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Review Article

Bone marrow cells as precursors of the tumor stroma

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ABSTRACT

Cancer is a systemic disease. Local and distant factors conspire to promote or inhibit tumorigenesis. The bone marrow is one important source of tumor promoting cells. These include the important mature and immature hematopoietic cells as well as circulating mesenchymal progenitors. Recruited bone marrow cells influence carcinogenesis at the primary site, within the lymphoreticular system and even presage metastasis through their recruitment to distant organs. In this review we focus on the origins and contribution of cancer-associated fibroblasts in tumorigenesis. Mesenchymal cells present an important opportunity for targeted cancer prevention and therapy.

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Introduction

Cancer is defined by changes within the neoplastic epithelium, however, it is the dynamic stroma that heralds many of the earliest events in carcinogenesis. Stromal cells in cancer arise from all germ layers. The mesoderm provides mesenchymal, hematopoietic and endothelial cells. The ectoderm contributes additional mesenchymal cells as well as cancer-associated nerves via the neural crest. Ultimately, the endoderm provides invading neoplastic cells in malignant solid tumors, many of which adopt

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a mesenchymal phenotype [1–3]. Like the epithelial compartment, the tumor microenvironment has disturbed differentiation. There is an expansion of stromal stem and progenitor cells with distinct functions compared to their more differentiated counterparts. Many stromal cells in cancer emerge from a tissue resident population, but a substantial portion of cancer stroma is recruited from distant sites [1,4,5]. In this review we focus on the organization, recruitment and contribution of mesenchymal cells to solid organ cancer.

Mesenchymal organization in normal tissues

Before considering mesenchymal stromal cells in cancer, it is worth exploring mesenchymal stromal cells in the normal tissues, in which the tumors develop, and the normal bone marrow, which is the quintessential mesenchymal organ. Bone marrow mesenchymal cells are heterogeneous [6,7]. They may be considered in terms of their functional mesenchymal lineages: chondrocyte, adipocyte, osteoblast and marrow stromal cells. But, even within these discrete lineages there is considerable diversity in terms of cellular differentiation that may be related to the specific cellular and extracellular context of these cells (referred to as their specific "niche"). These differences in differentiation affect both the multilineage potential of a given cell as well as its biological function [7,8].

The bone marrow is structurally organized to achieve its two chief responsibilities: hematopoiesis and skeletal integrity. In the bone marrow, an intricate network of small endothelial-lined fenestrated vessels, known as sinusoids, are woven into a lattice of trabecular bone. Niche specific microenvironmental gradients, involving calcium and oxygen tension, exist between these endosteal and perivascular zones to help regulate local hematopoietic stem cells (HSCs) [8]. The key niche cells contributing to the support of HSCs include osteolineage cells, endothelium, macrophages and perisinusoidal stromal cells, along with the sympathetic nerves that innervate them [8–10].

The perivascular bone marrow mesenchymal cells are believed to contain true mesenchymal stem cells (MSCs) [6–9]. Interestingly, perivascular stromal cells are also believed to contain MSC-like cells within extramedullary organs [11,12]. Given the lack of specific fibroblast and MSC markers the functional assays of clonogenicity (number of colony forming fibroblasts per cells plated) and multipotentiality *in vitro* are often used to stratify mesenchymal stromal cells. *In vitro* MSCs (those that exhibit multipotentiality and clonogenicity) may be enriched from the bone marrow through initial depletion of non-stromal populations (CD45 (hematopoietic), CD31 (endothelial), Ter119 (erythrocyte) triple negative) and then positively selected for using specific perivascular or stem cell markers such as, in the mouse, Sca1, CD105 or CD140a [6,7] or in humans CD105, CD73 and CD90 [13]. Even within these enriched populations of stromal cells only 2%–5% of cells prove to be clonogenic [6,7]. This suggests that true MSCs are extremely rare, which is likely, but also perhaps that our attempts to enrich for them and to cultivate them could be significantly improved. Nevertheless, the *Nestin*-GFP transgenic line is expressed in perisinusoidal cells and identifies a population enriched for clonogenic cells with multilineage potential *in vitro* [9]. A separate perisinusoidal transgenic line (Lepr-Cre), however, does not lineage trace osteoblasts during development [10]. These conflicting results suggest that there is heterogeneity even within similar cells in similar parts of the bone marrow. As proof of concept it is possible to transplant mesenchymal progenitors cells and achieve engraftment in the bone marrow and other organs [1,14] and tracing from some transgenic lines suggests the presence of a true MSC [9,15]. But the key issue remains the exact nature of the engrafted or the transgenically recombined cell. Which cells within an often heterogeneous population of transplanted or recombined cells are the true MSCs, which are lineage restricted mesenchymal progenitors and which are only differentiated cells that have lost the capacity for self renewal and multipotentiality *in vivo*? As newer, more specific transgenic markers and methods develop we will ultimately be able to reconcile the evolution, function and specific potential of these cells.

Interestingly many of the fundamental features of mesenchymal organization in the bone are replicated in extra-medullary organs. In the intestine, for instance, the microenvironment also consists of an integrated community of stromal cells with extracellular matrix interspersed by pericyte-covered endothelium [1–3,16]. Whilst extramedullary organs lack the obvious osteoblasts and chondrocytes of the bone marrow, the intestinal myofibroblast sheath that rests just beneath the epithelial basement membrane is proposed to have an important role in intestinal stem cell function and throughout the mucosa perivascular stromal cells may also contribute important niche signals [1,4,5,16,17]. Extramedullary organs may even contain stromal cells with *in vitro* MSC capabilities [6,7,11]. In the gastrointestinal tract MSCs are recognized to be important cells in colonic wound healing, perhaps through the direct regulation of intestinal re-epithelialization [7,8,18,19]. It has been speculated that mesenchymal cells surrounding the intestinal crypts, vessels or within the intestinal serosa are the source of these MSCs [8,20]. Whether they also contribute to ongoing mesenchymal renewal in health or injury, however, is currently unknown. An interesting hypothesis is that stromal mesenchymal cells behave similarly in the tumor microenvironment: vital supportive cells nurturing the cancer stem cell niche [8–10,21].

Mesenchymal organization in cancer

In the tumor microenvironment, many of the mesenchymal subtypes remain the same, albeit that the number, distribution and even their compartment of origin are altered. Attempts to resolve stromal heterogeneity in cancer are complicated by the same lack of specific markers that have limited the classification of normal mesenchyme. Without the capacity to mark, measure and modify specific subsets of fibroblasts using discrete gene expression profiles, we are left with a more descriptive vocabulary. Thus the term cancer-associated fibroblast (CAF) is used for cells purely on the basis of their spindle-shaped morphology and their peritumoral context. Immunohistochemistry has been used to try and resolve CAF heterogeneity as well as to exclude other potential cell types such as inflammatory cells, endothelium, nerves and muscle. Several markers have been reported to help define distinct subpopulations of CAFs. The activated fibroblast markers of αSMA and Fibroblast Activation Protein (FAP) have been used extensively to examine the tumor promotion of mesenchyme in co-injection tumorigenicity studies [6–9,22,23]. Others have shown that certain markers such as FSP1+ label