

Cancer-Associated Fibroblasts Drive the Progression of Metastasis through both Paracrine and Mechanical Pressure on Cancer Tissue

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Abstract

Neoplastic cells recruit fibroblasts through various growth factors and cytokines. These "cancer-associated fibroblasts" (CAF) actively interact with neoplastic cells and form a myofibroblastic microenvironment that promotes cancer growth and survival and supports malignancy. Several products of their paracrine signaling repertoire have been recognized as tumor growth and metastasis regulators. However, tumor-promoting cell signaling is not the only reason that makes CAFs key components of the "tumor microenvironment," as CAFs affect both the architecture and growth mechanics of the developing tumor. CAFs participate in the remodeling of peritumoral stroma, which is a prerequisite of neoplastic cell invasion, expansion, and metastasis. CAFs are not present peritumorally as individual cells but they act orchestrated to fully deploy a desmoplastic program, characterized by "syncytial" (or collective) configuration and altered cell adhesion properties. Such myofibroblastic cohorts are reminiscent of those encountered in wound-healing processes. The view of "cancer as a wound that does not heal" led to useful comparisons between wound healing and tumorigenesis and expanded our knowledge of the role of CAF cohorts in cancer. In this integrative model of cancer invasion and metastasis, we propose that the CAF-supported microenvironment has a dual tumor-promoting role. Not only does it provide essential signals for cancer cell dedifferentiation, proliferation, and survival but it also facilitates cancer cell local invasion and metastatic phenomena. *Mol Cancer Res*; 10(11); 1403–18. ©2012 AACR.

Introduction

According to the classical overview of tumor development (Fig. 1), epithelial cancers initially grow *in situ* whereby basement membranes supporting the epithelium remain intact, efficiently separating the tumor population from the adjacent stromal compartment. During the first step of invasion, the basement membrane is degraded by extracellular proteases. Consequently, motile carcinoma cells with altered cell-to-cell, cell-to-basement membrane, and cell-to-ECM adhesion properties may migrate and translocate through basal lamina stroma. Subsequent steps, including invasion in neighboring tissues and lymphatic or blood vessels, require the modification and remodeling of both the architecture and the molecular

constituents of the host stroma (1). Activated fibroblasts, along with immune and endothelial cells have a central role in this process. In fact, fibroblasts comprise a variable proportion of most carcinomas, constituting in many cases the dominant cell population of the tumor stroma. In an exaggerated paradigm, the fibroblastic population in pancreatic cancers may comprise more than 90% of the overall tumor mass (2, 3).

Recruited fibroblasts, however, do not always retain their phenotype. Rather, they become reprogrammed variants resembling *myofibroblasts*. The latter are normal cellular elements of many mucosal surfaces and basic structural components of the periglandular sheaths. They are also a basic component of the granulation tissue with an important role in wound healing and chronic inflammation (4). Also known as cancer-associated fibroblasts (CAF), these recruited myofibroblasts tend to aggregate peritumorally and encircle carcinoma cells invading the adjacent normal tissues (Fig. 1; ref. 5).

In addition to fibroblasts, many different types of progenitor cells may differentiate into CAFs. For instance, bone marrow-derived circulating cells and myeloid precursors are able to localize and proliferate in the peritumoral stroma, specifically contributing to the myofibroblasts of the desmoplastic response, as well as angiogenesis (6, 7). Of note, the phenotypic switching of endothelial cells seems to also be context dependent, as various cytokines present in their

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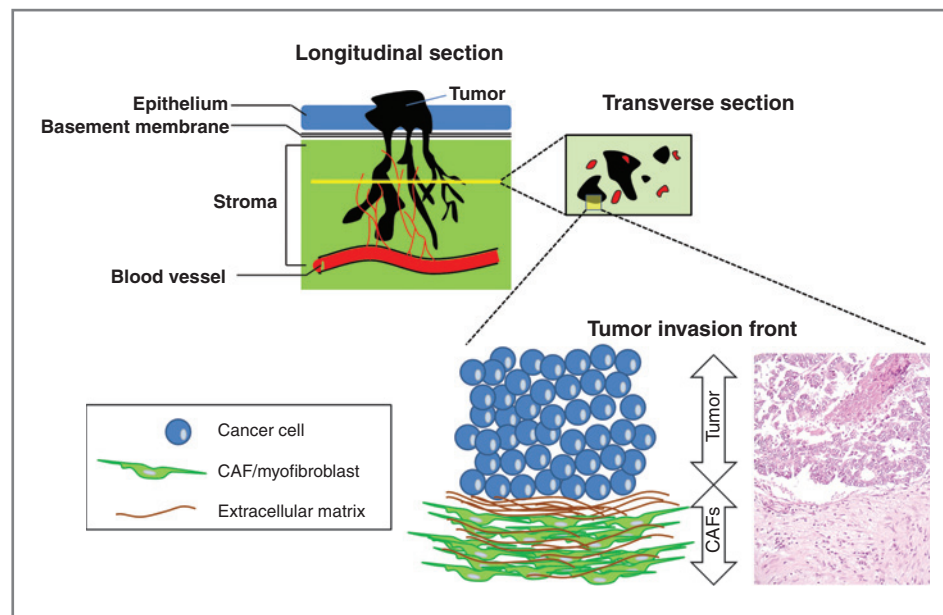


Figure 1. The tumor invasion front. Observations on longitudinal sections (top-left corner) of cancers penetrating the host stroma show an octopus-like configuration of cancer spread. After penetrating the basement membrane, epithelial cancers migrate toward the underlying lymphatic or blood vessels, patterned in a branching morphology. In such areas within the stroma, transverse section (yellow line) may reveal patterns of cancer cell "islets"/"cohorts" (top-right corner). In this cartoon, cancer cell cohorts are depicted with black, blood vessels with red roundish shapes and stromal cells (CAFs) are depicted with the background green color. A magnification of the tumor-host cell interface area reveals 2 clearly distinguished subpopulations, the cancer population, and the myofibroblasts. The magnified area is depicted through both a cartoon (left schematic) and a histologic figure (right schematic), obtained from our archive. This interaction area is described as the "tumor invasion front" and it is characterized by "desmoplasia," or "desmoplastic reaction," a histopathological lesion defined as the peritumoral accumulation of CAFs with parallel deposition of ECM components.

microenvironment, such as TGF- β , have been shown to induce a biologic program termed endothelial-to-mesenchymal transition (8). Indeed, a significant proportion (up to 40%) of CAFs may share endothelial markers such as PECAM/CD31, which implies they originate from an endothelial subpopulation (8). Remarkably, a special case of the epithelial-mesenchymal transition (EMT) program which is deployed by cancer cells to efficiently assist their invasive/migratory behavior, may sequentially lead to the formation of CAFs, given that a permissive microenvironment exists. For instance, Petersen and colleagues (2001) showed that breast cancer cells may typically undergo an EMT event that transforms them into myoepithelial cells and a subsequent transdifferentiation event, which results in the generation of a nonmalignant stroma consisting of CAFs (9, 10).

Several lines of evidence indicate that CAFs are recruited by cancer cell-secreted factors, such as TGF- β and platelet-derived growth factor (PDGF; refs. 5, 11). CAFs are identified by expression of smooth muscle-like gene- and protein-expression machinery, which is primarily characterized by α -smooth muscle actin (α -SMA). Although myofibroblasts are beneficial in wound healing, their persistent presence in chronic inflammation and cancer contributes to pathological fibrosis and desmoplasia, respectively. The latter is a cancer-specific type of fibrosis, characterized by peritumoral presence of CAF aggregates (cohorts) and abundant deposition of ECM proteins, such as collagen types I and III, glycosaminoglycans and proteoglycans (4, 12).

Tumor transplantation studies show that CAFs enhance cancer cell proliferation, angiogenesis, invasion, and metastasis. Indeed, tumors formed in mice after transplanting cancer cells admixed with CAFs are more malignant than those formed by transplanting cancer cells alone or cancer cells with normal fibroblasts (13, 14).

