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Review

Molecular biology of cancer-associated fibroblasts: Can these cells be targeted in anti-cancer therapy?

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ABSTRACT

It is increasingly recognized that the non-neoplastic stromal compartment in most solid cancers plays an active role in tumor proliferation, invasion and metastasis. Cancer-associated fibroblasts (CAFs) are one of the most abundant cell types in the tumor stroma, and these cells are pro-tumorigenic. Evidence that CAFs are epigenetically and possibly also genetically distinct from normal fibroblasts is beginning to define these cells as potential targets of anti-cancer therapy. Here, we review the cell-of-origin and molecular biology of CAFs, arguing that such knowledge provides a rational basis for designing therapeutic strategies to coordinately and synergistically target both the stromal and malignant epithelial component of human cancers.

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1. Introduction

The original concept of cancer as a non-healing wound has been dramatically extended by recent research on the cancer stroma. Although the mesenchymal proliferation that surrounds carcinoma cells shares some features with the proliferative fibroblastic reactions seen in benign ulcers and wounds, accumulating evidence suggests that cancer-associated fibroblasts (CAFs) are a special cell type that actively contributes to tumor growth and malignant behavior. A fast moving and sometimes controversial area of investigation concerns the origins of CAFs and the genetic and epigenetic changes that may account for the tumor-promoting phenotype of these cells. Here we argue that research on the molecular biology of CAFs is beginning to suggest approaches for targeting these pro-tumorigenic stromal cells in anti-cancer therapy.

2. The phenotype of CAFs

In addition to their plump spindle-shaped mesenchymal appearance, several specific molecular markers have been used to define CAFs in tissue sections. The terms 'peri-tumoral fibroblast', 'cancer-associated fibroblast' and 'cancer-associated myofibroblast' are often used interchangeably [1]. The presence of mesenchymal markers (alpha-smooth muscle actin—ASMA, vimentin, paladin 4Ig, podoplanin) and absence of epithelial (cytokeratin), endothelial (CD31) and fully differentiated smooth muscle (smoothelin) markers have been used to define myofibroblasts

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(both tumor-associated and non-tumor associated), but there may be a population of morphologically similar cells in the peri-tumoral microenvironment that does not express all these markers yet shares certain functional and lineage traits with CAFs [2,3]. In addition none of these markers by themselves are unique to CAFs. In this review we use the term CAFs and include under this term cells that are found in the tumor stroma, are morphologically fibroblast-like, express ASMA, and are negative for epithelial markers such as cytokeratin and E-cadherin.

3. Origin and function of myofibroblasts in tissue repair and cancer

In wound healing, the differentiation of local precursor cells into myofibroblasts results in a new phenotype with enhanced contractile and secretory abilities, that allows mobility of these cells for wound contraction (hence the importance of ASMA expression) and synthesis and deposition of extracellular matrix (ECM) [3]. Among the important signals driving this transition are local cytokines and inflammatory mediators [4]. While it is believed that myofibroblastic transdifferentiation of resident fibroblasts is numerically most important at many tissue sites of injury, there is also evidence that a smaller sub-population of myofibroblasts is recruited from the bone marrow. Although engraftment of bone-marrow derived cells (BMDCs) was seen in tissue stroma even in the absence of injury [5], it has been shown that the percentage of BMDCs among ASMA-positive cells increases after injury. It appears that there may be tissue specific differences in the degree to which BMDCs contribute to the stroma in wound healing. In one early study, which examined this question in the mouse small intestine and colon by a protocol of male bone marrow transplantation in females followed by Y-chromosome in the recipients, 40–60% of pericryptal ASMA-positive cells after radiation injury were found to be bone-marrow derived [6]. The numbers have been somewhat lower, though still significant, in injury paradigms with transplantation of tagged bone marrow cells followed by examination of the lung, kidney and pancreas [5]. Although in certain tissues (stomach) BMDCs have been shown to contribute significantly to both epithelial [7] and stromal cells after injury, in other tissue (pancreas), the majority of BMDCs were seen only in the reactive stroma [8].

In terms of cell numbers, data from mouse models of cancer suggest that in some situations, such as gastric cancer, bone-marrow derived mesenchymal cells may contribute as much as 15–25% to the CAF population [9,10]. Bone-marrow derived stem cells are divided into hematopoetic stem cells and mesenchymal stem cells (MSCs). The latter group makes a contribution, both in health and disease, to connective tissue like bone, cartilage, muscle and adipose tissue [11]. After early development it appears to be chronic inflammation and tissue remodeling that drives these cells to target tissues. Thus, the recruitment of BMDCs to cancer or precancerous tissue is another and perhaps newest evidence of the similarity between benign and malignant wound healing [12,13]. Similar to the ability of cancer cells to transform resident fibroblasts or myofibroblasts to tumor-promoting CAFs, it has been shown that cancer-derived soluble factors (conditioned media or malignant ascites) and direct co-culture with carcinoma cells can transform mesenchymal stem cells to a CAF phenotype [14,15].

