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Review article

The hallmarks of CAFs in cancer invasion

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ABSTRACT

The ability of cancer cells to move out of the primary tumor and disseminate through the circulation to form metastases is one of the main contributors to poor patient outcome. The tumor microenvironment provides a niche that supports cancer cell invasion and proliferation. Carcinoma-associated fibroblasts (CAFs) are a highly enriched cell population in the tumor microenvironment that plays an important role in cancer invasion. However, it remains unclear whether CAFs directly stimulate cancer cell invasion or they remodel the extracellular matrix to make it more permissive for invasion. Here we discuss paracrine communication between cancer cells and CAFs that promotes tumor invasion but also stimulates CAFs to remodel the matrix increasing cancer dissemination.

Fibroblasts are a population of cells characterized by their elongated spindle-like shape, their similarities to mesenchymal and smooth muscle cells and, in the context of a wound, their role in tissue contraction (Gabbiani et al., 1971; Hirschel et al., 1971; Majno et al., 1971). There have been many attempts to further define this cell population. However, to date, there is still no specific marker of fibroblasts. Experimentally, fibroblasts are defined based on their shape, the absence of markers of other cell types, as well as the expression of a combination of smooth muscle and mesenchymal cells' markers such as α-smooth muscle actin, the platelet derived growth factor receptor β (PDGFrβ), vimentin and desmin.

Fibroblasts are the main generators of extracellular matrices (ECM), scaffolds that other cells are anchored to. They are at their most active state during embryonic development when all matrices in the human body are being created (Powell et al., 2005). In adult normal tissues, they are quiescent residents of the stroma. Normal fibroblasts have never been successfully isolated and kept in a 'non-activated' form. To study fibroblast's functions it is possible to either immortalize normal adult fibroblasts or use embryonic fibroblasts that retain a proliferative capacity, albeit these two populations do not always recapitulate normal fibroblasts' functions.

In tissue inflammation, fibrosis, and during wound healing, fibroblasts get 'activated' (Gabbiani et al., 1971; Powell et al., 2005). In those conditions, they are often called myofibroblasts because of their increased capacity to contract and remodel the matrix which is necessary to heal the wounded tissue (Majno et al., 1971). Once wound is healed, they either revert back to a normal state or undergo apoptosis as activated fibroblasts are not present in significant amounts in normal adult tissues. The presence of activated fibroblasts in adult tissues suggests the existence of a disease. At the tumor site, activated fibroblasts are known as cancer-associated fibroblasts (CAFs) (Kalluri and Zeisberg, 2006). CAFs were initially thought to be a consequence of tumor formation but later it has been shown that they actively contribute to tumor growth, invasion and metastasis (Bissell and Hines, 2011). Therefore, targeting CAFs seems to be a good clinical strategy to fight cancer. However, as promising as it sounds, targeting CAFs in clinics remains a complicated task. Their origin is still unknown and the lack of a specific fibroblast marker makes it difficult to discriminate what cell(s) population(s) give rise to CAFs. Do they emerge from resident normal fibroblasts that are activated by neighboring cancer cells? (Albrengues et al., 2015; Avgustinova et al., 2016; Calvo et al., 2013; Kojima et al., 2010) Are CAFs the progeny of mesenchymal stem cells that are recruited from the bone marrow to the site of the tumor? (Karnoub et al., 2007; Lu et al., 2013; Shinagawa et al., 2013; Talele et al., 2015) Can other cell types harboring some similar characteristics, such as pericytes, transform into CAFs in tumors? (Ross et al., 1974) Or do they originate from cancer cells that have undergone epithelial to mesenchymal transition (EMT)? (Radisky et al., 2007; Rowe et al., 2009; Schulte et al., 2012) And if they have multi-

Abbreviations: CAF, carcinoma-associated fibroblast; ECM, extracellular matrix; TGF-β, transforming growth factor beta; TβR, transforming growth factor beta receptor; Timp, tissue inhibitor pf metalloproteinase; PDGF, platelet derived growth factor; MMP, matrix metalloproteinase; FAP, fibroblasts activation protein; JAK, janus kinase; YAP, yes-associated protein; LOX, lysyl oxidase.

