Journal of Bone Oncology 5 (2016) 128–131

Contents lists available at ScienceDirect

Journal of Bone Oncology

journal homepage: www.elsevier.com/locate/jbo

Research Paper

Cancer-associated fibroblasts – Not-so-innocent bystanders in metastasis to bone?

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ARTICLE INFO

Article history:
Received 11 January 2016
Received in revised form
12 May 2016
Accepted 20 May 2016
Available online 16 May 2016

1. Introduction

Metastatic dissemination of cancer cells to local and distant sites such as bone requires a complex interaction of cancer cells with their surrounding microenvironment to allow invasion, immune evasion and spread via the vascular or lymphatic systems. The tumour microenvironment is composed of an array of cells embedded in a complex extracellular matrix (ECM) (Fig. 1). The predominant cell type of the tumour microenvironment, the fibroblast, becomes corrupted by cues derived from malignant cells and other cells present in the tumour stroma to become cancer-associated fibroblasts (CAF). The presence of CAF correlates with poor disease outcome in a number of tumours, leading to the suggestion that they may present a viable novel therapeutic target. In this review we summarise the roles of CAF in the metastatic cascade and focus on the emerging understanding of their contribution to dissemination and growth of tumours in bone.

2. Cancer associated fibroblasts – bad neighbours in the tumour microenvironment

Malignant tumours grow and spread by corrupting the surrounding stroma, composed of cells such as fibroblasts, endothelial cells, and immune cells (Fig. 1), to encourage cancer cells to proliferate, evade the host's immune system, and metastasise. There exists considerable evidence highlighting the prognostic importance of various components of the modified cancer microenvironment, including cancer-associated fibroblasts (CAF) [1–3]. CAF are the most abundant cell type in the tumour microenvironment but reliably identifying them remains challenging due to their heterogeneity [4]. CAF express mesenchyme specific markers such as fibroblast activating protein (FAP), fibroblast specific protein 1 (FSP1/S100A4), vimentin, platelet derived growth factor receptors, podoplanin and the most commonly used marker, alpha smooth muscle actin (α-SMA) [5], but many of the markers used to identify them are expressed by other cell types; for example podoplanin is found in lymphatic vessels, as well as some cancer cells or, platelet-derived growth factor receptor β (PDGFRβ) is expressed by pericytes . Some of the heterogeneity observed in CAF populations may result from subpopulations arising from different origins; resident fibroblasts, mesenchymal stem cells, fibrocytes, stellate cells (pancreas, liver), Kuppfer cells (liver), endothelial cells, smooth muscle cells, myoepithelial cells (breast), pericryptal myofibroblasts (gastrointestinal tract) have all been demonstrated to give rise to CAF [6,7]. It is notable, however, that the heterogeneity of CAF is generally accepted to arise not from genomic changes (ie alterations in DNA sequence) but rather from epigenetic and other modifications of gene expression. This is significant as the genomic stability of CAF make them less likely to acquire resistance to therapy frequently encountered when pharmacologically targeting genomically unstable cancer cells, and therefore an attractive target for intervention [4].

The presence of an inflammatory infiltrate is associated with poor prognosis in a number of malignancies, including those with a predilection for metastasis to bone. CAFs are capable of promoting, and maintaining an inflammatory environment by recruiting immune cells; in particular monocyte/macrophage through secretions of cytokines and chemokines. Recruited macrophages are known to polarise towards mostly the tumour promoting M2 type which encourage angiogenesis, immune suppression and metastasis. Evidence demonstrates that CAFs recruit monocytes mostly through CCL2-CCR2 in breast cancer and melanoma, and $CD68^{+}$ macrophages through CXCL14 in prostate

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http://dx.doi.org/10.1016/j.jbo.2016.03.008