CAFs have been documented to contribute a wide spectrum of secreted factors, including chemokines and cytokines to the invasive margins of desmoplastic cancers, thus promoting invasive and metastatic phenomena (15). Therefore, CAFs participate in a heterotypic cross-talk with the cancer cells lining the desmoplastic invasion front. This contributes to the accumulation of important traits of metastasis by the cancer cells, including increased local growth, invasiveness, and EMT (16–18). In addition to their paracrine signaling effects, CAFs appear to exert a direct physical impact on tumor tissues (19), resulting in increased peritumoral ECM stiffness and consequently mechanical stress. This may affect the malignant phenotypes and the metastatic behavior of the cancer cells (20). Therefore, the exact tumor-promoting mechanisms of the desmoplastic microenvironment seem to be multifaceted and for that only partially understood.

Conceptual progress in the last decade suggests that CAFs should not be seen as single cellular elements but instead as stromal collectives/cohorts, as a *de novo* homotypic cell adhesion program propagates their "syncytial" configuration and behavior (21, 22). We propose that an orchestrated collective configuration allows CAFs to first, formulate a

cancer cell niche and second, achieve coordination of their own contractile and migratory behavior. In this review, current advances in the paracrine and mechanical impact of CAFs on tumor tissue are both explained in detail, to bring together an integrative model of CAF-directed metastasis.

The CAF niche: "Paracrine" Pressure of CAFs on Cancer Tissue

To date, it has been suggested that cancers do not necessarily deploy *de novo* biologic programs for the development and progression of neoplastic disease. Instead, they may activate various biologic programs, encoded already in their genomes, for physiological or developmental processes. For instance, cancer cells elicit the developmental program of EMT, to acquire less differentiated phenotypes, and obtain mesenchymal properties, essential for the completion of the metastatic cascade (23, 24). In a respective manner, the observed stromal responses against tumor-bearing stimuli are not considered as *de novo* reactions. They could be conceptualized as parallels of normal biologic programs that normally support tissue homeostasis. In particular, CAFs seem to deploy a myofibroblast-like gene and protein expression machinery. For that, CAFs are considered by many as myofibroblasts (25). The term "myofibroblast" was first coined to describe fibroblastic cells with contractile properties located within granulation tissue. These cells possess an important cytoplasmic microfilamentous apparatus and are considered responsible for the phenomenon of wound contraction (26, 27). As then, many laboratories have reported the presence of cells with myofibroblastic features in normal tissues, whereby they exert a mechanical function and support polarized mucosal epithelia, as they reside at submucosal layers or subepithelially (27, 28). In this article, we present evidence to support the notion that CAFs exert paracrine effects on the adjacent tumor population (CAF niches), and that these effects are comparable to the paracrine impact of the myofibroblasts (myofibroblastic niches) on tissues they normally support, either these are wounded or nonwounded, polarized epithelia.

CAF niches mimic myofibroblastic niches during wound healing

In cancerous microenvironments, CAFs behave as if they are responding to tissue damage and inflammatory stimuli. This is in line with view of cancer as "*a wound that never heals*" (29). As this response could be paralleled with prolonged wound healing, it could be also speculated that desmoplasia shares the nature of typical fibrotic diseases. Here, we describe the permissive microenvironments typically seen in fibrotic conditions (e.g., wound healing and cancer) as myofibroblastic or CAF niches. It appears that paracrine signaling supports both the epithelial cell restoration after injury in the case of the myofibroblastic niches and the development and progression of cancer in the case of the CAF niches. Here, we propose that CAF niches mimic the wound healing-responsive myofibroblastic niches, in 4 different aspects: (i) the mechanism of niche recruitment, (ii)

the induction of a fibrillar network (desmoplasia), (iii) the paracrine support for the epithelial compartment, and (iv) the induction of various stromal responses (e.g., angiogenesis).

The mechanism of niche recruitment. During wound healing, platelets aggregate at injured epithelia and secrete PDGF, a cytokine responsible for fibroblast chemotaxis and proliferation (30). The underlying resident fibroblasts are subsequently attracted at the wound site, where they undergo fibroblast-to-myofibroblast transdifferentiation under the influence of other platelet-derived cytokines, such as TGF- β 1 (31, 32). The presence of such "recruiter" cytokines (i.e., PDGF and TGF- β 1) at the wound site guarantees the induction and maintenance of an activated myofibroblastic network, which supports the wound healing process for as long as the injury persists (31). The myofibroblastic response during wound healing is a reversible process; once the recruiter cytokine production is ceased and the neoformation of healthy epithelium is achieved, the myofibroblastic cohort undergoes apoptosis (33). The described recruitment of the myofibroblast cohort during wound healing is mimicked, at least in part, by most solid cancers during migration and local growth. In tumorigenesis, TGF- β 1 and PDGF production is principally mediated by the cancer cells and to a lesser extent by other types of cells within the tumor microenvironment (34, 35). A striking difference between cancer progression and wound healing is that recruiter cytokines persist at the cancer microenvironment, with cancer cells being the permanent source. This may explain why the CAF niche is often found peritumorally throughout the entire course of metastasis, constantly exerting a paracrine impact on the cancer tissue. Taken together, CAFs and myofibroblasts share a similar recruitment-signaling network (Fig. 2).

The induction of desmoplasia (fibrillar network). During wound healing, the cohort-forming myofibroblasts are directly connected with the ECM by means of specialized structures called *fibronexi* (36). Their cytoskeletal component assumes the architecture of tensegrity structure, during which microtubules and intermediate filaments exert resistance on the tension produced by contractile elements, such as α -SMA and myosin (37). This contractile myofibroblast cohort resides in juxtaposition to the injured epithelium and participates in the construction of the fibrotic stroma through the secretion of collagens type I and III, fibronectin, proteoglycans, and glycosaminoglycans, which all provide structural framework for the restoration of the damaged epithelium (38, 39). Interestingly, peritumoral CAFs contribute to the induction of desmoplasia, by secreting an identical-to-wound healing fibrillar network (39). In many cancers, the initially present stromal cells progressively disappear from the peritumoral site and are substituted by a dense, acellular, and collagenous ECM, which resembles scar tissue after wound healing (39). Taken together, both CAF and myofibroblastic niches share a common mechanism for mature connective tissue construction (Fig. 2).

The paracrine support on the epithelial compartment. It has been shown that myofibroblast cohorts assist

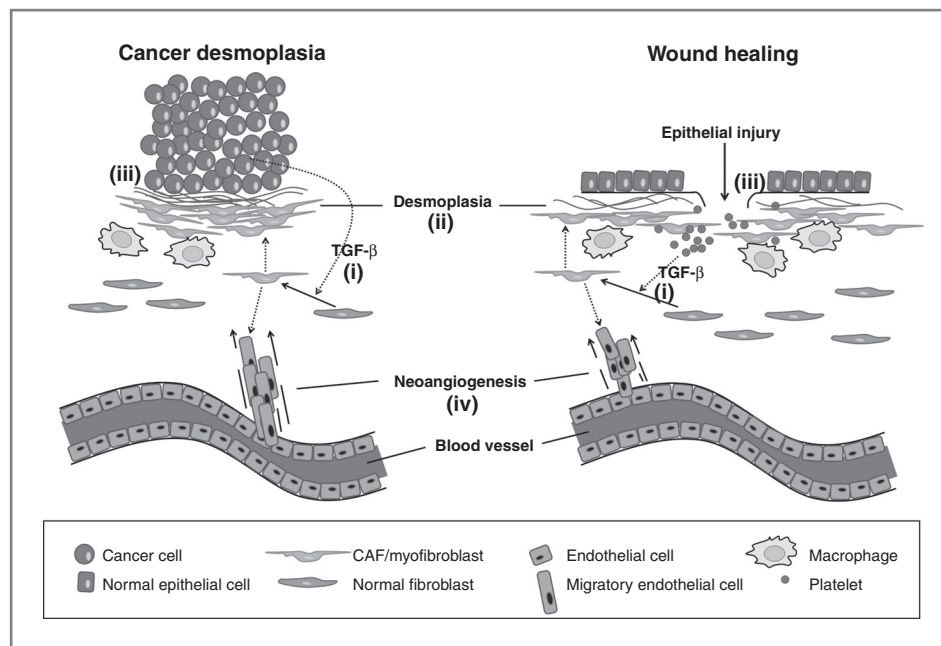


Figure 2. The CAF niche mimics the myofibroblastic niche during wound healing. Four distinct molecular concepts between cancer desmoplasia (left) and wound healing (right) show that the paracrine impact of the CAF niche on cancer tissue is highly similar to the paracrine impact of the myofibroblastic niche on damaged epithelia. The 4 molecular concepts shown are: (i) the fibroblast-to-myofibroblast transdifferentiation by recruiter cytokines (e.g., TGF- β), either by cancer cells or platelets, (ii) construction of desmoplasia (fibrillar network) around the tumor or the damaged epithelium, (iii) paracrine signaling from the CAF or myofibroblastic niche on the tumor invasion front cancer cells or wound edge cells respectively, and (iv) induction of stromal reactions such as neoangiogenesis.

