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Review

Carcinoma-associated fibroblasts provide operational flexibility in metastasis

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ABSTRACT

Malignant cancer cells do not act as lone wolves to achieve metastasis, as they exist within a complex ecosystem consisting of an extracellular matrix scaffold populated by carcinoma-associated fibroblasts (CAFs), endothelial cells and immune cells. We recognize local (primary tumor) and distant ecosystems (metastasis). CAFs, also termed myofibroblasts, may have other functions in the primary tumor versus the metastasis. Cellular origin and tumor heterogeneity lead to the expression of specific markers. The molecular characteristics of a CAF remain in evolution since CAFs show operational flexibility. CAFs respond dynamically to a cancer cell's fluctuating demands by shifting profitable signals necessary in metastasis. Local, tissue-resident fibroblasts and mesenchymal stem cells (MSCs) coming from reservoir sites such as bone marrow and adipose tissue are the main progenitor cells of CAFs. CAFs may induce awakening from metastatic dormancy, a major cause of cancer-specific death. Cancer management protocols influence CAF precursor recruitment and CAF activation. Since CAF signatures represent early changes in metastasis, including formation of pre-metastatic niches, we discuss whether liquid biopsies, including exosomes, may detect and monitor CAF reactions allowing optimized prognosis of cancer patients.

1. Introduction

Malignant tumors consist of cancer cells and tumor-associated host cells. Spatial heterogeneity of cancer cells has long been recognized by pathologists and forms the basis of the Gleason score tumor grading prognostic classification system for prostate cancer. This morphological intratumoral heterogeneity of cancer cells has been confirmed at the genetic level using deep sequencing technologies [1–3]. It has long been recognized that genomic instability is a hallmark of cancer cells [4]. Campbell et al. [2] detected specific chromosomal fold-back inversions, which are duplicated chromosomal DNA regions following a breakage, in both the primary and metastatic lesion suggesting that fold-back inversions occur early in tumorigenesis and probably drive metastasis. However, cancer cells do not act as lone wolves, as they exist within a complex ecosystem consisting of an extracellular matrix (ECM) scaffold populated by stromal cells including fibroblast-like cells, endothelial cells, and immune cells, morphologically defined by desmoplasia, angiogenesis, and inflammation and immune response respectively. There is increasing histopathological and genetic evidence to suggest that tumor-associated stroma proportions or signatures may refine the prognostic assessment of tumors [5–7]. Stromal components, such as endothelial and immune cells are considered as emerging hallmarks indispensable for metastasis [4]. The specific role of the apparently innocuous-appearing desmoplasia (from the Greek word desmos, *fettering or restraining*; and plasis, *formation*) has received relatively little attention, primarily because of a lack of vital function being attributed to this accumulation of fibroblast-like cells and ECM proteins. This is particularly bemusing, since hard cancers amongst soft organs have been a critical diagnostic feature taught to all medical students [8]. Fibroblast-like cells contributing to desmoplasia often express α-smooth muscle actin (α-SMA), an important marker for myofibroblasts. Indeed, α-SMA expression in tumor-associated stroma is an independent prognostic marker in multiple cancer types including colon, pancreas, and breast [9–11]. In addition, gene expression signatures of tumor-associated fibroblast-like cells not only hold prognostic information [12] but also predict resistance to neo-adjuvant chemotherapy in breast cancer [13]. Clinical correlation data combined with experimental modeling suggests that tumor-associated fibroblast-like cells are not idle bystanders but rather corruptive drivers of metastatic progression. To avoid misunderstanding we shall use the term carcinoma-associated fibroblast (CAF) in this review which may refer to peritumoral fibroblasts, myofibroblasts, tumor-associated mesenchymal cells, tumor-associated fibroblast-like cells, mesenchymal stromal cells and other definitions described in literature.

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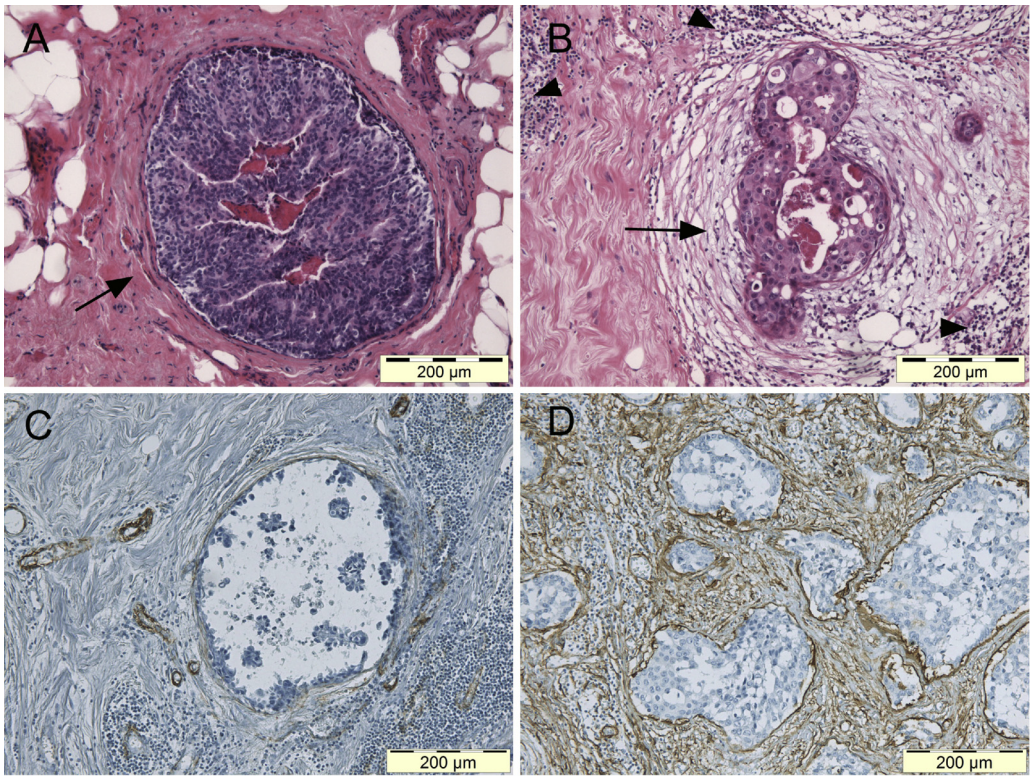

