INSIGHTS

Deciphering cancer fibroblasts

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In this issue of *JEM*, Raz et al. (https://doi.org/10.1084/jem.20180818) identify a subset of bone marrow–derived cells that uniquely promotes breast cancer angiogenesis and tumor growth. The existence of functional heterogeneity among stromal populations motivates further fundamental and therapeutic inquiries.

Stromal cells constitute the tumor microenvironment (TME), a niche where neoplastic cells reside and progress. While the genetic and epigenetic drivers of cancer cells have been extensively investigated, the mechanisms governing the recruitment and activation of a major stromal cell type, cancer-associated fibroblasts (CAFs), are largely unknown (Öhlund et al., 2014; Kalluri, 2016). Investigating the origin and developmental lineage of CAFs is essential for determining their functions and designing means to impede their tumor-supportive roles.

CAFs may be globally viewed as the chief architects of the TME due to their multiple functions. Indeed, they are considered the major source of extracellular matrix components that alter physical-chemical properties, concomitantly impairing vascular function and, therefore, drug delivery (Olive et al., 2009; Provenzano et al., 2012). Furthermore, CAFs secrete paracrine ligands that promote tumor growth, angiogenesis and drug resistance (Öhlund et al., 2014; Kalluri, 2016), and directly blunt T cell cytotoxicity (Feig et al., 2013) while recruiting immunosuppressive populations. Therefore, a multitude of preclinical and clinical studies have attempted to antagonize CAFs as a treatment modality for cancer. However, the classical view of uniformly pro-tumorigenic CAFs has been modified by the recent identification of subsets with tumor-suppressive properties (O'Connell et al., 2011; Rhim et al., 2014). This new appreciation that CAFs are a heterogenous population in the TME (Öhlund et al., 2017; Costa et al., 2018; Su et al., 2018) prompts a reevaluation of CAF identities and functions in efforts to develop more effective therapies.

Raz et al. contribute to a deeper understanding of this issue by probing the origin of stromal subtypes in mouse models of breast cancer. Using reporter alleles and bone marrow (BM) transplantation, the authors show that a unique stromal population in primary breast tumors and lung metastases migrates as precursors from the bone marrow and is differentiated into CAF-like cells within the TME. This BM-derived stromal subtype lacks extracellular matrix–producing characteristics typical of myofibroblasts, and features of inflammatory fibroblasts (Erez et al., 2010; Öhlund et al., 2017). The transcriptional signature of BM-derived CAF-like cells is distinct from that of resident fibroblasts, and this is reflected in differential promotion of tumor progression and remodeling of the tumor architecture through stimulation of angiogenesis. Interestingly, the BM-derived population also lacks platelet-derived growth factor receptor α (PDGFRα) expression, which is commonly considered a canonical marker of CAFs. Further characterization of this BM-derived subset, and of the tumor-specific factors responsible for its reprogramming, may enable its specific targeting in breast cancer.

With the discovery that CAFs are a complex, heterogeneous cell population, several critical questions need to be addressed to clarify their properties. While it has been reported that the same CAF precursors can acquire distinctive phenotypes depending upon local tumor cues (Öhlund et al., 2017), the role of the cell lineage in CAF functional specification had not previously been reported. The work from Raz et al. (2018) begins to address this topic by showing that the molecular and functional heterogeneity of

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CAFs may to some extent be predetermined by the tissue and cell of origin of the CAF precursors. Therefore, to achieve a deeper understanding of CAF biology in all cancers, the differential impact of the tumor niche and the cell lineage should be carefully ascertained for each subtype of CAFs. Such work will be enabled by the development of new mouse models that reveal the unambiguous origin and cell lineage of the diverse CAFs that populate the tumor stroma. Indeed, CAFs have been suggested to derive from a multitude of cell types, including resident quiescent fibroblasts, stellate cells, BM-derived mesenchymal cells, such as those reported by Raz et al. (2018), adipocytes, mesothelial cells, and endothelial cells (Öhlund et al., 2014; Kalluri, 2016). However, the supporting evidence for this is rather limited and was largely gathered from cell culture and transplantation assays. As a unique and specific CAF marker has not been reported yet, the use of multiple markers may enable the generation of new mouse models to identify, trace, and ultimately ablate, different subsets of CAFs. The combination of reporter alleles and intravital imaging techniques would directly reveal CAF recruitment, physical interactions with non-CAF cells, and CAF population dynamics present in the TME.