the induction and maintenance of the EMT of epithelial cells residing at the wound edges. When this physiological EMT process is disrupted, wound healing fails. For instance, cutaneous wound reepithelialization is compromised in mice lacking functional *Slug*, a transcription factor involved in TGF- β -induced EMT (40). To achieve EMT at the wound edge, myofibroblast cohorts secrete extracellular proteolytic enzymes, such as matrix metalloproteinases (MMP), which cleave ECM components and release TGF- β and other EMT-inducing cytokines (4, 41, 42). This is an interesting parallel with EMT in cancer, which is also regulated by cytokines (i.e., TGF- β) that are "trapped" within the tumor microenvironment. CAFs in this case participate in their release and bioavailability through secretion of extracellular proteases and ECM-remodeling enzymes (4). We have previously shown that normal colonic fibroblasts transform into α -SMA-positive CAFs and secrete enhanced amounts of MMP2 and urokinase-type plasminogen activator (uPA), upon their coculturing with various colon cancer cell lines (43). In general, *in vitro* transdifferentiation of fibroblasts into myofibroblasts is accompanied by changes in MMP2, MMP9, and uPA secretion, as shown in several studies (36, 44, 45). These proteolytic enzymes are believed to cleave various ECM components such as decorin, which covalently binds to TGF- β and consequently prevents the latter from binding to the TGF- β receptor in adjacent cancer cells and initiate EMT (46–48). All these lines of evidence suggest that both CAF and myofibroblast niches may exert paracrine signaling regulation of epithelial phenotype plasticity (i.e., EMT; Fig. 2).

Stromal responses at the interface area. The successful termination of the wound-healing program is strongly dependent on the cross-talk of various stromal cells with the myofibroblasts at the wound site. For instance, myofibroblasts induce the formation of new blood vessels

(angiogenesis) from preexisting parental vessels or circulating endothelial precursor cells (EPC; ref. 49). Myofibroblasts secrete a potent chemokine, the stromal-derived factor-1 (SDF-1), also known as CXCL12, which assists in the recruitment of EPCs at the wound site (50). Once EPCs are chemotactically attracted, they may transdifferentiate into endothelial cells with the assistance of VEGF, also secreted by myofibroblasts (51, 52). Interestingly, neutralizing VEGF antibodies caused a striking reduction in wound angiogenesis in a pig wound model (53). In a relative context, CAFs are documented to support pathological angiogenesis by shifting the switch toward an angiogenesis-promoting phenotype in most cancers. Most desmoplastic tumors are highly vascularized (13), although there are exceptions. In pancreatic cancer, for example, hedgehog signaling coordinates the acquisition of a nonvascularized, desmoplastic microenvironment that prevents efficient drug delivery (2). Interestingly, as in the case of wound healing, the CAF niches use the CXCL12/CXCR4 axis and VEGF production to stimulate formation of novel vasculature at the tumor-host cell interface area (54, 55). In the case of breast cancer, both breast cancer cells and endothelial cells retain an active receptor (i.e., CXCR4) for SDF-1. As a result, neoangiogenesis may either be driven by the direct binding of CAF-produced CXCL12 at EPCs, or by the indirect production of VEGF upon CXCL12/CXCR4 activation in breast cancer cells (56). Collectively, both CAF and myofibroblast niches may support angiogenesis through similar signal transduction pathways (Fig. 2).

Collectively, these observations suggest that CAF niches may support malignancy, through production of a desmoplastic fibrillar network and through regulation of critical tumor-promoting events such as EMT and angiogenesis. These key paracrine mechanisms may organize the tumor

cell cohorts in a similar way to epithelia undergoing re-epithelialization during wound healing (Fig. 2).

CAF niches mimic normal myofibroblastic niches that support polarized epithelia

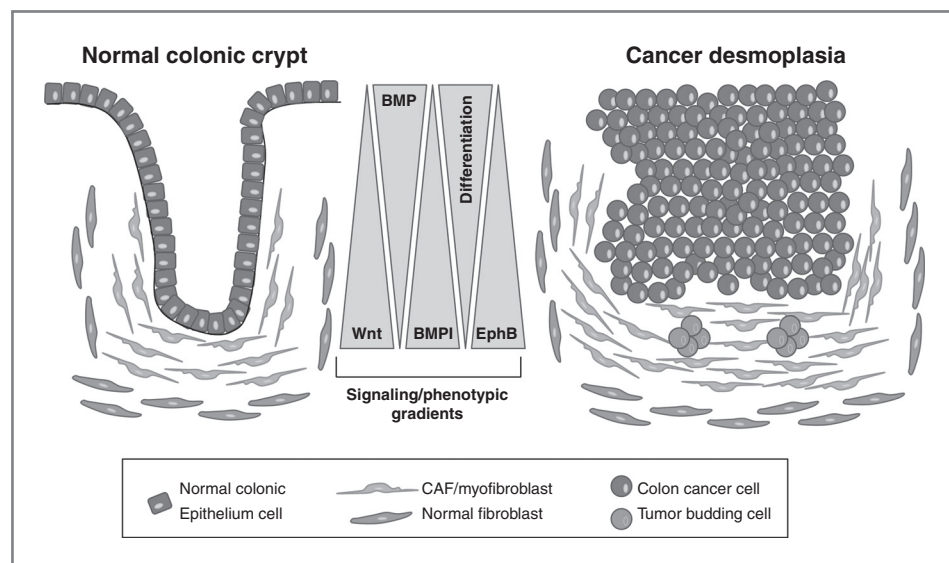
CAF and myofibroblastic niches are not only encountered as supportive niches for specific pathologies, such as cancer and epithelial injury respectively, but also as a homeostatic mechanism of epithelial integrity in normal tissues (4). In many strictly polarized and organized normal epithelia, such as in mucous membranes, there is a reported subpopulation of normal subepithelial myofibroblasts supporting the stem cells that are lined in close proximity to the subepithelial layers. For instance, subepithelial myofibroblasts are positioned at the bottom of intestinal crypts (57, 58), the uterine subepithelial stroma (59), and beneath epithelial layers lining various body cavities (60). In this review, we focus on the colonic crypt as a model to show the resemblance of the CAF niche with physiological subepithelial myofibroblastic niches.

Kosinski and colleagues (2007; ref. 61) compared transcriptomic signatures between horizontally dissected upper and lower human colonic crypt compartments and found 2 major clusters of genes upregulated in the lower compartments. The first cluster involved genes implicated in cell proliferation, whereas the second involved secretory proteins implicated in cell matrix or matrix remodeling. Notably, most of the genes overexpressed at the bottom cryptal compartments were genes expressed by cryptic stromal cells, including subepithelial myofibroblasts. This analysis suggested that a myofibroblastic niche supports the nondifferentiated state and proliferation of stem cell-like cells present at the bottom of the colonic crypt, which are responsible for generating the overall crypt epithelial progeny (61). This particular myofibroblastic niche resembles the CAF niche of desmoplastic cancers, as there are similar phenotypic/signal transduction "gradients" across both the myofibroblast/

colonic crypt axis and the CAF/cancer cohort axis (Fig. 3). Here, we propose 3 specific signal transduction pathway gradients: (i) Wnt/ β -catenin pathway, (ii) bone morphogenetic protein (BMP) pathway, and (iii) Eph/ephrin pathway, which regulate malignancy in both normal and cancerous microenvironments in a similar context.

