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Review

Complexity in the tumour microenvironment: Cancer associated fibroblast gene expression patterns identify both common and unique features of tumour-stroma crosstalk across cancer types

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ABSTRACT

Cancer is a complex disease, driven by the accumulation of several somatic aberrations but fostered by a two-way interaction between tumour cells and the surrounding microenvironment. Cancer associated fibroblasts (CAFs) represent one of the major players in tumour-stroma crosstalk. Recent *in vitro* and *in vivo* studies, often conducted by employing high throughput approaches, have started unravelling the key pathways involved in their functional effects. This review focus on open challenges in the study of CAF properties and function, highlighting at the same time the existence of common mechanisms as well as peculiarities in different cancer types (breast, prostate and lung cancer). Although still limited by current experimental models, which are unable to deal with the full level of complexity of the tumour microenvironment, a better understanding of these mechanisms may enable the identification of new biomarkers and therapeutic targets, to improve current strategies for cancer diagnosis and treatment.

1. Introduction

Tumorigenesis has been classically considered as a cell autonomous mechanism triggered by the accumulation of mutations able to confer a growth advantage to transformed cells and the capability to invade surrounding tissues and eventually metastasize. However, cancer cells are integrated in a complex microenvironment and the importance of stromal cells in contributing to tumour initiation and progression is increasingly recognized [1–5].

Tumour microenvironment (TME) is an ensemble of different cellular and structural factors including vasculature and immune-related cells, fibroblasts and the extracellular matrix (ECM), showing either tumour promoting or anti-tumour activity in an intricate network of signals hard to dissect and to characterize experimentally. However, its relevance in several aspects of tumour progression, including response to pharmacological treatments and its potential role as a direct therapeutic target [6,7] has motivated growing research efforts to investigate the key molecular mechanisms involved in tumour-stroma crosstalk. Such crosstalk is mediated either by cell–cell interactions or soluble factors, but more recently other key players have been identified, as secreted miRNAs, metabolites and exosomes [8–12].

In this review, we mainly focus on one of the major players in the TME, usually referred to as cancer associated fibroblasts (CAFs). Fibroblasts, beside their involvement in development, tissue repair and inflammatory response, have been shown to participate in human tumorigenesis by providing a permissive environment for proliferation and survival of epithelial cells, and by remodelling ECM to promote tumour growth and invasiveness [13–16]. Rising evidence however also informs on phenotypic and functional heterogeneity within the stromal compartment of the TME [17], indicating that, even for a single cell type such as fibroblast, different subpopulations might exist and exert distinct functions [18]. Intriguingly, recent studies support the notion that cells other than normal fibroblasts are a possible source of CAFs, redefining this microenvironment component more like a cell state rather than a cell type. Among them are mesenchymal stem cells, smooth muscle

Abbreviations: CAF, cancer associated fibroblast; NF, normal fibroblasts; AF, adjacent fibroblasts; ECM, extracellular matrix; TME, tumour microenvironment; BC, breast cancer; PC, prostate cancer; LC, lung cancer; EMT, epithelial-mesenchymal transition.

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cells, endothelial cells and cancer cells themselves after undergoing epithelial-mesenchymal transition (EMT) [19,20].

Complexity of interactions within the microenvironment is also exemplified by recent data centred on selective ablation of specific stromal elements in pancreatic cancer. Selective targeting of cells expressing the fibroblast activation protein (FAP) with FAP-targeted chimeric antigen receptor T cells was able to reduce desmoplasia and impair growth of murine models of pancreatic cancer [21]. However, strategies targeting cells expressing another key protein involved in CAF function, α-smooth muscle actin (α-SMA) [22], or reducing sonic hedgehog signalling [23] (both resulting in reduction of stromal elements and fibrosis) resulted in more aggressive tumours. These novel findings further strengthen the notion that a number of different aspects should be evaluated when investigating tumour-stroma crosstalk.

The role of CAFs in tumour progression, the regulation of response to therapies and the prognostic relevance of markers associated with their activity in different tumours have been recently reviewed [17,24–26]. This review will largely concentrate on the evaluation of the complexity of the interaction between CAFs and cancer cells across different cancer types to highlight similarities and specificities of this crucial tumour promoting crosstalk.

