Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Cell Biology

Cancer associated fibroblasts: is the force the path to the dark side?

Jorge Barbazán and Danijela Matic Vignjevic

The most abundant cell type in the tumor microenvironment are cancer-associated fibroblasts (CAFs). CAFs play an important role in tumor growth and progression. Besides direct communication with cancer cells via secreted molecules or cell–cell adhesions, CAFs also indirectly affect cancer cell behavior by remodeling the extracellular matrix (ECM). Here, we summarize recent findings on the distinct mechanisms that CAFs use to modify ECM, specifically, their proteolytic versus force-dependent activity. We then review the consequences of CAF force transmission on the physico-chemical properties of the matrix, focusing on the deposition of new matrix components, and the alteration of the organization and stiffness of the ECM. CAFs promote tumor invasion by creating the roads cancer cells use to escape the tumor mass. However, there is also evidence that CAFs can prevent invasion, possibly by forming a physical barrier around the tumor edge. We discuss the controversial role of CAFs in tumor progression.

Address
Institut Curie, PSL Research University, CNRS UMR 144, F-75005 Paris, France

Corresponding author: Barbazán, Jorge (Jorge.barbazan@curie.fr)

Current Opinion in Cell Biology 2019, **56**:71–79

This review comes from a themed issue on **Cell architecture**

Edited by **Johanna Ivaska** and **Manuel Théry**

https://doi.org/10.1016/j.ceb.2018.09.002

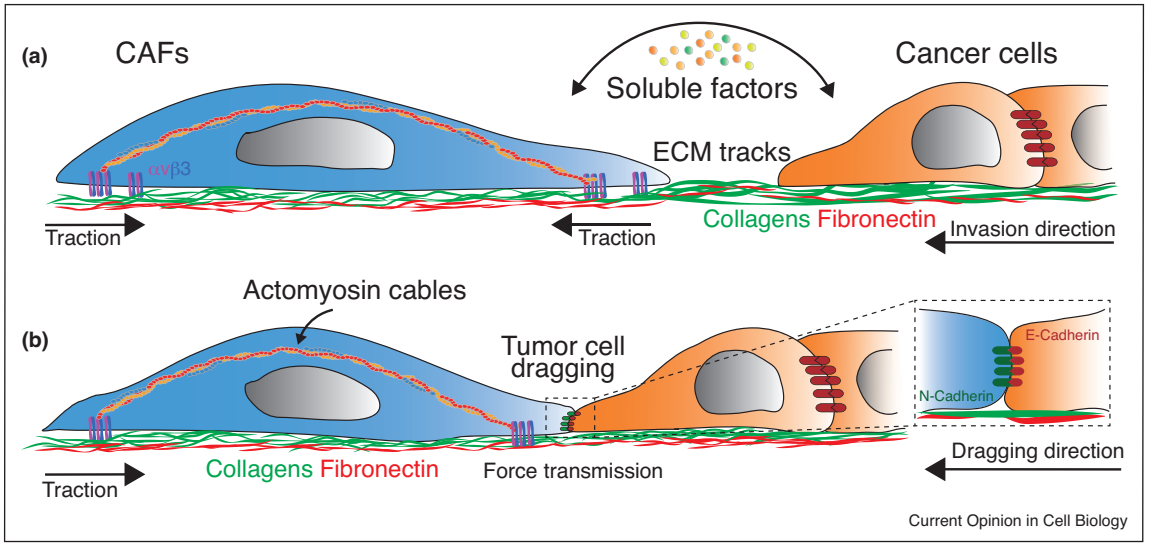
Introduction

In tumors, cancer cells are not alone. They are intercalated with myriad cell types, which together form the tumor microenvironment. During formation of carcinomas, cancer-associated fibroblasts (CAFs) accumulate close to tumor cells and generate a specific extracellular matrix (ECM) in a process known as a desmoplastic reaction. A range of structures become embedded in this ECM, including blood and lymphatic vessels, made of endothelial cells and pericytes, and the tumor associated immune component, established by T-lymphocytes and B-lymphocytes, dendritic cells, NK cells, macrophages and others. Tumor cells actively interact with these cell types in many different ways and such interactions determine the dynamics of tumor behavior over time.

CAFs constitute a heterogeneous population of mesenchymal cells whose origin and specific characteristics remain unclear. Among potential CAF origins are recruitment of bone marrow mesenchymal stem cells, differentiation from adipose stem cells, or conversion from tumor or endothelial cells through a process of epithelial/endothelial to mesenchymal transition (EMT/EndMT) [1–3]. However, EMT/EndMT are controversial CAF sources that still need to be further explored. To date, the best-documented source of CAFs is the activation of 'normal' resident fibroblasts. Soluble factors produced by tumor cells such as leukemia inhibitory factor (LIF) [4], CCL2 [5] or oncostatin M [6], drive fibroblasts activation through JAK/STAT signaling. In addition, the relocalization of cell-matrix receptor integrin α5β1 from the membrane to endosomes leads to the same phenotype [7]. Once activated, CAFs acquire a series of particular features such as the expression of αSMA, PDGFR, FSP1 or FAP, and increased cell contractility. Those, together with the absence of epithelial, endothelial or immune markers are currently used for the identification of CAFs. However, CAF-specific markers are still lacking. The development specific functional tests together with the analysis of the absence of driver oncogenic mutations could serve as platforms to determine which fibroblasts can be considered as bona fide CAFs.