Fig. 1. Photomicrographs showing hematoxylin and eosin staining (A and B) and immunohistochemical staining of α-SMA (C and D) in DCIS lesions from the breast. Periductal sclerotic (S) stroma (A). Myxoid (M) stroma surrounding the affected ducts, areas with inflammatory cells are indicated by arrowhead (B). Periductal stroma with minor α-SMA expression, presumably pericytes of the blood vessels (C). Periductal stroma strongly positive for α-SMA expression (D).

Since previous reviews on the subject [14–18] many new findings have emerged to advance our understanding of the impact of CAFs on metastasis, but many questions remain unanswered and are important topics for further research. Key issues discussed in this review include: (i) Is there a molecular heterogeneity of CAFs in the primary tumor and at metastatic sites? (ii) What is the origin of corrupted CAFs? (iii) What are the instructive signals for CAF corruption? (iv) How do CAFs provide operational flexibility during metastasis? (v) Are CAFs implicated in metastatic dormancy? (vi) Do CAFs react to cancer management protocols? (Vii) Do liquid biopsies allow early detection of CAF?

2. Molecular heterogeneity of CAF

2.1. Within the primary tumor

Cancer cells within the primary tumor do not exist independently but interact dynamically with local and distant host cells, best conceptualized as a gradually evolving phenomenon termed microenvironment or ecosystem. Consequently, intratumoral heterogeneity of cancer cells will lead to heterogeneous local host reactions (Fig. 1). Indeed, some ductal carcinoma in situ (DCIS) lesions of the breast, which are considered to be a non-invasive precursor of invasive ductal carcinoma, have a mixed stromal architecture classified as either predominantly sclerotic or myxoid. Periductal myxoid stromal architecture is significantly associated with increased ipsilateral locoregional recurrence. Intriguingly, α-SMA expression is differentially expressed in periductal DCIS stroma but is not associated with the presence of myxoid stroma (unpublished data). In addition some DCIS regions contain an inflammatory component, others not. Even within one inflammatory component different subtypes of macrophages are described [19,20]. Macrophages are differentiated cells of the mononuclear phagocytic lineage and acquire a specific phenotype in response to signals present within individual microenvironments. The exact combination of such specific signals dictates both the differentiation and activation status of these cells. The diversity of macrophage subtypes raises the possibility that CAFs equally fall into different functional and molecular subtypes. CAFs closely resemble myofibroblasts, which are implicated in fibrosis and wound healing [16]. The most widely used molecular marker of CAFs in research and clinical diagnostics is the expression of α-SMA. The convenience of a unique molecular marker has fostered the misconception that a CAF must express α-SMA to be a CAF. However, the most important defining feature of CAFs compared to their precursors is their capacity to stimulate invasion and metastasis. In agreement, other common functional differences of CAFs compared to their cell of origin is an increased capacity to form colonies in soft agar [21], contract native type I collagen gels [22] and to acquire a nemosis response i.e. clustering of cells in culture leading to increased scatter factor/hepatocyte growth factor (SF/HGF) secretion [23]. Most importantly, we may consider CAFs, present at the primary tumor, as a heterogeneous population with different molecular identities that collaboratively stimulate metastasis (Fig. 2). Indeed, immunohistochemistry of prostate cancer CAFs showed mixed intensity of phosphorylated SMAD2, suggesting a heterogeneous transforming growth factor (TGF)-β responsiveness [24]. A computational model showed a 2-step mechanism in which proliferation and invasion of prostate cancer cells occur by distinct stromally derived paracrine signals produced by either TGF-β nonresponsive and responsive CAFs. In vitro cultures show that TGF-β nonresponsive CAFs secrete Wnt-3a which may cause epithelial hyperproliferation; the TGF-β responsive CAFs secrete CXCL12 (or stromal derived factor-1, SDF1) which support the invasion of proliferative epithelial cells through matrigel, a basement membrane mimic. In oral squamous cell carcinoma (OSCC)