Wnt/ β -catenin pathway. The Wnt/ β -catenin signaling pathway has been considered as major determinant of gene expression patterns along the colon crypt axis, as revealed in the study by Kosinski and colleagues (2007). The genes that were highly expressed in the upper part of the colonic crypt and induced by interruption of Wnt/ β -catenin signaling were *p21*, *BMP2*, *MAD*, and *CDH18* (61). The active Wnt/ β -catenin pathway at the lower compartment of the colonic crypt contributes to the enhanced proliferation of the stem cell-like cells responsible for generating the epithelial progeny of the upper parts. In particular, the Wnt/ β -catenin pathway acts through the transcription factor TCF4 to initiate transcription of *c-Myc* and repress *p21*, thus leading the cells toward the G₁ cell-cycle checkpoint. Following disruption of Wnt/ β -catenin signaling, the transcriptional expression of *c-Myc* is alleviated and *p21* leads the crypt cells to G₁ arrest and coincidental differentiation. Thus, when cells escape from the effect of Wnt/ β -catenin signaling, they move to the top of the colonic crypt, following a differentiation "gradient" across the axis (62). In concordance with this evidence, overexpression of the Wnt signaling inhibitor *Dkk1*, leads to the formation of elongated colonic crypts, as epithelial cells are entrapped in a persistent proliferative state (63). In the desmoplastic invasion front of intestinal cancers, a pattern of Wnt signaling activation, similar to that seen physiologically in the colonic crypt, is observed across the tumor. Specifically, expression of nuclear β -catenin (evidence of Wnt/ β -catenin activation) is primarily observed in areas of invasion fronts. Such cells are believed to have undergone or been in the process of an EMT program. In such areas, stromal signals further activate tyrosine receptor

Figure 3. The CAF niche mimics the myofibroblastic niche supporting the bottom of the colonic crypt. The CAF niche supports signaling and phenotypic gradients in the cancer cell cohort (right) in a relevant manner to the signaling gradients deployed in the colonic crypt (left). The triangles in between depict the signaling (Wnt, BMP/BMP1, and Eph/Ephrin) and the phenotypic (differentiation) gradients, as described in the manuscript, which are common to both cancer and colonic crypt positioning.



kinases in cancer cells which contribute to the phosphorylation of β -catenin tyrosine residues and the subsequent reduction of its affinity with the cytoplasmic parts of cadherins (64). CAFs are suggested to play a direct role by producing Wnt ligands for paracrine signaling in tumor invasion front cells. They may also have an indirect role by producing growth factors and cytokines which activate Wnt signaling in these cells through signal transduction cross-talk (65, 66).

BMP pathway. Kosinski and colleagues (2007) also showed differential expression of BMP ligands, receptors, and inhibitors along the colonic crypt axis (61). More specifically, *BMP1*, *BMP2*, *BMP5*, *BMP7*, *SMAD7*, and *BMP2* were highly expressed in colon tops, whereas BMP antagonists *GREM1*, *GREM2*, and *CHRD1* were highly expressed in the bottom of the colonic crypts. These authors showed that BMP antagonists were expressed by subepithelial myofibroblasts at the bottom of the crypt to shut down the BMP signaling at the stem cell-like colon cells and preserve their undifferentiated state; as such BMP signaling is highly active at the top part of the crypts (61). The effect of BMP signaling in stem cell self-renewal has been suggested and is speculated to occur by BMP-dependent inhibition of Wnt signaling (67). In addition, the overexpression of BMP antagonist noggin promotes the development of ectopic crypts in the intestine (68). Hence, BMP antagonists might represent an important aspect for maintenance of the subepithelial colonic crypt niche. BMP antagonists *GREM1* and *follistatin (FST)* are also found to be highly expressed by CAFs in desmoplastic lesions in various types of cancer including intestinal and basal cell carcinomas (69). Sneddon and colleagues (2006) suggested that *GREM1* might provide the advantage in the cancer invasion front cells to preferentially shift their differentiation state toward a more mesenchymal and stem-like phenotype (69). *GREM1* is not expressed by cancerous cells, which implies that the acquisition of a BMP-negative invasive phenotype is entirely dependent on microenvironmental regulation (70, 71). For instance, α -SMA-positive peritumoral CAFs are the major source for *GREM1*, and possibly other BMP antagonists in desmoplastic lesions of gastric cancer (72). BMP signaling is active away from the CAF-instilled microenvironment, namely at the core of the tumor cell aggregates (70, 71). Consistent with such observations, we have recently characterized a desmoplastic signature of secreted proteins in an *in vitro* coculture system of colon cancer cell lines and CAFs and we found that BMP antagonists, such as *GREM1* and *FST*, were exclusively present in the coculture conditions (43).

Eph/Ephrin pathway. Kosinski and colleagues (2007) noticed an expression gradient of multiple members of the Eph/ephrin A (EPHA) and Eph/ephrin B (EPHB) family of tyrosine kinase receptors and their respective ligands in the colonic crypt axis (61). Expression of *EPHB1*, *EPHB2*, *EPHB3*, *EPHB4*, and *EPHB6* is generally noted in the base of the colonic crypts (61). The expression of EPHB receptors and their ligands have been documented to play significant roles in the polarization, the bidirectional migration and the

correct positioning and orientation of proliferation of the progenitor part of the murine colonic crypt (73). In particular, Smad3 regulates the expression of *EPHB2* and *EPHB3* at the lower part of the colonic crypt that results in a proliferating zone in this region. The importance of Smad3 in this process is highlighted by the fact that mice lacking Smad3 present scattered proliferation patterns across the crypt axis (74). Interestingly, *EPHB2* is overexpressed in colorectal cancers including colorectal adenomas, in a manner that parallels Wnt signaling activation and β -catenin nuclear accumulation (75).

Collectively, these observations indicate that the CAF niche may support various malignant phenotypes, by providing a frame of key signaling pathway gradients across tumor cell foci invading the stroma (Fig. 3). These key regulating pathways (Wnt, BMP, and EPHB) of cell proliferation, migration, polarization, and differentiation organize the tumor cell cohorts in a context similar to that of normal epithelia (Fig. 3).

The CAF Migratory Cohesive Unit: "Mechanical" Pressure of CAFs on Cancer Tissue

For a long time, the tumor stroma, and especially the myofibroblastic accumulation around the tumor cells, was simply viewed as a reactive tissue, whose architecture is shaped as a response to the expansive pressure from the cancerous compartment. However, DeWever O. and colleagues (76) proposed that CAFs may not necessarily be a passive component of the microenvironment, but instead they may behave as a particularly motile cohesive unit, capable of penetrating the cancerous compartment, as well. Their data suggest that the molecular signaling between cancer cells and CAFs stimulates migration of both cell types against each other and modifies the adjacent ECM and basement membranes. Along these lines, Kumar and colleagues (2009) conceptualize metastasis as a "force journey" of the tumor cell, by which mechanical forces play a major part in the onset and progression of the disease (20).