Box 1: Challenges in defining and studying cancer associated fibroblasts and their interplay with tumour cells

- **Multiple players in the TME:** An increasing bulk of literature demonstrates the relevance of tumour-CAF crosstalk, however many other cell types (e.g. adaptive and innate immune cells or endothelial cells) populate primary or metastatic sites contributing to a complex network of heterotypic interactions resulting in a global tumour-promoting or inhibiting effect. Such a complexity is still hard to reproduce or dissect with current available models and techniques.
- **Limits of available models:** Although studies on co-culture models are generating significant advances in the understanding of the biology of cancer, thus providing a controllable technique to study tumour-stroma interactions, models with increasing complexity will be needed to improve our knowledge on heterotypic interactions and clarify the role of other important factors such as ECM stiffness and mechanical forces and the relevance of the 3D architecture of the tissue [127].
- **Identification of CAFs:** Detecting fibroblasts having a so-called 'activated' phenotype able to promote cancer progression is still a challenge. Although several markers have been proposed and some of them largely used across different cancer types (e.g. α-SMA and FAP), none of them seems to be specific enough and further research is needed to identify new markers or the best combination of them [19].
- **Quantity vs quality:** Gene expression profiling has been extensively used to identify or validate CAF-derived signatures. However it is challenging to distinguish variations in expression levels caused by the presence of specific cell phenotypes from variations ascribable to variability of tumour sampling and therefore mainly reflecting a higher or lower amount of stroma. Unfortunately, although potentially useful datasets are nowadays available, only a minority has information on tumour cellularity, which might help during data analysis and interpretation.

2. The CAF-tumour interplay in different cancers types

In several different solid cancers, CAFs have been shown to often display properties associated with the activated myofibroblasts found at sites of wound healing [14,27] Accurate definition of markers of CAF activation and functional dissection of the molecular bases of their pro-tumorigenic properties are however still hampered by several experimental challenges (Box 1). Nonetheless, what we have learned so far is detailed in the following paragraphs, principally for breast, prostate and lung cancer. For these tumour types, an extensive characterization of the CAF component exists in the literature and they were also the most studied in our laboratories, with several in-house datasets available (see Section 3). Furthermore, they could be seen as representative of different major etiological factors (or drivers) in their predominant histologies: i.e. heavy exposure to environmental carcinogens (lung), hormone dependency (breast and prostate) and possibly organ senescence (prostate). Investigation of stromal components in these three entities may therefore offer the opportunity to investigate common versus unique features in different tumour types.

2.1. Breast cancer

In the mammary gland, fibroblasts are one of the most important components of the connective tissue that contribute to structural integrity. Similarly to the wound healing process, with the onset of breast cancer (BC), fibroblasts adjacent to tumour cells (the so called CAFs) become activated and acquire myofibroblast features. CAFs have indeed a drastically different phenotype with respect to their normal counterpart, in terms of morphology, immunophenotype, proliferation rate, released cytokines, deposition of ECM proteins and gene expression profiles [28–30]. Such acquired phenotype makes them able to sustain tumour progression and affect response to treatment [14,31].

BC CAFs represent a very heterogeneous cell population, and different hypotheses have been formulated to explain their origin [32–34], with no evidence on a main dominant derivation.

One of the open challenges is the identification of reliable markers able to identify the CAF population. In addition to α-SMA [35], the characteristic fingerprint of myofibroblasts, several CAF markers have been proposed, such as Platelet-Derived Growth Factor Receptor-β (PDGFR-β) and FAP, a type II integral membrane protein with peptidase, gelatinase and collagenase activity, highly expressed in reactive stroma of solid tumour and wounded tissue [36,37]. However, the combination of different markers seems to be advantageous compared to any single marker for their identification. High expression of PDGFR-β was associated with shorter recurrence and survival in a cohort of BC patients [38]. On the contrary, FAP was significantly associated with longer disease-free and overall survival [39].

In keeping with the prominent changes affecting the ECM during carcinogenesis, other proposed CAF markers are ECM-related molecules as Tenascin-C (TNC) and different matrix metalloproteinases (MMPs), the latter responsible for the degradation of the basement membrane during tumour invasion. Several other CAF markers have been proposed, such as neural/glial antigen 2 (NG2), podoplanin (PDPN) and fibroblast specific protein (FSP), but their specificity for CAFs is questionable. Such a lack of consensus might be a consequence of intrinsic phenotype heterogeneity of CAFs.

The mechanisms of fibroblasts activation in BC are still elusive. The major tumour cell-derived factors that have been reported to activate stromal fibroblasts are TGFβ and CXCL12/SDF1. Kojima et al. showed an elegant mechanism by which TGFβ, released by carcinoma cells [40], enhances endogenous TGFβ and SDF-1 production via TβR-Smad signalling and induces *CXCR4* expression in stromal fibroblasts, generating two autocrine signalling loops which cross-communicate to maintain the myofibroblastic phenotype [32]. Other pro-fibrotic factors released by breast tumour cells, such as PDGF-α/β [41], or IL6 [42] can also act on resident fibroblasts and induce their activation.

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