CAFs play a variety of roles in tumor development. For example, CAFs at the vicinity of tumors feed cancer cells through nutrient-rich exosomes [8]. Reciprocal signaling of CAFs with cancer cells promote cancer cell survival and proliferation [2,9]. CAFs as well interact with tumor immune cells, generating a pro-tumorigenic inflammation through cytokine production, and creating at the same time an immunosuppressive environment that limits antitumor immunity [2,10]. Cancer cell invasion is one of the hallmarks of tumor progression as it determines tumor spreading, impacting dramatically patient survival. CAFs influence the invasion of cancer cells in multiple ways (Figure 1). They secrete growth factors, such as HGF and PDGF, that increase the migration of tumor cells [11,12••]. CAFs also lead cancer cell invasion in a contact-mediated fashion [13,14••]. By establishing heterotypic contacts with cancer cells via N-E cadherins, CAFs pull cancer cells out of the tumor bulk [14]. Finally, CAFs excavate passageways in the ECM that cancer cells use to disseminate [13,15••,16].

Figure 1



Interactions between cancer cells and CAFs during tumor invasion. **(a)** Production of soluble factors by CAFs and cancer cells has a bidirectional effect. Factors produced by CAFs promote cancer cell migration, and at the same time, cancer cells secrete molecules that stimulate CAF activation. Once activated, CAFs actomyosin contractility generates force that is transmitted to the ECM via integrins. Rearrangement of matrix components leads to collagen and fibronectin fibers alignment. This creates ECM tracks that cancer cells use to invade. **(b)** CAFs can also establish direct contacts with epithelial cancer cells. These contacts are heterotypic, formed by CAF's N-cadherin and cancer cell's E-cadherin. CAFs generated force can be transmitted to cancer cells through those contacts, dragging tumor cells out of the tumor bulk.
For simplification, CAFs and cancer cells were depicted contacting the ECM only ventrally, but on a 3D *in vivo* scenario, ECM contact will be present both ventrally and dorsally.

The molecular changes that CAFs undergo during tumor progression determine their function. For instance, gain of proteolytic activity endows CAFs the capacity to degrade the ECM. At the same time, fibroblasts activation increases their contractile properties and consequently their ability to generate force. This turns CAFs into masters of ECM remodeling. Altogether, CAF acquired functions result in a dramatic change in chemical and physical properties of the tumoral ECM with consequences for tumor cell behavior. Here, we review recent advances in understanding the distinct mechanisms that CAFs use to modify ECM, focusing on their proteolytic versus force-dependent activity, and how this affects cancer cell properties.

Running with scissors: CAF-driven proteolytic ECM degradation

The ability of cancer cells to invade has traditionally been attributed to their capacity to degrade surrounding ECM. Tumor cells form actin-based protrusions called invadopodia, which are enriched in matrix metalloproteinases (MMPs) that cut the fibers in the matrix. This opens a passage in the surrounding stroma through which cancer cells move [17,18]. However, CAFs, but not normal fibroblasts, can also form invadopodia and use MMPs to degrade the ECM [17]. Invadopodia are formed upon Twist1 translocation into the nucleus, which upregulates the expression of the actin-binding protein palladin (isoform 4) [19], contributing to the activation of a small GTPase Cdc42 [20]. CAFs also form degradative protrusions that differ from classical invadopodia. These tubular/reticular structures do not contain actin or cortactin and are independent of Cdc42 activity, but are still MMP positive [21], and so could be used by CAFs to degrade the ECM. Whether CAFs forming invadopodia differ in their origin from CAFs lacking these invasive structures is still unknown. Although it is appealing to hypothesize that invadopodia-rich CAFs could directly derive from tumor cells undergoing EMT, this needs to be explored. Lineage tracing experiments could help addressing this question.

The expression of MMPs in CAFs is regulated by a number of different factors. For instance, under hypoxic conditions, reactive oxygen species (ROS) produced by the metabolic activity of tumor cells [22] downregulate MMP-3 in CAFs [23]. Hypothetically, this could result in degraded matrix at the tumor edges that are well-oxygenated, and intact matrix on poorly vascularized and likely hypoxic tumor necrotic cores. But, this still needs to be experimentally tested.

Transforming growth factor beta (TGFβ) is another example of a regulator of MMPs in CAFs. For instance, it is required for the maintenance of MMP-9 activity [24]. In turn, binding of MMP-9 to lysil hydroxylase 3 (LH3)