The CAF cell-motility programming

In the cancer microenvironment, the fibroblast-to-myofibroblast transdifferentiation program is dictated by a variety of wound healing- and fibrosis-related cytokines, the most prominent ones being TGF- β , PDGF, and IL-6 (11, 35). CAFs migrate faster in the presence of the recruiter cytokines compared with resident quiescent fibroblasts (5, 12, 77, 78). In one study, Commandeur and colleagues (2011) compared the profiles of stromal fibroblasts retrieved either from desmoplastic lesions of cutaneous squamous cell carcinoma or from healthy dermis, and showed that CAFs had increased migratory potential compared with normal fibroblasts (NFs; ref. 79). The increased CAF migratory behavior has also been observed *in vitro* in a number of studies (80, 81). Moreover, TGF- β -recruited CAFs have been found to form an aberrant type of filopodia. According to De Wever and colleagues (2004), this allows them to migrate and invade into cancerous cell subpopulations (82). TGF- β acts

through *c-jun*-NH2-kinase (JNK) to overexpress N-cadherin in neofomed filopodia. It also induces phenotypical hallmarks of directional migration, such as front-to-rear polarization and Golgi-complex reorientation in myofibroblasts. Importantly, pharmacologic, or chemical inhibition of either TGF- β 1 or N-cadherin/JNK upon TGF- β 1 stimulation, inhibits the formation of this migratory myofibroblastic phenotype (82).

Overall, these observations suggest that CAFs harbor an efficient migratory gene/protein expression programming (Fig. 4A).

Homotypic cell-adhesion programming within the CAF cohort

This altered migration programming in CAFs cannot fully explain the collective and coordinated nature of CAF migration. A concerted myofibroblastic reaction has been noticed in early wound healing studies (83), in which pathologists described the neofomation of adherens and tight junctions in polarized "syncytia" of myofibroblasts. Indeed, in contrast to quiescent fibroblasts that travel as individual cells (21), CAFs have been documented to migrate through collectives (22, 84), whose formation is now presumed to be assisted by various types of specific junction apparatuses, such as tight, gap, and adherens junctions (85).

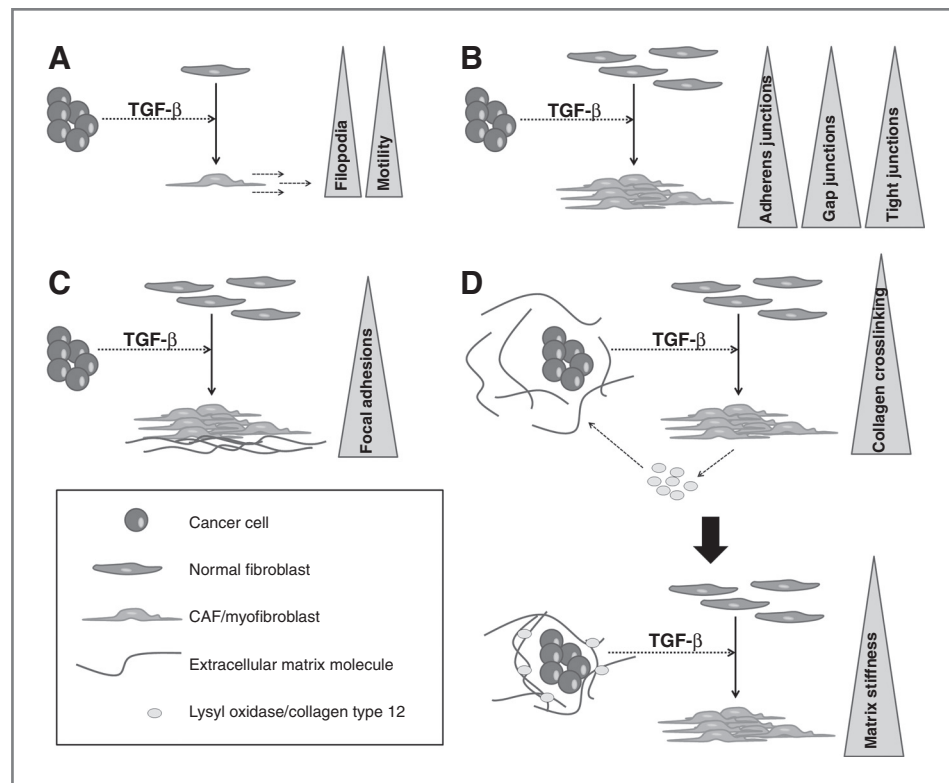
Cohort configuration in myofibroblasts is primarily mediated by formation of cell-cell adherens junctions that intercellularly couple contractile stress fibers (85, 86). In particular, myofibroblasts highly express N-cadherin, which

further allows them to acquire a migratory phenotype (82, 87). It has been suggested that fibronectin matrix assembly affects the organization, composition, and function of N-cadherin-based adherens junctions, by recruiting integrin- α 5/ β 1 (ITGA5/B1) and tensin into sites of cell-to-cell adhesion (88). This recruitment is also associated with the presence of fibronectin fibrillogenesis and RhoA in the adhesions, which may also mediate the migratory behavior of the cells (89, 90). Taken together, these data suggest that the integrity of the CAF cohort could also be dependent on both cell-to-cell and cell-to-matrix specialized adhesion programming.

Interestingly Hinz and colleagues (2004) showed that fibroblasts may also shift the adherens junction composition from N-cadherin (cadherin-2) to OB-cadherin (cadherin-11) during myofibroblast transition in both contractile wounds and in *in vitro* conditions. As a consequence, the coupling of OB-cadherin to α -SMA may reinforce the mechanical stability of the myofibroblast cohort (86). Cadherin switching may also be involved in heterotypic interactions between the CAF cohort and other stromal or cancer cells. For instance, it has been shown that suburothelial myofibroblasts adhere with smooth muscle cells through OB-cadherin-based adherens junctions in the bladder (91).

An altered gap junction adhesion programming has been observed in a mouse model of colon adenoma, where connexin-43 was not found to be abnormally expressed in neither normal, nor neoplastic epithelium. In contrast, it was highly expressed in CAFs surrounding the invasive cancer

Figure 4. The CAF population accumulates phenotypic properties to behave as a migratory cohesive unit. CAFs need to achieve 4 prerequisite properties to exert a mechanical impact on the cancer tissue: A, recruited CAFs need to gain an enhanced migratory potential (filopodia formation), (B) recruited CAFs need to gain an altered cell-to-cell adhesion programming (adherens-, gap-, tight-junction apparatuses), resulting in the enhancement of their homotypic adhesions, (C) recruited CAFs need to gain an altered cell-to-matrix adhesion programming (focal adhesions), resulting in their capability of moving onto and through ECM, (D) recruited CAFs need to gain the capability of remodeling the peritumoral ECM by causing collagen cross-linking (secretion of lysyl-6-oxidase or collagen type XII), resulting in enhanced matrix stiffness.



(92). In a wound healing model of *in vivo* endothelial injury, the knockdown of connexin-43 resulted in a reduced α -SMA-positive myofibroblast yield at the wound site, which further prevented fibrous membrane formation (93). Therefore, cell-cell adhesion within the CAF cohort may be additionally achieved by the formation of connexin-43-containing gap junctions within the myofibroblast collectives. It has been speculated that these may serve toward the mutual Ca^{2+} exchange and balance, through which coordination in the contractile activity is achieved (85, 94, 95). For instance, gap junctional intercellular communication uncouplers heptanol and endosulfan were administered daily into polyvinyl alcohol sponge implants in rats. Seven days posttreatment, the uncoupler-treated implants had increased fibroblast density and diminished numbers of myofibroblasts (96). In addition, the uncouplers reduced the deposition and organization of collagen in the implants and the penetration of the sponge by the myofibroblasts (96). These data suggest that gap junctional intercellular communication is essential for both myofibroblast phenotype induction and acquisition of migratory phenotype.

Given the above, it is unclear whether the coordination of myofibroblast contractility is gap junction- or adherens junction-dependent. Follonier and colleagues (2008) proposed that individual cell contraction is transmitted via adherens junctions and leads to the opening of mechanosensitive ion channels in adjacent cells. The resulting Ca^{2+} influx induces a contraction that can feed back on the first cell and/or stimulate other contacting cells. This mechanism could facilitate the remodeling of cell-dense tissue by coordinating the activity of myofibroblasts (84). Thus, the migratory behavior of CAFs may actually be dependent on the formation of a coordinated contractile cohort with alterations in the adherens and gap junction gene and protein expression machinery.

