune surveillance of tumors. J Clin Invest. 2007; 117:1137–1146. [PubMed: 17476343]

93. Brundula V, Rewcastle NB, Metz LM, Bernard CC, Yong VW. Targeting leukocyte MMPs and transmigration: minocycline as a potential therapy for multiple sclerosis. Brain. 2002; 125:1297–1308. [PubMed: 12023318]

94. Seibert K, Masferrer JL. Role of inducible cyclooxygenase (COX-2) in inflammation. Receptor. 1994; 4:17–23. [PubMed: 8038702]

95. Nakagawa H, Liyanarachchi S, Davuluri RV, Auer H, Martin EW Jr, de la Chapelle A, Frankel WL. Role of cancer-associated stromal fibroblasts in metastatic colon cancer to the liver and their expression profiles. Oncogene. 2004; 23:7366–7377. [PubMed: 15326482]

96. Baggiolini M. Chemokines and leukocyte traffic. Nature. 1998; 392:565–568. [PubMed: 9560152]

97. Raingeaud J, Pierre J. Interleukin-4 downregulates TNFalpha-induced IL-8 production in keratinocytes. FEBS Lett. 2005; 579:3953–3959. [PubMed: 16004996]

98. Mueller L, Goumas FA, Affeldt M, Sandtner S, Gehling UM, Brilloff S, Walter J, Karnatz N, Lamszus K, Rogiers X, Broering DC. Stromal fibroblasts in colorectal liver metastases originate from resident fibroblasts and generate an inflammatory microenvironment. Am J Pathol. 2007; 171:1608–1618. [PubMed: 17916596]

99. Tang DG, Patrawala L, Calhoun T, Bhatia B, Choy G, Schneider-Broussard R, Jeter C. Prostate cancer stem/progenitor cells: identification, characterization, and implications. Mol Carcinog. 2007; 46:1–14. [PubMed: 16921491]

100. Bonnet D, Dick JE. Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. Nat Med. 1997; 3:730–737. [PubMed: 9212098]

101. Singh SK, Hawkins C, Clarke ID, Squire JA, Bayani J, Hide T, Henkelman RM, Cusimano MD, Dirks PB. Identification of human brain tumour initiating cells. Nature. 2004; 432:396–401. [PubMed: 15549107]

102. Morrison SJ, Spradling AC. Stem cells and niches: mechanisms that promote stem cell maintenance throughout life. Cell. 2008; 132:598–611. [PubMed: 18295578]

103. Barclay WW, Axanova LS, Chen W, Romero L, Maund SL, Soker S, Lees CJ, Cramer SD. Characterization of adult prostatic progenitor/stem cells exhibiting self-renewal and multilineage differentiation. Stem Cells. 2008; 26:600–610. [PubMed: 18055450]

104. Sneddon JB, Zhen HH, Montgomery K, van de Rijn M, Tward AD, West R, Gladstone H, Chang HY, Morganroth GS, Oro AE, Brown PO. Bone morphogenetic protein antagonist gremlin 1 is

widely expressed by cancer-associated stromal cells and can promote tumor cell proliferation. Proc Natl Acad Sci U S A. 2006; 103:14842–14847. [PubMed: 17003113]

