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Review

Functional subsets of mesenchymal cell types in the tumor microenvironment

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ABSTRACT

In the field of tumor biology, increasing attention is now focused on the complex interactions between various constituent cell types within the tumor microenvironment as being functionally important for the etiology of the disease. The detailed description of tumor-promoting properties of cancer-associated fibroblasts, endothelial cells, pericytes, and immune cells, introduces novel potential drug targets for improved cancer treatments, as well as a rationale for exploring the tumor stroma as a previously unchartered source for prognostic or predictive biomarkers. However, recent work highlights the fact that cellular identity is perhaps too broadly defined and that subdivision of each cell type may reveal functionally distinct subsets of cells. Here, we will review our current understanding of the diversity of different subsets of mesenchymal cells, *i.e.*, cancer-associated fibroblasts and pericytes, residing within the tumor parenchyma.

1. Introduction

Cancer accounted for an estimated 1.75 million deaths in Europe alone and 8.2 million deaths worldwide during 2012 [1]. With the projected increase of the elderly population, the incidence of cancer is expected to rise steadily. Our knowledge base about cancer has exploded ever since the discovery of oncogenes during the 1970's and the recent specification of the traits of a tumor cell is the distillate of several decades of research dedicated to the malignant cell [2]. Despite this dramatic development in information, the death rate of cancer in the population has not decreased accordingly during the same time period. The need for new and effective strategies for cancer therapy is thus imperative.

Cancer is in its essence a disease of miscommunication. The failure of tumor cells to communicate correctly internally is caused by genetic and epigenetic events that lead to excessive cell growth. In addition, and perhaps equally important for the etiology of cancer, malignant cells engage in intercellular communication with various cell types populating their micro- and macroenvironment [3,4]. Thus, a tumor should be considered as a communicating organ in its own right comprising multiple cell types that collectively evolve into a clinically manifested and deadly disease. With this proposition follows that targeting of the web of intercellular communication within tumors in order to attenuate the support from accessory cell types holds promise as a viable strategy to achieve long-term therapeutic benefit.

Studies of the tumor microenvironment continuously alert us to new important functions performed by accessory cell types during malignant conversion. Moreover, increasing evidence points to that cellular identity is more plastic than previously thought. Indeed, subsets of various cell types within tumors can be distinguished by differential marker expression and may hold functional significance. The increasing awareness that we must consider a higher order of cellular organization in tumors, leads to the companion conclusion that we need to study the cellular context of cancer with a higher resolution. With the development of novel methodologies, such as genome-wide transcriptional analysis and proteome-wide description of the cellular distribution of gene products in various tissues, comes the ability to more accurately define cellular subsets. In the context of tumorigenesis, the most striking example is the identification of an exclusive subset of malignant cells harboring tumor-initiating capacity, as opposed to the bulk of tumor cells [5]. In addition, the concept of cellular subtypes also applies to the tumor microenvironment. Endothelial cells engaged in sprouting angiogenesis come in flavors of specialized tip cells, stalk cells and phalanx cells, each with its own specific function and marker distribution [6]. Macrophages infiltrate tumors either as polarized towards an inflammatory (M1) or a tissue remodeling (M2) phenotype; again a subdivision of functional importance since the M2 macrophage is considered a superior tumor promoter [7]. Thus, it is

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likely that in-depth analysis of different cellular compartments in tumors with higher resolution will reveal subsets of additional cell types that hold utility as drug targets and/or biomarkers. Herein, we summarize the state-of-the-art on functional subsets of mesenchymal cells, *i.e.* cancer-associated fibroblasts (CAF) and pericytes (PC), residing in the tumor microenvironment. For the purpose of this article, a subset of CAF or PC is delineated as a group of cells within the widely defined cell type that is distinguished by one particular marker. We acknowledge that using this definition may identify two seemingly discreet cellular subsets that in reality are similar or identical. Moreover, the plasticity and hierarchical relationship between different subsets of mesenchymal cell types residing in the tumor microenvironment is still largely unchartered, resulting in uncertainty about the distinction of various cellular subsets of the stroma.

2. Cancer-associated fibroblasts

Cancer-associated fibroblasts are known to support many different aspects of tumor initiation, growth and progression by secretion of growth stimulatory, pro-survival and angiogenic factors [3,8]. Due to a paucity of specific and all-encompassing markers, CAF have traditionally been localized simply by their widespread expression of α-smooth muscle actin (α-SMA) [9]. A myofibroblastic tumor microenvironment, as identified by α-SMA immunostaining, has been demonstrated to hold prognostic significance in, among others, colorectal carcinomas, breast carcinomas and gastric carcinomas [10–12]. However, it is now becoming evident that α-SMA expression in itself is neither sufficient to identify all subsets of CAF, nor able to clearly distinguish CAF from other cell types [13–16]. Here, we will focus on the functional aspects of CAF expressing some of the more widely used markers (Fig. 1). Cancer-associated fibroblasts will be considered as a single entity without regarding potential multiplicity caused by the source for recruitment, such as resident fibroblasts, mesenchymal stem cells [17], bone marrow-derived mesenchymal progenitors [18] or circulating fibrocytes [19].