Myofibroblastic syncytia form tight junctions in wound healing processes (83). Gene expression meta-analysis reveals that cells resembling CAFs (e.g., activated hepatic stellate cells) present altered tight junction machinery (97). Surprisingly, a similar study has not been conducted in the context of neoplastic disease. In general, tight junctions are hallmarks of epithelial cells. Their regulation has been primarily investigated in the epithelial compartment of cancers, as their disruption is closely associated with the induction of EMT and loss of cell-to-cell adhesion. Therefore, many types of cancer are characterized by severe down-regulation of tight junction proteins (98). Tight junctions are also implicated in the maintenance of the epithelial polarity of cells lining basement membranes by regulating polarity complexes and cytoskeletal proteins (99). Thus, it would be reasonable to hypothesize that the tight junctions may offer epithelial-like polarity and the ability of polarized migration to the CAF cohorts.

Taken together, the *de novo* expressed homotypic cell-adhesion machinery may potentially allow CAFs to act as a cohesive unit (CAF cohort), during their migratory behavior (Fig. 4B).

Altered cell-to-matrix adhesion programming of the CAF cohort

The aforementioned homotypic cell-adhesion programming of CAFs and the ability of collective configuration could not fully explain their highly significant impact on cancer development. Presumably, CAF collectives would not be able to migrate and invade the cancer and other tissues upon retaining strong anchoring to resident ECM components and basement membranes, such as collagens and laminins. Significant molecular insight on cell-to-matrix adhesion programming in CAFs was provided by a recent study by Navab and colleagues (2011). The authors conducted a microarray gene-expression analysis of 15 matched CAF and NF primary cell lines derived from non-small cell lung cancer. They identified 46 differentially expressed genes, encoding for proteins that were significantly enriched for extracellular proteins regulated by TGF- β . Interestingly, focal adhesion-related proteins belonged to the most over-represented pathways in this analysis (100). The altered focal adhesion programming in CAFs/myofibroblasts, compared with the quiescent fibroblasts, has been also experimentally validated through immunohistochemistry and transmission electron microscopy (85).

Data from Navab and colleagues (2011) suggest that in CAFs, the altered focal adhesion network might be implicated in the regulation and activation of integrin- and FAK-signaling pathways (100). Focal adhesion kinase (FAK) is a cytoplasmic protein tyrosine kinase recruited to and activated at sites of integrin-receptor binding to fibronectin at focal adhesions. As such, FAK acts as a principal effector of fibronectin-stimulated cell motility (101). In a complementary fashion, Stokes and colleagues (2011) showed that FAK is activated in both tumorous and stromal components during pancreatic ductal adenocarcinoma metastasis. This group further showed that cancer-associated stromal cell migration was abrogated upon stimulation with a FAK inhibitor (102). Tomar and colleagues (2009) additionally showed that FAK not only promotes the migration of fibroblasts and other stromal cells such as endothelial cells, but also establishes an anteroposterior polarized cell axis, by forming a complex with p120RasGAP and p190RhoGAP (103). Overall, these data point out that an altered focal adhesion programming in CAFs is essential to provide increased migratory potential.

In a variety of fibrotic diseases, myofibroblasts present elevated expression of focal adhesions such as integrin- β . Consequently, the use of neutralizing integrin- β 1 antibodies alleviates the adhesive properties and migration of myofibroblasts onto ECM (104, 105). Also, during wound healing, dermal fibroblasts show increased expression levels of proadhesive proteins such as integrin- α 2, α 4, and FAK. Unlike skin, oral gingiva does not scar in response to fibrotic cytokines and injury; as such, lower expression of the aforementioned proadhesive molecules as well as less FAK and p38 phosphorylation are typically found in the gingival myofibroblasts (106). These contrasting observations between skin and oral gingival myofibroblasts may suggest that desmoplastic/fibrotic lesions

are not induced unless myofibroblasts migrate onto ECM, through an integrin- and FAK-associated signaling circuitry (106).

In a wound-healing model of cutaneous repair in response to injury, Vi and colleagues (2011) observed that TGF- β 1-dependent fibroblast-to-myofibroblast differentiation required the expression of a focal adhesion molecule, integrin-linked kinase (ILK; ref. 107). Specifically, ILK-deficient fibroblasts failed to complete the transdifferentiation program. Moreover, ILK-deficient fibroblasts had attenuated Smad 2 and Smad 3 phosphorylation. Consequently, TGF- β 1-targeted genes such as α -SMA failed to transcribe, resulting in severe cytoskeletal abnormalities (107). In a similar manner, FAK participates in the regulation of cytoskeletal dynamics, as FAK-deficient mouse embryonic fibroblasts have destabilized cytoskeletons and reduced adhesive properties (108). Collectively, these data indicate that focal adhesion programming is not only important for the migratory capabilities of the CAFs but is also essential for the successful completion of the fibroblast-to-myofibroblast differentiation program.

Several other focal adhesion molecules have been found in myofibroblasts of the tumor invasion front. For instance, Gullotti and colleagues (2011) observed that α -SMA-positive myofibroblasts in peritumoral desmoplastic tissue of both sporadic large bowel carcinomas and hereditary non-polyposis colorectal cancer showed an overexpression of the four and a half LIM-domain protein-2 (FHL2). FHL2 interacts with both cytoplasmic and nuclear proteins and has been documented to play a role in modulating various cellular processes, such as cell proliferation, transcription, and signal transduction (109). The authors, additionally observed a positive correlation between TGF- β 1 expression in colon cancer cells and FHL2 in peritumoral CAFs, indicating that the recruitment of the latter is most probably tumor dependent (110).

Collectively, these observations suggest that CAFs use a specialized focal-adhesion machinery, with the assistance of which they mediate cell-to-matrix interactions, to fully exert a migratory program (Fig. 4C).

Tissue integrity of the continuously disrupted stroma

The development of many cancers is accompanied by progressive sclerosis of peritumoral ECM. For instance, mammographic density measurements in human patients showed that mammary tumor tissue and tumor-adjacent stroma were 5 to 20 times harder than the normal mammary gland (111). The enhancement of tumor stroma durability is regulated by CAFs and allows tumors to overcome the mechanic pressure of the peritumoral desmoplastic reaction (112). In this section, we focus on the causes and consequences of ECM sclerosis. We first present available evidence that support the CAF-regulated ECM sclerosis model. Then we elaborate on the fact that ECM sclerosis has an impact in both biochemical and biophysical properties of the tumor cells. These data will favor the hypothesis that the CAF cohort enforces the persistent growth and metastatic potential of the

cancer cells, allowing them to overcome the topographic restrictions of the desmoplastic microenvironment.

Collagen is the most abundant ECM scaffolding protein in the stroma and the main contributor of tissue tensile strength. Its metabolism is aberrantly deregulated during development of cancer, where increased collagen expression, deposition, remodeling, and organization are deployed to support the neoplastic tissue (113). Fibroblasts, along with other recruited stromal cells such as immune and endothelial cells, elaborate a plethora of growth factors, cytokines, and chemokines (112). Recent evidence suggests that this milieu promotes rather than suppresses tumor growth. It has been suggested that collagen cross-linking in tumor boundaries, could increase ECM density and interstitial pressure for the confinement of the tumor and the preservation of tissue homeostasis. CAFs seem to be the key elements participating in collagen cross-linking, resulting in remodeled and reoriented collagen in fibrotic lesions, which has been termed "cancer-associated collagen" (112, 114–116). A widely explored mechanism by which CAFs achieve collagen cross-linking is via production of lysyl-6-oxidase (LOX; Fig. 4D; ref. 117), a copper-dependent amine oxidase, which initiates the process of covalent intra- and intermolecular cross-linking by oxidatively deaminating specific lysine and hydroxylysine residues located in telopeptide domains (118). Using rheological measurements and second harmonics generation imaging, Levental and colleagues (2011) showed that mammary glands conditioned with LOX-expressing fibroblasts were harder in consistency than those containing LOX-negative fibroblasts. LOX-positive fibroblasts enhanced the deposition of fibrillar and linearized (cross-linked) collagen (117). Presumably, LOX is a direct product of the desmoplastic reaction and its expression occurs at the tumor-host cell interface. LOX can be induced by both fibrogenic pathways such as TGF- β , as well as by hypoxia, which often occurs during cancer progression (119, 120). Therefore, CAFs could directly produce LOX in desmoplastic lesions, regardless of the presence of hypoxia, given they are mainly recruited by TGF- β -producing cancer cells.