105. Calabrese C, Poppleton H, Kocak M, Hogg TL, Fuller C, Hamner B, Oh EY, Gaber MW, Finklestein D, Allen M, Frank A, Bayazitov IT, Zakharenko SS, Gajjar A, Davidoff A, Gilbertson RJ. A perivascular niche for brain tumor stem cells. Cancer Cell. 2007; 11:69–82. [PubMed: 17222791]
106. Sipkins DA, Wei X, Wu JW, Runnels JM, Cote D, Means TK, Luster AD, Scadden DT, Lin CP. *In vivo* imaging of specialized bone marrow endothelial microdomains for tumour engraftment. Nature. 2005; 435:969–973. [PubMed: 15959517]
107. De Toni F, Racaud-Sultan C, Chicanne G, Mas VM, Cariven C, Mesange F, Salles JP, Demur C, Allouche M, Payrastre B, Manenti S, Ysebaert L. A crosstalk between the Wnt and the adhesion-dependent signaling pathways governs the chemosensitivity of acute myeloid leukemia. Oncogene. 2006; 25:3113–3122. [PubMed: 16407823]
108. Risbridger GP, Taylor RA. Minireview: regulation of prostatic stem cells by stromal niche in health and disease. Endocrinology. 2008; 149:4303–4306. [PubMed: 18535102]
109. Sneddon JB, Werb Z. Location, location, location: the cancer stem cell niche. Cell Stem Cell. 2007; 1:607–611. [PubMed: 18371402]
110. Sandler RS, Halabi S, Baron JA, Budinger S, Paskett E, Keresztes R, Petrelli N, Pipas JM, Karp DD, Loprinzi CL, Steinbach G, Schilsky R. A randomized trial of aspirin to prevent colorectal adenomas in patients with previous colorectal cancer. N Engl J Med. 2003; 348:883–890. [PubMed: 12621132]
111. Loeffler M, Kruger JA, Niethammer AG, Reisfeld RA. Targeting tumor-associated fibroblasts improves cancer chemotherapy by increasing intratumoral drug uptake. J Clin Invest. 2006; 116:1955–1962. [PubMed: 16794736]
112. De Wever O, Nguyen QD, Van Hoorde L, Bracke M, Bruyneel E, Gespach C, Mareel M. Tenascin-C and SF/HGF produced by myofibroblasts *in vitro* provide convergent pro-invasive signals to human colon cancer cells through RhoA and Rac. FASEB J. 2004; 18:1016–1018. [PubMed: 15059978]
113. Reardon DA, Akabani G, Coleman RE, Friedman AH, Friedman HS, Herndon JE 2nd, McLendon RE, Pegram CN, Provenzale JM, Quinn JA, Rich JN, Vredenburgh JJ, Desjardins A, Gururangan S, Badruddoja M, Dowell JM, Wong TZ, Zhao XG, Zalutsky MR, Bigner DD. Salvage radioimmunotherapy with murine iodine-131-labeled antitenascin monoclonal antibody 81C6 for patients with recurrent primary and metastatic malignant brain tumors: phase II study results. J Clin Oncol. 2006; 24:115–122. [PubMed: 16382120]
114. Scott AM, Wiseman G, Welt S, Adjei A, Lee FT, Hopkins W, Divgi CR, Hanson LH, Mitchell P, Gansen DN, Larson SM, Ingle JN, Hoffman EW, Tanswell P, Ritter G, Cohen LS, Bette P, Arvay L, Amelsberg A, Vlock D, Rettig WJ, Old LJ. A Phase I dose-escalation study of sibrotuzumab in patients with advanced or metastatic fibroblast activation protein-positive cancer. Clin Cancer Res. 2003; 9:1639–1647. [PubMed: 12738716]
115. Yang F, Tuxhorn JA, Ressler SJ, McAlhany SJ, Dang TD, Rowley DR. Stromal expression of connective tissue growth factor promotes angiogenesis and prostate cancer tumorigenesis. Cancer Res. 2005; 65:8887–8895. [PubMed: 16204060]
116. Davies B, Miles DW, Happerfield LC, Naylor MS, Bobrow LG, Rubens RD, Balkwill FR. Activity of type IV collagenases in benign and malignant breast disease. Br J Cancer. 1993; 67:1126–1131. [PubMed: 8494711]
117. Micke P, Ostman A. Tumour-stroma interaction: cancer-associated fibroblasts as novel targets in anti-cancer therapy? Lung Cancer. 2004; 45(Suppl 2):S163–S175. [PubMed: 15552797]
118. Fingleton B. Matrix metalloproteinase inhibitors for cancer therapy:the current situation and future prospects. Expert Opin Ther Targets. 2003; 7:385–397. [PubMed: 12783574]
119. Hurwitz H, Fehrenbacher L, Novotny W, Cartwright T, Hainsworth J, Heim W, Berlin J, Baron A, Griffing S, Holmgren E, Ferrara N, Fyfe G, Rogers B, Ross R, Kabbinavar F. Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. N Engl J Med. 2004; 350:2335–2342. [PubMed: 15175435]

120. Kong HL, Hecht D, Song W, Kovesdi I, Hackett NR, Yayon A, Crystal RG. Regional suppression of tumor growth by *in vivo* transfer of a cDNA encoding a secreted form of the extracellular domain of the flt-1 vascular endothelial growth factor receptor. Hum Gene Ther. 1998; 9:823–833. [PubMed: 9581905]