From experiments in transgenic mouse models it also appears that some CAFs can arise locally from an endothelial–mesenchymal transformation at the invasive edge of the cancer [16]. A related possibility is that at least a sub-population of these cells is derived from epithelial cells via epithelial–mesenchymal transition (EMT). There is evidence that *in vitro* epithelial cells can transdifferentiate into myofibroblasts [17] and that in fibrosing diseases of the lung and kidney several cells with myofibroblast markers also share epithelial markers. In a TGF-β1 induced model of lung fibrosis using β-gal tagged epithelial cells it was concluded that the majority of myofibroblasts were epithelial derived [18]. Nonetheless, this field of research remains incomplete. So far, it has been more exciting to probe the contributions of non-resident cells, but it will be interesting to see future experiments directly evaluating the more mundane contribution of tissue-resident fibroblasts to populations of CAFs in various tumor models.

PDGF (platelet derived growth factor) and TNF-α are examples of cytokines that induce proliferation of resident fibroblasts, but they are not sufficient to induce expression of ASMA. TGF-β1 has been shown to induce differentiation to myofibroblasts [19,20] but it appears that this process is dependent on the expression ECM proteins, including ED-A fibronectin [21]. In addition, expression of other proteins involved in cell adhesion (vinculin, paxillin, tensin) is increased [2]. TGF-β1 controls both fibronectin production and signalling, as it also induces the focal adhesion-associated kinase FAK, which is important for integrin signalling. Inhibition of FAK signalling inhibited the ability of TGF-β1 to mediate ASMA production and myofibroblastic differentiation [22]. Secretion of ECM proteins and matrix metalloproteases (MMPs) also increases, leading to restructuring of the ECM. The source of TGF-β1 in tumors may not only be the epithelial cells but also macrophages and other cells in the tumor microenvironment. Another important regulator of transdifferentiation is endothelin, which is produced by the tumor neo-vasculature [23].

The term CAF in the broadest sense refers simply to myofibroblasts that are found physically associated with carcinoma cells, but this term is also appropriately used with the more specific functional connotation of "tumor promoting cancer-derived myofibroblasts" [24,25]. In addition to the circumstantial evidence that these cells constitute a substantial volume of the tumor mass (in certain epithelial tumors such as pancreatic, gastric and breast cancers as much as 50–70% [1]) there are now multiple lines of functional evidence supporting an active role for CAFs in tumor formation. In particular, experiments in multiple laboratories have shown that: (i) CAFs are synergistic with epithelial cell and accelerate carcinogenesis in xenograft models; and (ii) CAFs can influence the invasiveness and metastatic pattern of tumors. The evidence for synergies between CAFs and malignant epithelial cells in tumor initiation, proliferation and invasion has been reported in cell mixing studies with tumor xenografting, and in other *in vivo* studies. One of the earliest observations was that polyoma virus induced transformation of epithelial cells into malignant neoplastic cells (that is, carcinoma cells) occurred only when these cells were co-cultured with induced mesenchymal cells, but not when they were cultured alone [26]. In the prostate, CAFs from human tumors were able to induce transformation of non-tumorigenic prostatic epithelial cell lines (SV40T-expressing, immortalized but non-tumorigenic BPH-1 cell lines) while normal prostate fibroblasts were not. While non-tumorigenic fibroblasts may play a role in enhancing proliferation of malignant and pre-malignant epithelial cells, they are not able to initiate the malignant transformation of epithelial cells de novo [27]. Furthermore, co-culturing CAFs with induced epithelial cells resulted in a tumorigenic epithelial cell phenotype that persisted even in the absence of CAFs [24]. In addition to irradiation of stromal fibroblasts, which has been shown to enhance the tumor promoting ability of CAFs in some cancers [28], senescence and an inflammatory microenvironment may also contribute to this functional CAF phenotype [29,30].

Transformation or expansion of local mesenchymal cells occurs under the influence of cytokines and as noted above several other precursors of CAFs have been demonstrated or postulated, including smooth muscle cells, endothelial cells, fibroblasts, stellate cells (in the liver) or adipocytes via transdifferentiation, and even epithelial cells via EMT. In the gastrointestinal tract myofibroblasts are already present in low but easily detectable numbers in