Collagen cross-linking is a complicated process, which cannot be fully explained by the exclusive function of LOX. For instance, it has been shown that fibril-associated collagens with interrupted triple helices (FACIT), such as collagen type XII and XIV are in fact fibrillar collagen organizers, and participate in collagen cross-linking (Fig. 4D). FACITs have 4 domains, 1 that anchors the molecule to the surface of other fibrils (e.g., collagens type I and III), and 3 "finger-like" domains. By residing in collagen I fibrils, collagen type XII participates in fibril structure interaction and organization, by further stabilizing them (121, 122). Interestingly, collagen type XII has been previously linked to various fibrotic diseases in the lung (123, 124) and we have recently linked its high expression to CAF populations in desmoplastic lesions of colorectal cancer (43). These findings indicate a major role for the CAFs in ECM sclerosis, through secretion of desmoplastic proteins and maintenance of other pathophysiological processes such as collagen cross-linking.

The impact of such an increased matrix stiffness on the cancerous compartment itself remains to be elucidated.

Tension-dependent matrix remodeling promotes the linear reorientation of collagen fibrils, surrounding the invasion front of the tumor. It is now postulated that such tractional forces may influence morphological and mechanical properties of the cells by altering signaling pathway behaviors (20). Lee and colleagues (2011) showed that fibroblast activation protein (FAP)-overexpressing fibroblasts produce an ECM that enhances the invasive velocity and directionality of pancreatic cancer cells in an integrin- β 1/FAK-dependent manner (125). In particular, FAP remodels and reorients the produced ECM in *in vivo*-like models of 3-dimensional matrices. It follows that disruption of FAP enzymatic activity impairs tumor invasion and progression, because of matrix disassembly and chaotic organization (125). Through *real-time* monitoring in cell motility assays, Lee and colleagues (2011) verified that the ECM sclerosis is the major prerequisite event for integrin- β 1/FAK-dependent cell migration (125, 126).

In a comparative context, Levental and colleagues (2009) showed that LOX-mediated collagen cross-linking and linearity is associated with increased activation of focal adhesions in breast cancer cells, both *in vitro* and *in vivo*. Integrin clustering promoted focal adhesions to drive invasion of mammary tumor cells, through PI3K signaling; as such, pharmacological inhibition of PI3K repressed all malignant phenotypes induced in LOX-mediated collagen fibril organization (117). Therefore, increased ECM density, as induced by enhanced collagen cross-linking and maturation of focal adhesions could promote invasiveness of breast and possibly other cancers by enhancing the integrin signaling (127).

Collectively, these observations suggest that CAFs participate in the progressive ECM sclerosis through secretion of collagen cross-linking molecules, which then, in turn, alters biochemical pathways in cancer cells (i.e., increased integrin signaling; Fig. 4D).

Interdigital Migration: A Working Model of CAF-Directed Metastasis

Description of the working model

We propose a novel working model of metastatic growth progression, based on both the *paracrine* and the *mechanical* impact of the CAF cohort at the tumor-host cell interface. Here, the interaction between cancer cells and normal host stroma, results in the induction of desmoplastic reaction, characterized by the emergence of a responsive myofibroblastic tissue, the CAF cohort. The CAF cohort has the capability of acting both as a myofibroblastic-signal source niche and as a migratory cohesive unit, interacting mechanically with the tumor compartment. At the tumor invasive front, it creates a complex and dynamic framework, in which stromal populations invade the tumorous ones and vice versa. The invasive properties of each population, together with the mechanical stress of the microenvironment may actually direct (and to some extent enforce) these popula-

tions to migrate against each other in an interdigital pattern (Fig. 5, left column).

The "paracrine" component of the proposed model postulates that CAFs may secrete paracrine soluble factors at the tumor invasion front, which may affect the various layers of the neoplastic population, generating phenotypic, and signaling pathway gradients across the tumor cohort. CAFs may form niches similar to the ones occurring in various physiologic and pathophysiological conditions in the human body. The major biologic prerequisites that CAFs must deploy to achieve this, are summarized below.

- CAFs are recruited through TGF- β and PDGF, participate in the construction of a desmoplastic fibrillar network, affect the phenotype of cancer cells lining the invasion front through secretion of EMT-promoting factors and finally cause aberrant stromal responses such as SDF-1 and VEGF-dependent neoangiogenesis (Fig. 2). As such, CAFs niches mimic the myofibroblastic niches during wound healing.
- CAFs generate striking signal transduction (e.g., Wnt, BMP, and EPH) gradients, spanning from the tumor periphery to the tumor core, thus affecting specific phenotypes in a parallel manner (Fig. 3). CAF niches mimic the myofibroblastic niches that naturally occur in subepithelial areas of various tissues.
- In the interdigital model of metastasis, the CAF niches and the signaling gradients are retained throughout, as the CAF cohort invades into the cancer cohort and vice versa (Fig. 5, right column).

The "mechanical" component of the proposed model postulates that CAFs migrate through cohort formation and exert a mechanical pressure on the tumor invasion front, capable of changing the tissue-tension dynamics of the tumor population. Consequently, they may force the tumor to migrate toward less dense stromal regions. The major biologic prerequisites that CAFs must deploy to achieve this, are summarized below.

- CAFs must obtain a specialized cell migration program, characterized by accumulation of invasive properties (e.g., filopodia formation), a phenotype that is clearly discriminated from the quiescent (nonmigratory) phenotype of the normal fibroblasts (Fig. 4A).
- CAFs must obtain a specialized cell-to-cell adhesion program, characterized by specific expression and localization of adherens, tight and gap junction apparatuses. Such an adhesion program should be deployed in a highly sophisticated way in the neoformed CAF cohort, as it will: (i) allow the CAFs to behave as a syncytium (adherens junctions), (ii) will not allow the disruption of the cohort because of noncoordinated movements (gap junctions), and (iii) will provide directionality (or polarity) in the migratory route of the CAF cohort (tight junctions; Fig. 4B).
- CAFs must obtain a specialized cell-to-matrix adhesion program, characterized by specific expression of focal