121. Posey JA, Ng TC, Yang B, Khazaeli MB, Carpenter MD, Fox F, Needle M, Waksal H, LoBuglio AF. A phase I study of anti-kinase insert domain-containing receptor antibody, IMC-1C11, in patients with liver metastases from colorectal carcinoma. Clin Cancer Res. 2003; 9:1323–1332. [PubMed: 12684400]

122. Kobayashi H, Eckhardt SG, Lockridge JA, Rothenberg ML, Sandler AB, O'Bryant CL, Cooper W, Holden SN, Aitchison RD, Usman N, Wolin M, Basche ML. Safety and pharmacokinetic study of RPI.4610 (ANGIOZYME), an anti-VEGFR-1 ribozyme, in combination with carboplatin and paclitaxel in patients with advanced solid tumors. Cancer Chemother Pharmacol. 2005; 56:329–336. [PubMed: 15906031]

123. Zukiel R, Nowak S, Wyszko E, Rolle K, Gawronska I, Barciszewska MZ, Barciszewski J. Suppression of human brain tumor with interference RNA specific for tenascin-C. Cancer Biol Ther. 2006; 5:1002–1007. [PubMed: 16775434]

124. Adams S, Miller GT, Jesson MI, Watanabe T, Jones B, Wallner BP. PT-100, a small molecule dipeptidyl peptidase inhibitor, has potent antitumor effects and augments antibody-mediated cytotoxicity via a novel immune mechanism. Cancer Res. 2004; 64:5471–5480. [PubMed: 15289357]

125. Wuest T, Moosmayer D, Pfizenmaier K. Construction of a bispecific single chain antibody for recruitment of cytotoxic T cells to the tumour stroma associated antigen fibroblast activation protein. J Biotechnol. 2001; 92:159–168. [PubMed: 11640985]

126. Samel D, Muller D, Gerspach J, Assohou-Luty C, Sass G, Tiegs G, Pfizenmaier K, Wajant H. Generation of a FasL-based proapoptotic fusion protein devoid of systemic toxicity due to cell-surface antigen-restricted Activation. J Biol Chem. 2003; 278:32077–32082. [PubMed: 12773535]

127. Leighl NB, Paz-Ares L, Douillard JY, Peschel C, Arnold A, Depierre A, Santoro A, Betticher DC, Gatzemeier U, Jassem J, Crawford J, Tu D, Bezjak A, Humphrey JS, Voi M, Galbraith S, Hann K, Seymour L, Shepherd FA. Randomized phase III study of matrix metalloproteinase inhibitor BMS-275291 in combination with paclitaxel and carboplatin in advanced non-small-cell lung cancer: National Cancer Institute of Canada-Clinical Trials Group Study BR.18. J Clin Oncol. 2005; 23:2831–2839. [PubMed: 15837997]

128. Aikawa T, Gunn J, Spong SM, Klaus SJ, Korc M. Connective tissue growth factor-specific antibody attenuates tumor growth, metastasis, and angiogenesis in an orthotopic mouse model of pancreatic cancer. Mol Cancer Ther. 2006; 5:1108–1116. [PubMed: 16731742]

129. Kondo S, Tanaka N, Kubota S, Mukudai Y, Yosimichi G, Sugahara T, Takigawa M. Novel angiogenic inhibitor DN-9693 that inhibits post-transcriptional induction of connective tissue growth factor (CTGF/CCN2) by vascular endothelial growth factor in human endothelial cells. Mol Cancer Ther. 2006; 5:129–137. [PubMed: 16432171]

130. Bramhall SR, Hallissey MT, Whiting J, Scholefield J, Tierney G, Stuart RC, Hawkins RE, McCulloch P, Maughan T, Brown PD, Baillet M, Fielding JW. Marimastat as maintenance therapy for patients with advanced gastric cancer: a randomised trial. Br J Cancer. 2002; 86:1864–1870. [PubMed: 12085177]

131. Hirte H, Vergote IB, Jeffrey JR, Grimshaw RN, Coppieters S, Schwartz B, Tu D, Sadura A, Brundage M, Seymour L. A phase III randomized trial of BAY 12–9566 (tanomastat) as maintenance therapy in patients with advanced ovarian cancer responsive to primary surgery and paclitaxel/platinum containing chemotherapy: a National Cancer Institute of Canada Clinical Trials Group Study. Gynecol Oncol. 2006; 102:300–308. [PubMed: 16442153]