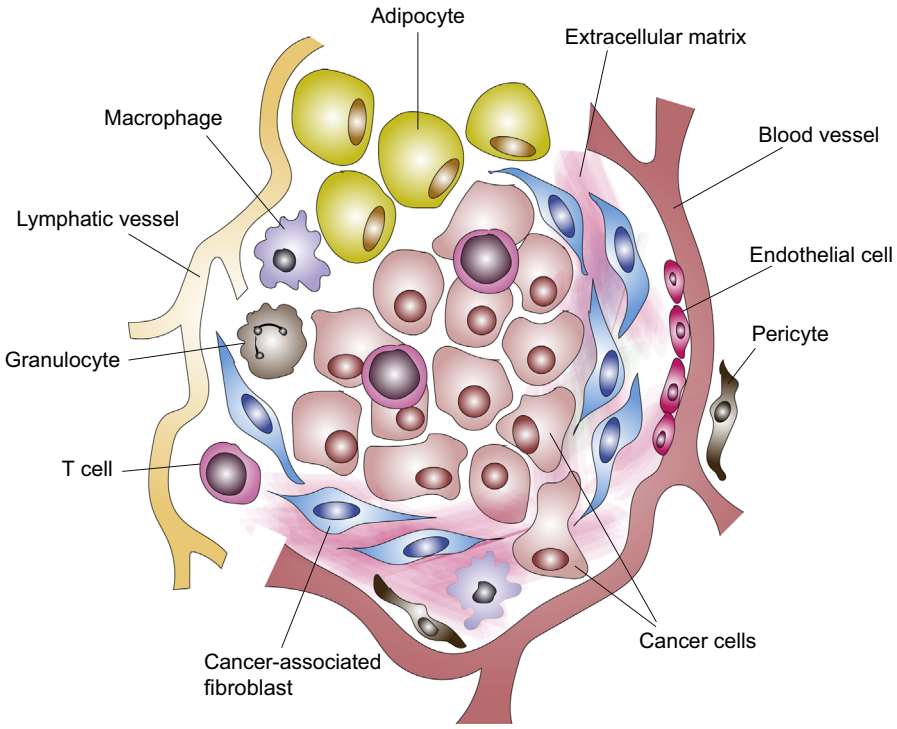

Fig. 1. Cancer associated fibroblasts in the tumour microenvironment. Schematic depicting the major components of the tumour microenvironment, including cancer cells (pink), cancer associated fibroblasts (blue), adipocytes (yellow), granulocytes (grey), pericytes (brown), macrophages (purple) and T-cells (dark pink).

cancer [8]. IL-6 secreted by CAFs encourages differentiation of $CD14^{+}CD1a^{-}$ monocytes to macrophages . CAFs also recruit mast cells through secreting IL-6, and T lymphocytes via CXCL9, CXCL10, and CXCL12 which promote angiogenesis and modulate immune responses to tumour cells .

One of the major roles of normal fibroblast is to secrete components of extracellular matrix (ECM) and this property is retained in CAFs, however the ECM secreted is altered to support tumour growth and invasion. Many tumours are surrounded by a desmoplastic stroma; that is, one rich in collagen, fibronectin, and other ECM components, which has been associated with poor prognosis [9]. Early breast tumours show ECM stiffening due to cross linking of collagen catalysed by lysyl oxidase (LOX) expressed by CAFs, and stiffer ECM is known to promote aggressive growth in hypoxic cancer cells [9]. CAFs have an enhanced expression of fibronectin which has demonstrated a role in cell adhesion and increased metastatic potential in Lewis lung cancer, ovarian cancer, and melanoma [10]. Their role in maintaining ECM homeostasis makes fibroblasts an important component of mechanisms regulating tissue mechanics. Changes in ECM stiffness are frequently observed in the tumour microenvironment and are known to influence tumour cell and fibroblast phenotype (reviewed in Bonnans et al. [11]). Wong and colleagues demonstrated activation of focal adhesion kinase (FAK) in fibroblasts by mechanical forces leading to secretion of CCL2, consequently recruiting macrophages [12]. Evidence also exists supporting knockdown of FAK in CAFs in oral squamous cell carcinoma inhibited metastasis by minimizing CCL2 secretion [13]. The immune cells recruited as a result secrete cytokines and MMPs and may further modulate the ECM to promote metastasis.

CAF in primary tumours also secrete a variety of factors that can directly influence the behavior of cancer cells and encourage distant metastasis. For example, stromal derived factor 1 (SDF-1/CXCL12), which interacts with cancer cells through CXCR4 inducing tumour growth and metastasis (including to bone) [14]. Other factors released by CAF, such as epidermal growth factor receptor (EGFR) ligands also promote tumour growth and metastasis by interacting directly with cancer cells [15]. Extracellular vesicles (EV, frequently erroneously referred to generically as exosomes) are also reported to play a role in the paracrine signaling between CAF and cancer cells [16], possibly through the delivery of microRNA which influence specific gene expression profiles in recipient cells [17].

3. The malignant stroma – conspirator in bone metastasis?

Bone metastasis is a common and often devastating feature of several cancers; some subtypes of breast cancer and prostate cancer in particular show a predilection for dissemination to bone [18]. Once present in the bone microenvironment, malignant cells can stimulate bone destruction or formation leading to pain, fracture, hypercalcaemia, and spinal cord compression. The determinants of bone-tropic metastatic dissemination are poorly understood, but it is becoming evident that the primary tumour microenvironment may play a key role. CAF in particular are known to secrete elevated levels of several cytokines and growth factors found in the bone marrow microenvironment, suggesting that the primary stroma may be able to select for cancer cells able to thrive in bone. This hypothesis was given credence by the work of Zhang et al., who provided evidence that CAF in the primary tumour microenvironment of an aggressive and bone-tropic subclass of triple negative (TN; negative for estrogen receptor, progesterone receptor and HER2 amplification) breast cancers secrete factors such as IGF-1 and CXCL12 [19]. These cytokines select for cancer cells with high src activity and PI3K-Akt signaling which subsequently thrive in the bone microenvironment (illustrated in Fig. 2). In prostate cancer, TGFβ–dependent signals such as CXCL1 and CXCL16 derived from stromal CAF have been shown to promote growth of metastatic lesions in bone [20].