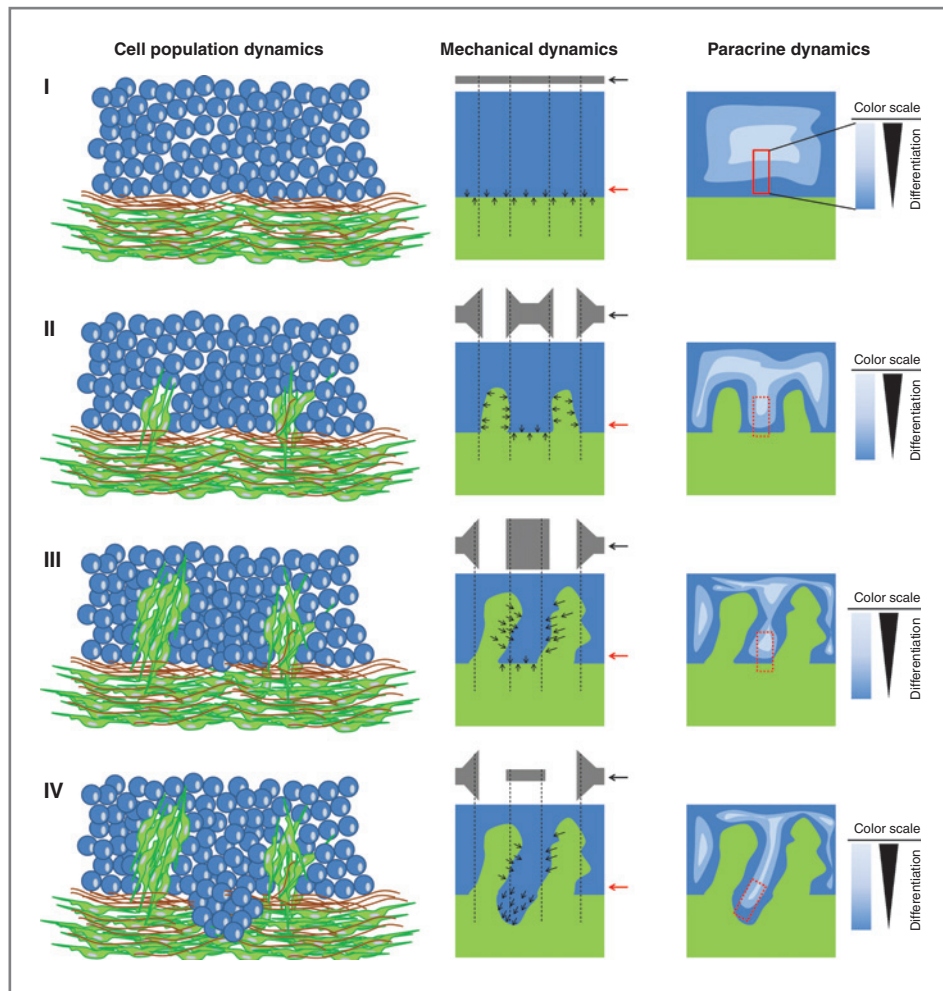


Figure 5. "Interdigital migration," a working model of CAF-directed metastasis. The cartoon illustrates 4 (I–IV) sequential steps of the proposed model of interdigital CAF-directed metastasis and briefly shows how the myofibroblastic cohort invades the cancer population and vice-versa. The illustration is followed by the reader vertically in each step (I–V), whereby the description of cell-population (left column), mechanical (middle column), and signaling/paracrine (right column) dynamics is provided for each specific snapshot during the metastatic process. The first column shows the population movements: cancer cells, blue roundish; CAFs, green elongated; ECM, brown curvy lines. The second column represents the exact same snapshot as the first column: cancer cells, blue; CAFs, green; arrows within the snapshot show mechanical tensile forces inflicted by one population over the other; the thickness of the gray line illustrated over the snapshot and shown by black arrow is relative to the mechanical pressure on the cancer population at the level of the red arrow. Therefore, before invasion both subpopulations rest in quiescence (step I), so the thickness of the gray line is very small. After the first CAF digits invade the cancer population (steps II and III), the thickness of the gray line significantly and progressively grows. This mechanical force allows the cancer cell cohort to invade the CAF cohort in an antiparallel manner, reducing the mechanical tension at the level of the invasion front (step IV), so the thickness of the gray line in the last snapshot is again reduced. The third column represents the exact same snapshot as the first column: cancer cells, blue scale; CAFs, green. The blue scale in the cancerous population is proportional to the differentiation status of the cells in each step (I–V) of metastasis, the darker the blue, the less differentiated the cancer cells. Note that the paracrine impact of the CAF cohort translocates the signaling gradients (rectangular box) to retain the undifferentiated state of the cancer cells lining the invasion front.

adhesions, capable of: (i) avoiding strong attachments of the cohort with the ECM, and at the same time, (ii) facilitating the migratory behavior of the cohort in the matrix (Fig. 4C).

- CAFs must induce peritumoral stiffness by exacerbating collagen cross-linking and/or ECM enzymatic remodeling (Fig. 4D).
- In the interdigital model of metastasis, the CAF cohorts cause progressive sclerosis of the peritumoral matrix (between 2 CAF digits), which eventually results in the antiparallel protrusion and invasion of the cancer cohort

toward areas that are less dense, a forced action that alleviates the increased mechanical tension (Fig. 5, middle column).

Assumptions and limitations of the working model

The model implies that the CAF cohort is the only stromal participant at the tumor-host cell interface. However, a heterotypic signaling interplay between cancer cells and other cell types, such as endothelial cells, various immune/inflammatory cells, bone marrow-derived cells, adipocytes, pericytes, and smooth muscle cells, is well

documented (128). All these stromal cells might actually depict very crucial roles in the progression of the metastatic cascade and in general, their impact should not be underestimated.

In addition, the model assumes that the CAF cohort is a nonheterogeneous cohort by itself, and therefore, it is tempting to speculate that CAFs affect with the same paracrine and mechanical signals all cancer types in all possible biologic contexts. However, such an assumption is oversimplified, as CAFs may originate from a huge plethora of precursor cells. Despite resident quiescent fibroblasts may give rise to the vast majority of peritumoral CAFs, it has been suggested that CAFs may also be derived from mesenchymal stem cells, bone marrow stem cells/hematopoietic progenitors, endothelial cells, and possibly even cancer cells upon EMT (19), as already described. The concept that there are different subtypes of CAFs at the tumor microenvironment is now well accepted (129, 130). Therefore, it seems likely that not all CAF cohorts will behave identically, although they will retain the basic myofibroblastic properties outlined in previous sections.

The major focus of this working model is both the paracrine and the mechanical impact of the CAF cohort on the cancer cell population. However, redox-dependent interactions such as, for instance, the generation of reactive oxygen species (ROS) by CAFs under low pH conditions should not be neglected, as it is now well shown, they could act as a mutagen to the surrounding cancer cells (131). Specifically, it has been shown that the effects of CAF oxidative stress can be laterally propagated, amplified and spread from cell-to-cell, creating an oncogenic/mutagenic field promoting widespread DNA damage and EMT (132). Along the same lines of evidence, ROS may also have a direct effect on the stromal compartment itself. Toullec and colleagues showed that junD inactivation was able to cause a ROS-dependent migratory potential in CAFs (133). Taken together, our working model should be seen as a platform attempting to bridge together all current knowledge on the tumor-promoting impact of CAF cohorts on cancer tissue, either these signals are physical or biochemical or even chemical.

Conclusion and future perspectives

In view of the emerging concept of the heterotypic nature of cancer, several models of microenvironmental regulation of metastasis have been proposed (134, 135). However, our working model of interdigital metastasis should be eyed as the first attempt to provide mechanistic insight for CAF-directed cancer cell growth and metastasis, based on integration of biologic and physical properties of stromal tissues during cancer development and progression. Therefore, the

proposed model should be viewed as working hypothesis bringing together the most recent and rationalized advances in the CAF biology, rather than a *de novo* concept.

Certain questions need to be addressed in the future to provide a more stable framework for our working model. For instance, because of technological limitations, it still remains a challenging task to monitor cancer progression in *real-time* fashion and be able to capture an interdigital pattern of metastasis. Therefore, there is a profound difficulty in the direct observation and proof of our proposed concept, as current technologies usually provide molecular snapshots (e.g., immunostaining methods) of the tumor-host cell interface. However, emerging computational and imaging technologies, such as multiphoton microscopy, have provided the first steps toward identifying "tracks" of the CAF and cancer cohorts as they migrate within the tumor microenvironment (136–138). Such technologies are likely to advance our understanding of the complicated nature of microenvironmental dynamics in various types of cancer.

The conceptualization of the interdigital model of CAF-directed metastasis may have prognostic and/or therapeutic implications. With a growing emphasis on a "hallmark-targeting" strategy for cancer therapy, the tumor microenvironment now appears as a promising target for metastasis prevention (4). Indeed, there is now strong rationale for targeting specific subtypes of CAFs, as exemplified by inhibition of the metastatic cascade through neutralization of the FAP-expressing CAF subpopulation (139). Thus, in the next decade, we anticipate that the signaling circuitry and intercommunication between the cancer cells and CAFs will be mapped in far greater detail and clarity and novel key-therapeutic molecules will emerge.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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