2.1. Fibroblast activation protein α

Fibroblast activation protein α (FAP) was first identified as a tumor-specific antigen expressed by cells in the stroma of various epithelial cancers, including breast, pancreas and colon carcinomas [20]. Further characterization has identified FAP as a member of the serine protease subfamily of dipeptidyl peptidases, which is selectively expressed by stromal cells and mesenchymal stem cells during embryogenesis, wound healing, fibrotic reactions and inflammatory conditions [21–24]. Little is currently known about the substrate specificity of the proteolytic activity of FAP. Nevertheless, in keeping with the well-recognized role of CAF as providers of extracellular matrix, a recent study demonstrated modulation of the composition and organization of the substrate by CAF^{FAP}, resulting in enhanced invasiveness of pancreatic adenocarcinoma cells [25]. Prominent presence of CAF^{FAP} in the stroma of solid tumors is correlated to a poor prognosis in colon carcinoma and pancreatic adenocarcinoma [26,27], indicative of a functional role for FAP and/or the CAF^{FAP} subset of stromal cells during the development of tumors. This notion is supported by gene expression analyses indicating that FAP is part of a stromal profile, in which each gene by itself predicts for advanced stages and poor outcome of invasive esophageal carcinomas [28]. Based on the likely contribution of CAF^{FAP} to the tumorigenic program, various efforts to therapeutically target FAP have been made. To establish proof-of-principle, Kraman et al. depleted CAF^{FAP} from mice by means of a transgenic approach in which the diphtheria toxin receptor was expressed under the FAP promoter [29]. The study elegantly demonstrated that depletion of CAF^{FAP} effectively removes the immunosuppression exerted by stromal cells, thus resulting in an immune-mediated rejection of the tumor. In support of the immunomodulatory properties of CAF^{FAP}, a DNA vaccine targeting FAP was found to shift the polarization of the immune response within 4T1 breast carcinomas from Th2 to Th1; an effect which potentiated the response to doxorubicin chemotherapy [30]. Direct targeting of CAF^{FAP} has also been achieved by using pharmacological inhibitors. Treatment of transgenic mice carrying K-ras-induced lung carcinomas with PT630, an inhibitor of FAP enzymatic activity, resulted in a severe retardation of tumor growth, accompanied by depletion of α-SMA^+ cells and a blunted angiogenic response [31]. Clearly, CAF^{FAP} represent a functional subset of mesenchymal cells within the tumor stroma with a diverse repertoire of tumor-promoting abilities, of which immunomodulation appears to be the most prominent.

2.2. Fibroblast-specific protein-1

Despite its name, fibroblast-specific protein-1 (FSP1, S100A4) is expressed by a variety of cell types within the tumor microenvironment, including CAF, macrophages and malignant cells [32]. Therefore, the functions of FSP1 are difficult to ascribe to a particular subset of cells, although progression of tumors that develop in FSP1-deficient mice is clearly blunted [33]. Nevertheless, a number of studies have attempted to isolate the effects of CAF^{FSP1} on the malignant phenotype. Firstly, Bhowmick et al. elucidated the function of TGF-β signaling in CAF^{FSP1} by specifically deleting the gene for TGFBRII using FSP1-Cre mice [34]. Non-functional TGF-β signaling in FSP1-expressing cells resulted in the formation of neoplasia at multiple sites, most prominently in the prostate and forestomach. The mechanism of transformation involved increased expression of hepatocyte growth factor by CAF^{FSP1}, which stimulated epithelial c-Met activity. Secondly, CAF^{FSP1} were ablated using ganciclovir treatment of transgenic FSP1-thymidine kinase mice [35]. While primary tumor growth was not affected, the absence of FSP1-expressing cells greatly diminished metastatic colonization by reducing the expression of VEGF-A and tenascin-C by stromal cells. Here, the functional impact was attributed to CAF^{FSP1}, and not to bone marrow-derived $FSP1^+$ cells. In a third study, CAF^{FSP1} were found to greatly enhance the infiltration of macrophages into the tumor microenvironment through secretion of monocyte chemotactic protein-1 [36]. Ablation of CAF^{FSP1} from mice decreased macrophage influx and resulted in a prolonged latency and reduced tumor formation following induction of skin tumors by DMBA/TPA. Interestingly, CAF^{FSP1} in this tumor model, and others, did not express α-SMA to a large extent, indicating that the myofibroblast phenotype is not a prerequisite for tumor-promoting CAF [36,37]. In yet another chemically induced tumor model, CAF^{FSP1} were found to limit the exposure of the carcinogen to the surrounding epithelial cells by encapsulating the foreign substance via collagen depositions, resulting in the formation of fibrosarcomas but no epithelial tumors [37]. By selective ablation of CAF^{FSP1}, the carcinogen was no longer encapsulated and was thus free to transform surrounding epithelial cells, leading to overt carcinomas.

While FSP1 is not a specific marker of CAF, CAF^{FSP1} carry out fundamental functions in the tumor microenvironment during malignant progression. However, more work is needed to fully elucidate the specific functional and prognostic capabilities of CAF^{FSP1} in human cancers.

2.3. Platelet derived growth factor receptor-α

Signaling by members of the platelet derived growth factor (PDGF) family is crucial for a diverse range of functions performed