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ple origins, can this explain CAFs' heterogeneity and their different functions?

This review discusses the markers used to characterize CAFs and the consequence(s) of their expression on tumor development; their direct effect on cancer cell invasion, and indirect effect through modification of the matrix.

1. Paracrine dialogue between CAFs and cancer cells

The transforming growth factor-β (TGF-β) signaling pathway is a major player in cancer development and is known to regulate tumor growth through multiple cellular mechanisms such as apoptosis, proliferation, angiogenesis, migration and invasion (Bierie and Moses, 2006; Siegel and Massague, 2003). Aberrant expression of the TGF-β receptors (TβRs) and their ligands is not reserved for cancer cells, as both overexpression (Calon et al., 2012; Tsushima et al., 2001) and silencing (Achyut et al., 2013; Bhowmick et al., 2004; Franco et al., 2011; Oyanagi et al., 2014) of the TGF-β signaling pathway in fibroblasts is important for tumor progression. TGF-β remains the most commonly used growth factor to activate fibroblasts in culture as it activates many downstream pathways lading to growth factor secretion and matrix remodeling (Desmouliere et al., 1993; Ronnov-Jessen and Petersen, 1993). However, it is still a debate whether loss of the TβR or its overexpression leads to a more aggressive tumor profile.

Conditional loss of the TGF-β type II receptor (TβRII) in fibroblasts increases cell proliferation of both fibroblasts and neighboring epithelial cells, and results in preneoplastic lesions that could eventually progress to invasive carcinomas (Bhowmick et al., 2004) (Fig. 1A). Upon silencing the TβRII, fibroblasts secrete upregulated amounts of HGF as a result of suppression of cell cycle regulators p21 and p27, and induction of transcription regulator c-Myc (Bhowmick et al., 2004; Oyanagi et al., 2014). Therefore, it seems that during cancer progression, CAFs can acquire conditional loss of TGF-β receptor II, which favors an increase of tumorigenicity.

Loss of the TβR does not abolish the secretion of the TGF-β ligand, allowing a paracrine effect on neighboring CAFs that do not have the same deletion of TβR (Franco et al., 2011) (Fig. 1A). In fact, the most optimal scenario for cancer progression is where CAFs are present in a heterogeneous manner; tumor cells could benefit from the growth factors secreted by CAFs that lack the TβR, and the activated state of CAFs that can respond to TGF-β activation. Of note, in some cancers the stroma is the only direct beneficiary of TGF-β as cancer cells display mutational deactivation of the TGF-β receptor (Calon et al., 2012). In other words, although they can secrete TGF-β, cancer cells cannot directly benefit from it, but only indirectly from the activated stroma.

TGF-β is a solid predictor of cancer recurrence and metastasis (Calon et al., 2012; Tsushima et al., 2001). It upregulates genes coding for gp130 binding cytokines, more specifically IL11. IL11 activates the phosphorylation of Stat3 in cancer cells, and this interaction renders cancer cells resistant to apoptosis and favors metastasis (Calon et al., 2012). TGF-β also stimulates expression of the stromal cell-derived factor 1 (SDF-1) in CAFs (Kojima et al., 2010), which is not only necessary for cancer cell proliferation and invasion, but also for recruitment of endothelial progenitor cells and angiogenesis (Izumi et al., 2016; Kojima et al., 2010; Orimo et al., 2005). A positive feedback loop is also established as SDF-1 has an autocrine effect on CAFs that leads to the secretion of TGF-β and the maintenance of CAFs' activated phenotype (Kojima et al., 2010).

