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Review

Fibroblasts as architects of cancer pathogenesis ☆

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ABSTRACT

Studies of epithelial cancers (i.e., carcinomas) traditionally focused on transformation of the epithelium (i.e., the cancer cells) and how aberrant signaling within the cancer cells modulates the surrounding tissue of origin. In more recent decades, the normal cells, blood vessels, molecules, and extracellular components that surround the tumor cells, collectively known as the "tumor microenvironment" or "stroma", have received increasing attention and are now thought to be key regulators of tumor initiation and progression. Of particular relevance to the work reviewed herein are the fibroblasts, which make up the major cell type within the microenvironment of most carcinomas. Due to their inherent heterogeneity, plasticity, and function, it is perhaps not surprising that fibroblasts are ideal modulators of normal and cancerous epithelium; however, these aspects also present challenges if we are to interrupt their tumor-supportive functions. Here, we review the current body of knowledge and the many questions that still remain about the special entity known as the cancer-associated fibroblast. This article is part of a Special Issue entitled: Fibrosis: Translation of basic research to human disease.

1. Introduction

1.1. Form and function — normal, activated, and cancer-associated fibroblasts

Fibroblasts are derived from the primitive mesenchyme, have an elongated, spindle-like morphology, and are metabolically active (the suffix "blast" typically denotes an active metabolism). As the most abundant cells of the connective tissue in animals, fibroblasts both synthesize and degrade extracellular matrix (ECM) components by expressing collagens, fibronectins, laminins, elastins, proteoglycans, integrins, matrix metalloproteinases (MMPs), tissue inhibitors of metalloproteinases (TIMPs), and a host of other ECM proteins that are expressed in a tissue-specific manner (reviewed in [1,2]). Consequently, fibroblasts are responsible for providing structural integrity to most tissues. Fibroblasts also produce the tissue-specific basement membrane that provides a protective barrier around the specialized epithelium, thereby contributing to specificity, polarity, and functionality of the epithelium [2]. There is also evidence indicating that fibroblasts communicate through the basement membrane to alter epithelial homeostasis, proliferation, and differentiation [3].

Fibroblast activity is crucial during processes of wound healing and inflammation. When tissue damage occurs, resident fibroblast populations proliferate and invade the injured area in response to platelet clotting. Platelets adhere to exposed subendothelium at sites of vessel injury and release their bioactive cargo (e.g., TGFβ1, PDGF, IL1-B, MMPs, TIMPs), predominantly from α-granules that degrade the basement membrane, induce cell proliferation and migration, and recruit inflammatory cells and fibroblasts (reviewed in [4]). Under such conditions, fibroblasts are generally considered to be "activated". In particular, as the healing process progresses, fibroblasts turn on expression of a filamentous actin, alpa-smooth muscle actin (αSMA), which enables them to exert contractile forces to close the wound. Local tissue contractility is mediated by focal adhesions between the activated fibroblasts – at this point called myofibroblasts – and the ECM. Moreover, contractile fibroblasts are known to regulate interstitial fluid volume and pressure via cytoskeletal infrastructure [5]. After wound closure, the balance of MMPs and TIMPs secreted by fibroblasts is changed to favor ECM degradation (as opposed to synthesis) which leads to massive apoptosis of the myofibroblast population. Consequently, only quiescent, non-contractile fibroblasts are left at the resolved wound site, and as such, myofibroblasts are only observed under pathological conditions.

The wound healing process seems to be co-opted by tumors; indeed, tumors have been likened to "wounds that never heal" [6]. However, unlike wound resolution in which fibroblasts "de-activate", the myofibroblast population persists during fibrosis or tumorigenesis for reasons that are not clear. It seems that normal fibroblasts have a bimodal effect on cancerous cells in that early in tumorigenesis,

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fibroblasts work against malignant progression, yet as the malignancy advances, fibroblasts are subverted to promote tumor growth — these tumor-supportive fibroblasts are referred to as cancer-associated fibroblasts (CAFs). In some cases, normal fibroblasts suppress malignant conversion of immortalized prostate epithelium [7], whereas in the breast, normal fibroblasts can induce the transition of already transformed ductal carcinoma in situ to invasive carcinoma [8]. The oncogenic transformation of the epithelium may subvert normal fibroblasts and potentiate their ability to promote tumor growth. Concordantly, one study has shown that suppression of the retinoblastoma (Rb) protein in pancreatic epithelium induces a selection pressure for fibroblasts that lack p53 and subsequently results in p53-inactivated epithelium [9]. Although the reasons why CAFs remain perpetually activated remain to be elucidated, it is very clear that fibroblasts participate in an elaborate, reciprocal cross-talk with the cancerous epithelium.

2. Cancer-associated fibroblasts — heterogeneity or a spectrum of phenotypes?

It is widely accepted that CAFs are a heterogeneous cell type and that this diversity may arise from their cell (s) of origin, the tissue in which they develop, or their activation state at any given time. This heterogeneity has presented challenges to precisely and exclusively identifying CAFs and to distinguishing them from other cell types that express similar markers upon histopathological analysis of tumors and tissues (Fig. 1). Instead, CAFs are more readily distinguished from their normal counterparts by their phenotype, proliferation rate, and differential expression of ECM constituents [10].