In addition to CAF in the primary tumour microenvironment, it is tempting to speculate that fibroblasts within the bone itself may

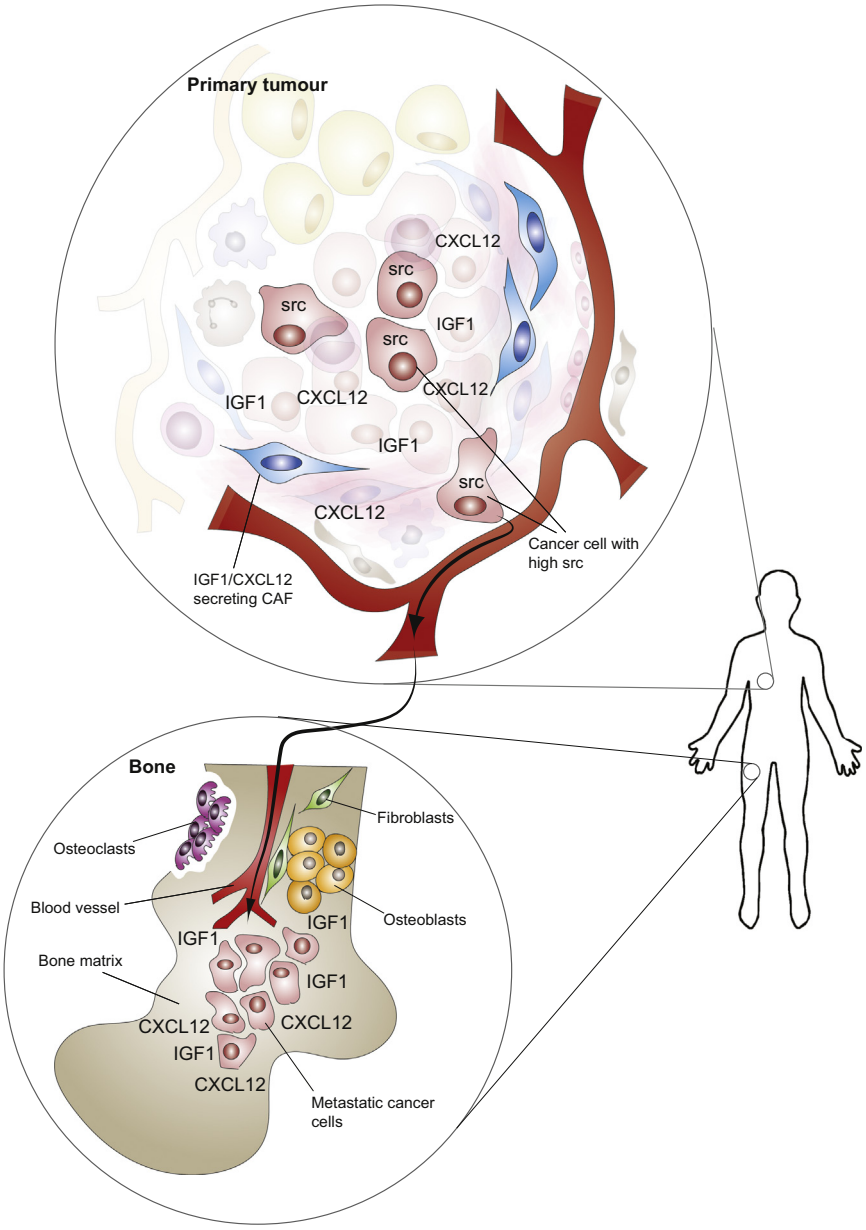

Fig. 2. Cancer associated fibroblasts can select cancer cells primed to thrive in the bone environment. Schematic illustrating the ability of a subset of CAF associated with the primary tumour (blue cells in the stroma) to select a sub-population of cancer cells with high src activity (brighter pink primary cancer cells), secreting a high level of IGF-1 and CXCL12, cytokines also present at high levels in bone. These cancer cells with high src activity are able to metastasise to and thrive in this bone environment [19].

play a role in the establishment of metastatic deposits. Fibroblasts form a major component of the bone marrow stroma and regulate haematopoiesis by secreting a range of cytokines, including those known to be involved in metastatic growth in bone [21]. Bone remodeling provoked by tumour growth (particularly in osteolytic lesions) causes the release of proteins such as TGFβ that may activate fibroblasts to secrete cytokines favouring further tumour growth [4,22].

4. Future perspectives

The tumour microenvironment is emerging as a key contributor to cancer metastasis to distant sites, including bone. Accordingly, the cells of the microenvironment, such as CAF, are increasingly recognized as a potential therapeutic target and prognostic biomarker. There remain, however, a number of barriers to translating this into the clinic. These include the complex heterogeneity of the CAF phenotype and the limited of understanding of the nature of CAF-derived signals contributing to metastasis. Greater understanding of CAF biology of bone-tropic tumours may identify markers which could be used for patient stratification, prognostics and as direct therapeutic targets to reduce metastasis to bone.

Conflicts of interest

The authors declare no conflicts of interest.

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