In conclusion, inactivation of the TGF-β receptor pathway in fibroblasts can induce the secretion of growth factors and promote tumor growth by activating cancer cell proliferation. Its activation is also crucial for the acquisition of a 'CAF phenotype' and for helping cancer cells invade and survive, especially during organ colonization at the metastatic sites.

Another important growth factor enriched in the tumor microenvironment is the platelet derived growth factor (PDGF). PDGF is mainly secreted by endothelial cells in order to recruit pericytes and stabilize blood vessels (Lindblom et al., 2003). In cancer, PDGF is secreted by both tumor cells and other components of the tumor microenvironment (Heldin and Westermark, 1999). Stromal cells, more particularly αSMA-positive mesenchymal cells (Bhardwaj et al., 1996), express PDGF receptors (PDGFR). PDGFR belongs to the family of tyrosine kinase receptors and exists in 2 isoforms α and β. PDGFRβ is expressed in higher levels during tissue inflammation, wound healing, in fibrosis, and in the tumor stroma (Alvarez et al., 2006; Heldin and Westermark, 1999). Upon activation, PDGFR dimerizes and activates multiple downstream pathways such as the phosphatidylinositol 3-kinase (PI3K) pathway, which leads to increased actomyosin activity, and the Ras pathway that induces cell proliferation (Cully et al., 2006; Schubbert et al., 2007). Besides acting as mitogen, PDGF exerts chemotactic effects on mesenchymal cells and increase their velocity and persistence (Martin et al., 2014; Osornio-Vargas et al., 1996). More specifically, PDGF serve as a cue to recruit fibroblasts to the tumor site (Cadamuro et al., 2013; Dong et al., 2004) where they are consequently activated to remodel the surrounding matrix (Kinnman et al., 2000; Pinzani et al., 1994; Yi et al., 1996). This appears to happen at early stages of cancer progression since cells expressing PDGFRβ are found in the stroma adjacent to *in situ* carcinomas (Bhardwaj et al., 1996). Finally, as found in clinical trials, PDGFR inhibitors successfully improved patient outcome. Blockade of PDGFR signaling in the stroma of mice bearing cervical tumors using the receptor tyrosine kinase inhibitor imatinib cancelled FGF secretion by fibroblasts (Pietras et al., 2008). This treatment impaired tumor angiogenesis and slowed the progression and growth of both non-invasive and invasive lesions. Similarly, in a colon cancer model, imatinib therapy impaired the recruitment of CAFs to the site of the tumor, which ultimately led to the inhibition of cancer growth and metastasis (Shinagawa et al., 2013)

Growth factors like TGF-β and PDGF can be either freely secreted in the tumor microenvironment or delivered via exosomes. Exosomes are cargo-carrying multi-vesicular bodies released in the extracellular milieu (Simons and Raposo, 2009; Thery et al., 2006). They have both autocrine and paracrine effects on the microenvironment they are released in. Although most studies focus on cancer cell-secreted exosomes, recent study highlighted the role of CAF-secreted exosomes (Fullar et al., 2012; Luga et al., 2012) (Fig. 1B). CAFs' exosomes specifically carry Wnt11, a ligand that is internalized by cancer cells through its receptor Fzd6, a component of the planar cell polarity (PCP) signaling pathway (Luga et al., 2012). Cancer cells' motility and metastatic potential is consequently stimulated.

The tissue inhibitors of metalloproteinases (*Timps)* are also involved in acquisition of a "CAF phenotype" and in exosome-mediated cancer cell invasion. *Timps* play a role in controlling the activity of matrix metalloproteinases (MMPs) by inhibiting their catalytic activity (Fullar et al., 2012). Exosomes of *Timp*-less mouse dermal CAFs are rich in a metalloproteinase ADAM10 that stimulates cancer cell migration and conserve cancer's stemness through activation of RhoA and Notch signaling cascade, respectively (Shimoda et al., 2014). Even though *Timp*-less fibroblasts are more contractile and secrete exosomes rich in ECM proteins, the role of *Timp*-less CAFs in matrix remodeling has never been addressed.