CAFs are most often denoted by expression of αSMA. Several additional markers are used to identify CAFs, including: vimentin, platelet-derived growth factor receptor alpha (PDGFR-α), platelet-derived growth factor receptor beta (PDGFR-β), fibroblast specific protein (FSP-1), and fibroblast activation protein (FAP) [11–14]. Nevertheless, no one marker specifically labels all CAFs or clearly distinguishes CAFs from normal fibroblasts or other closely-related cell types. These other cell types include pericytes (cells that line blood vessels, also known as mural cells), smooth muscle cells, epithelial cells that have undergone an epithelial-to-mesenchymal transition (EMT), myoepithelial cells (specifically in the breast), and some adipocytes (Fig. 1). Most often, in order to generally classify these various cell types, a combination of markers must be used. For example, αSMA-positive CAFs can be distinguished from pericytes, which stain positively for neuron glial antigen 2 (NG2) and regulator of G-protein signaling 5 (RGS5). RGS5 has been shown to be overexpressed in abnormal tumor vasculature and colocalizes predominantly with PECAM-1/CD31 and less so with PDGFR-α and αSMA [15]. Although some carcinoma cells express FSP-1, FSP-1-positive fibroblast sub-populations present in the tumor microenvironment have been shown to facilitate malignant progression. For example, in a syngeneic mouse model of melanoma, PDGF-CC signaling recruited fibroblasts with differential expression of FSP-1, PDGFR-α and αSMA [11]. Additionally, vimentin is expressed in most mesenchymal cell types as well as epithelial cells that have undergone an epithelial-to-mesenchymal transition (EMT). Due to the apparent heterogeneity of fibroblasts and their diverse origins, it has therefore been difficult to distinguish true fibroblasts from fibroblast-like cells. Moreover, identifying markers to label fibroblast sub-populations that exclusively contribute to cancer progression in various organs has presented challenges (Fig. 1).

Molecular profiling studies have also revealed the heterogeneity of fibroblast and CAF populations, yet have also suggested that core signatures, at least among sup-populations of fibroblasts, might predict tumor-supportive function. For example, gene expression analysis of fibroblasts isolated from breast cancer patient tumors yielded subtype-specific molecular signatures, especially with respect to expression of genes encoding cytoskeletal and integrin signaling proteins [16]. On the other hand, a study in which fibroblasts were isolated from ten different anatomical regions and exposed to serum (mimicking a wound response), revealed a common transcriptional signature, termed the fibroblast core serum response (CSR), that was also identified in CAFs isolated from various carcinomas and predicted metastatic progression in patients with breast, lung, and gastric cancers [16]. Similarly, differences in tumor-promoting ability were found between normal tissue fibroblasts and CAFs when examined for their prostaglandin (PGE2) secretory phenotype, which is elevated in tumors [17]. Two recent studies defined very similar CAF expression profiles that represented pro-inflammatory signatures also found in CAFs derived from cancer patients. In one study using a K14-HPV16 mouse model of multistep squamous skin carcinogenesis, this signature included: Cox2, IL-1β, OPN, IL-6, CXCL1/2 [18]. In the other study using a xenograft model of breast cancer progression, enhanced expression of many of these same proteins were found in CAFs relative to normal mammary fibroblasts [19]. Importantly, this second study also identified the molecular modulator that caused fibroblasts to adopt this pro-tumorigenic CAF signature — the secreted growth factor, granulin (GRN) [19]. Hence, common biological responses of fibroblasts to their microenvironmental cues (e.g., serum exposure) might reveal how fibroblasts acquire their CAF phenotypes. However, these responses seem restricted to different subpopulations of fibroblasts. Given this diversity of biological functions and their obvious heterogeneity, markers and methods to identify different CAF populations for therapeutic purposes, while challenging, would seem of utmost importance.

3. Fibroblasts in cancer pathophysiology

It has long been thought that fibroblast behavior is dictated by the epithelium, but recently more attention has been paid to the possibility that fibroblasts actively drive tumorigenesis and cancer progression [8–11,20,21]. There is now evidence to suggest that fibroblasts play important roles during the entire course of tumor development, from the pre-neoplastic state until the terminal stage of cancer progression — metastasis.

3.1. Cancer initiation — do fibroblasts direct tumorigenesis?

Tumor initiation is typically conceptualized as the accumulation of genetic and epigenetic mutations in the epithelium that results in recruitment of a reactive stroma. While the role of fibroblasts in de novo transformation or induction of carcinoma from epithelium lacking oncogenic mutation is currently debated, some studies have shown that fibroblasts facilitate carcinoma formation from epithelium that is cancer-prone.

Studies of prostate cancer have demonstrated that isolated CAFs, but not normal fibroblasts, can induce the transformation of immortalized epithelial cells [20,22]. Transgenic mouse models have provided some insights into CAF-derived factors that are responsible for tumor initiation. For example, Wnt1 overexpression in fibroblasts transforms mammary epithelial cells from C57BL/6 mice [23]. Additionally, overexpression of HGF and/or TGFβ in fibroblasts was demonstrated to be sufficient for inducing ductal carcinoma in situ (DCIS), adenocarcinoma, and poorly differentiated tumors in the breast [24]. Knockout models and depletion experiments have also demonstrated the importance of fibroblast activation in tumorigenesis. One study using FSP-1-deficient mice showed reduced tumor growth and attenuated metastatic potential of an otherwise highly metastatic murine mammary carcinoma cell-line, whereas injection of wild type fibroblasts partially rescued this effect [25]. Furthermore, knockout of TGFβRII in FSP-1-positive cells promoted prostate neoplasia and forestomach squamous cell carcinoma [10].

A recent study using mice containing conditional alleles of *Pten* and an *Fsp-cre* transgene, showed that inactivation of PTEN specifically in mammary fibroblasts significantly increased the incidence and rate of progression to adenocarcinoma of MMTV-ErbB2/Neu-driven tumors [21]. Upon examination of the pre-neoplastic mammary glands of the mice in this study, significant increases in ECM

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