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Breast tumor microenvironment
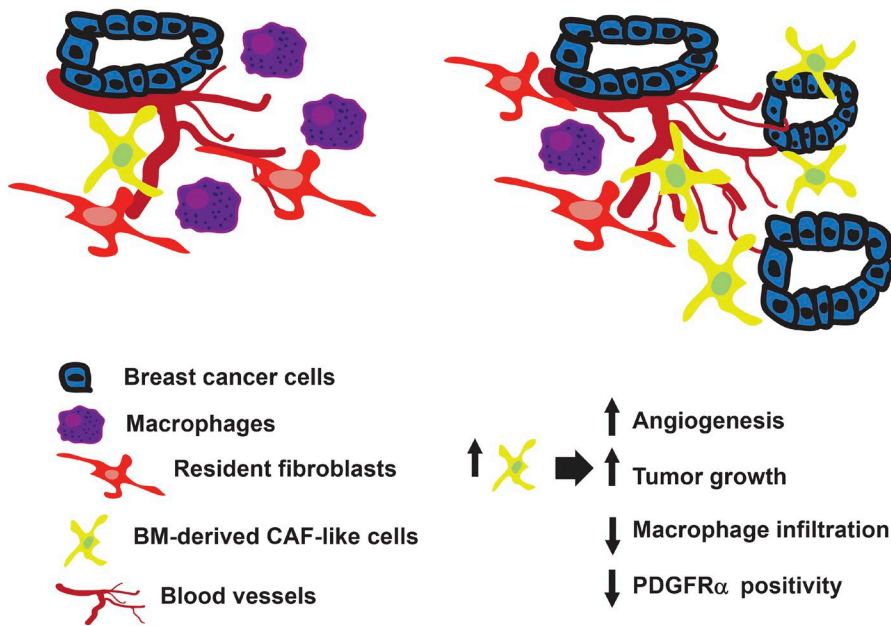
Model explaining the functional differences between two stromal populations in the breast tumor microenvironment. Increased presence of PDGFRα-negative BM-derived stromal cells induces angiogenesis and tumor growth, while reducing macrophage infiltration and overall PDGFRα positivity.

The complexity of CAFs has also been highlighted by single-cell RNA-sequencing analyses (Li et al., 2017; Bernard et al., 2018; Lambrechts et al., 2018). These studies reveal novel CAF subsets and highlight the need to establish a common nomenclature and classification system for the field. Since some CAF subtypes share transcriptional signatures across multiple cancers, future work should be directed at determining whether they also possess similar functions. In light of our current knowledge of stromal heterogeneity and of these emerging datasets at single-cell resolution, it is also pivotal to redefine classical CAF markers as subset specific, to develop panels of proteins unique to each CAF population, and to compare the stromal representation found at primary tumors and metastases. This work will complement and guide the development of new mouse models for lineage tracing to study CAF heterogeneity.

In addition to determining the developmental lineage of CAFs, the identification of tumor-secreted factors that recruit and convert precursor cells into CAF subsets is an opportunity for future investigation. Indeed, the definition of molecules that recruit and promote the differentiation of the BM-derived stromal population reported by Raz et al. (2018) could prevent their reprogramming into highly cancer-promoting cells. The search for tumor-derived signals that activate the stroma is especially relevant in light of recent findings showing that CAFs in pancreatic ductal adenocarcinoma interconvert into diverse phenotypic and functional subtypes, depending on the tumor cues they are exposed to (Öhlund et al., 2017). In pancreatic ductal adenocarcinoma, cancer cells secrete ligands that activate differential signaling pathways and play antagonistic roles in defining two distinct subsets of CAFs (Biffi et al., 2018). By exploiting the knowledge that the transition from one CAF state to the other is a dynamic event, driven by tumor-secreted factors, shifting of tumor-promoting inflammatory CAFs into potentially tumor-suppressive myofibroblasts was achieved by selective

targeting of the signaling pathways responsible for their formation (Biffi et al., 2018). An analogous approach was reported by the Evans group that used vitamin D receptor agonists to revert CAFs into quiescent fibroblasts (Sherman et al., 2014). Considering the impact that pharmacological agents could play in reprogramming the stroma, this newly identified CAF plasticity represents a potential therapeutic opportunity and should be investigated in other cancer types.

The work of Raz et al. (2018) strengthens the emerging view that distinct stromal populations play different roles in the TME by modulating cancer progression, immune infiltration, extracellular matrix remodeling, and angiogenesis. The identification of molecular, phenotypic, and functional differences between distinct CAF populations prompts a further dissection of stromal heterogeneity in order to design novel effective combination therapies for better patient outcomes.

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