132. Qu CF, Song EY, Li Y, Rizvi SM, Raja C, Smith R, Morgenstern A, Apostolidis C, Allen BJ. Pre-clinical study of 213Bi labeled PAI2 for the control of micrometastatic pancreatic cancer. Clin Exp Metastasis. 2005; 22:575–586. [PubMed: 16475028]

133. Kobayashi H, Sugino D, She MY, Ohi H, Hirashima Y, Shinohara H, Fujie M, Shibata K, Terao T. A bifunctional hybrid molecule of the amino-terminal fragment of urokinase and domain II of

bikunin efficiently inhibits tumor cell invasion and metastasis. Eur J Biochem. 1998; 253:817–826. [PubMed: 9654084]

134. Bleumer I, Knuth A, Oosterwijk E, Hofmann R, Varga Z, Lamers C, Kruit W, Melchior S, Mala C, Ullrich S, De Mulder P, Mulders PF, Beck J. A phase II trial of chimeric monoclonal antibody G250 for advanced renal cell carcinoma patients. Br J Cancer. 2004; 90:985–990. [PubMed: 14997194]

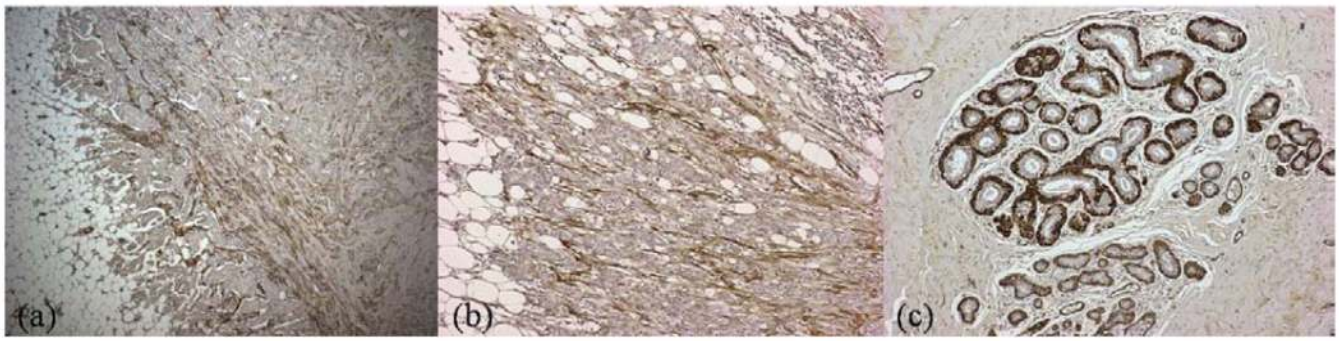

Figure 1.
Distinct alpha SMA expression in cancer and normal breast samples. (a) CAFs are abundantly present in invasive breast cancer. (b) Unique morphology of CAFs. (c) alpha SMA express in myoepithelial cells in normal tissue.

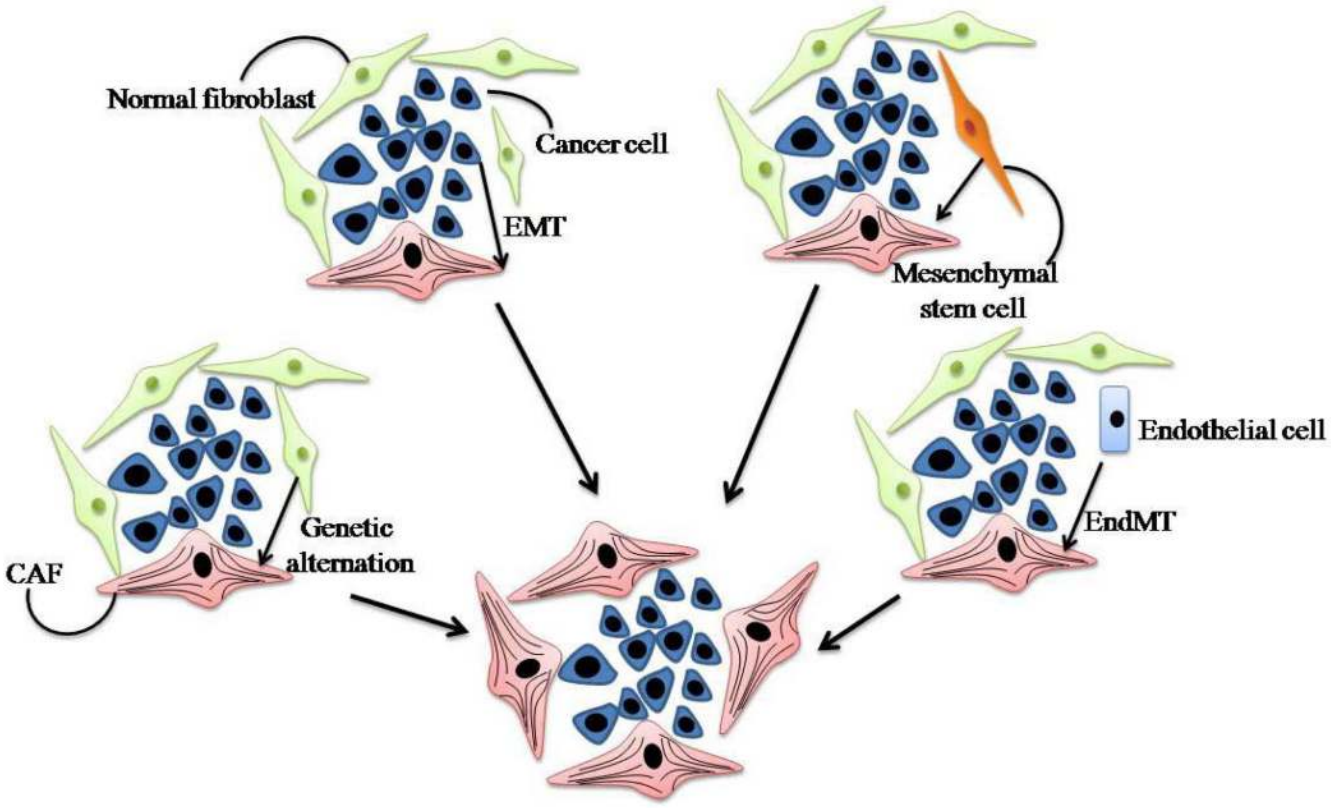

Figure 2.
Potential origins of CAFs.

Table 1

Drugs potentially targeting CAFs

Target factor	Expression cell	Function	Drug	Mechanism	Clinical Trial	Reference
VEGF	tumor cells CAFs, TAMs.	Angiogenesis	Bevacizumab	Neutralization VEGF	Phase II	(119)
			Adsflt	Interception of VEGF	Preclinical	(120)
			IMC-1C11	anit-VEGFR-2 antibody	Phase I	(121)
			RPI.4610	anti-VEGFR-1 ribozyme	Phase II	(122)
Tenascin-C	CAFs, cancer cells	cell adhesion	81C6	radioimmunotherapy	Phase II	(113)
			ATN-RNA	siRNA	Phase I	(123)
FAP	CAFs, TECs, cancer cells	Serine protease	PT-100	activity inhibitor	Phase I	(124)
			Sibrotuzumab	anti-FAP antibody	Phase I	(125)
			Sc40-FasL	induce apoptosis of FAP^+ cells	preclinical	(126)
			Rebimastat	activity inhibitor	Phase III	(127)
CTGF	CAFs, TECs, cancer cell, neural	Growth factor	FG-3019	anti-CTGF antibody	preclinical	(128)
			DN-9693	degrade mRNA	preclinical	(129)
MMPs	CAFs, TECs, TAMs, cancer cells	metalloproteinases	Marimastat	activity inhibitor	Phase III	(130)
			Tanomastat	activity inhibitor	Phase III	(131)
			Rebimastat	activity inhibitor	Phase III	(127)
uPA	CAFs, TAMs, cancer cells	Serine protease	PAI-2	activity inhibitor	preclinical	(132)
			uPA-UT1	activity inhibitor	preclinical	(133)
CA IX	CAFs, cancer cells	Carbonic anhydrase	Rencarex WX-G250	induce ADCC	Phase III	(134)