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Cortez, Eliane; Roswall, Pernilla; Pietras, Kristian

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PO Box 117
221 00 Lund
+46 46-222 00 00

Functional subsets of mesenchymal cell types in the tumor microenvironment

Eliane Cortez^a, Pernilla Roswall^b, Kristian Pietras^{a,b*}

^a Lund University, Department of Laboratory Medicine Lund, Division of Translational Cancer Research, Medicon Village, Building 404:A3, SE-223 81 Lund, Sweden

^b Karolinska Institutet, Department of Medical Biochemistry and Biophysics, Scheeles Väg 2, SE-171 77 Stockholm, Sweden

* Corresponding author

Lund University
Division of Translational Cancer Research
Medicon Village, Building 404:A3
SE-223 81 Lund
Sweden
Phone +46 709 209 709
E-mail Kristian.Pietras@med.lu.se

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 uncharted 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.

Keywords

Tumor microenvironment; Mesenchymal cells; Cancer-associated fibroblast; Pericyte

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 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 uncharted, resulting in uncertainty about the distinction of various cellular subsets of the stroma.

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].

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.

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 TGFBR2 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.

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 by mesenchymal cells during embryonic development [38, 39].

Specifically, activation of PDGFR- α appears instrumental in delivering mesenchymal-derived patterning information to forming epithelial structures in the lung, intestinal villus, palate and spine [38]. During tumorigenesis, PDGF-AA and PDGF-CC, both ligands of PDGFR- α , are abundantly expressed by the tumor epithelium and have been functionally implicated in a wide variety of malignancies [39]. In breast carcinomas, signaling by PDGF-AA induces a desmoplastic response by CAF^{PDGFR- α} , although overall tumor growth in this case was unaffected [40]. We have previously investigated the function of paracrine PDGF signaling to CAF^{PDGFR- α} in cervical carcinomas. In these studies, pharmacological blockade of PDGF signaling delayed tumor initiation and caused a diminished growth rate in a genetically engineered mouse model of squamous cell carcinoma of the cervix [41]. Mechanistic studies revealed that PDGF-stimulation of CAF^{PDGFR- α} provided growth-promoting cues to malignant cells (keratinocyte growth factor), as well as pro-angiogenic factors that stimulated blood vessel formation (fibroblast growth factor (FGF)-2). Similarly, signaling by PDGF-CC has been found to induce production of vascular

endothelial growth factor (VEGF)-A by recruited CAF^{PDGFR- α} , thereby acting to resume angiogenesis in tumors from VEGF-A^{-/-} malignant cells [9]. Similarly, we have also observed recruitment of CAF^{PDGFR- α} by PDGF-CC in experimental melanoma [15]. Tumors expressing PDGF-CC displayed a higher growth rate and an increased abundance of interstitial stroma and angiogenic vessels, induced by the combination of FGF-2 and osteopontin. Interestingly, different subsets of CAF were observed in melanomas engineered to express PDGF-CC, not all of which were CAF^{PDGFR- α} . Although all subsets of CAF expressed the growth-promoting mediator osteopontin, three distinct classes of CAF could be distinguished based on marker expression, *i.e.* CAF^{PDGFR- α (high)}, CAF^{PDGFR- α (low)/FSP1}, and CAF^{FSP1}, respectively. We hypothesize that CAF^{PDGFR- α (high)} may represent a progenitor CAF; a population which subsequently is differentiated into a fully mature and tumor growth-promoting CAF in response to the tumor microenvironment. In concordance with a more immature nature of CAF^{PDGFR- α} , signaling by PDGF-CC/PDGFR- α was recently demonstrated to be associated with reactivation of a developmental program in rhabdomyosarcomas [42]. In addition, education of normal dermal fibroblasts into mature CAF by squamous cell carcinoma cells of the skin indicates that the tumor microenvironment acts to differentiate and activate stromal cells [43]. In this genetically engineered mouse model of skin carcinoma, CAF^{PDGFR- α} were demonstrated to activate an inflammatory program during the progression from indolent to invasive tumors. Through signaling by the NF- κ B pathway, CAF^{PDGFR- α} induced tumor growth, macrophage recruitment and angiogenesis; a signaling program that appeared intact also in breast and pancreatic carcinomas [43].

Taken together, CAF^{PDGFR- α} are consistently involved in paracrine interactions within the tumor microenvironment with multiple cell types that concertedly orchestrate an enhanced angiogenic and pro-growth program in various tumor types.

Platelet derived growth factor receptor- β

Expression of PDGFR- β is mainly confined to vascular smooth muscle cells, PC^{PDGFR- β} and CAF^{PDGFR- β} within the tumor microenvironment and activation of the pathway promotes tumor initiation by the formation of a reactive stroma [44, 45]. In rhabdomyosarcomas, activation of CAF^{PDGFR- β} is associated with the more aggressive alveolar subtype and metastatic spread [42]. Likewise, signaling by PDGFR- β in CAFs has been demonstrated to promote metastasis of colorectal cancer [46, 47]. Functionally, CAF^{PDGFR- β} and PC^{PDGFR- β} promote the generation of a high interstitial fluid pressure in tumors, thus impairing the delivery of therapeutic agents [48-50]. Moreover, prominent occurrence of CAF^{PDGFR- β} is associated with shorter recurrence-free and disease-specific survival in human breast cancer patients [51]. Although the PDGFR- β pathway is clearly activated and tumor promoting in a range of malignant settings, more work is needed to distinguish the functional effects between CAF^{PDGFR- β} and PC^{PDGFR- β} in the tumor stroma.

Others

A wide variety of other markers are expressed by CAF, including TGFBR2, SPARC and components of the Hedgehog pathway; markers that have been demonstrated to hold both clinically and biologically meaningful information [34, 52-54]. More information is clearly needed to identify and functionally probe the full diversity of CAF subsets within the tumor microenvironment.

Pericytes

Pericytes are a heterogeneous population of mural cells in close contact with endothelial cells in small blood vessels and capillaries, distinguished from vascular smooth muscle cells (VSMC) both by physical location and marker expression [55]. In the normal microvasculature, PC regulate blood vessel formation and function [56]. However, their exact role in the tumor vasculature is not fully understood. Pericytes are largely defined based on morphology and location but also using various dynamic molecular markers. The expression of these markers appears to be tissue specific and vary during development and pathological conditions. The lack of markers that can clearly distinguish between normal and tumor-associated PC poses a particular challenge in studying these cells. Nevertheless, recent studies on PC in tumors, using different markers, suggest the existence of functional subsets that respond differently to both genetic and pharmacological impairment affecting tumor growth and dissemination in distinctive ways (Fig. 2). However, it remains to be clarified whether these markers represent distinct or overlapping populations of PC.

Nerve/glial antigen 2

Nerve/glial antigen 2 (NG2) is a chondroitin sulfate proteoglycan expressed by oligodendrocyte progenitors in the central nervous system and by PC^{NG2} in nascent microvessels both in normal and tumor tissue [57]. In tumors, NG2 is a marker denoting a mature population of PC [58]. By the use of transgenic mice expressing viral thymidine kinase under the control of the NG2 promoter, PC^{NG2} were selectively ablated from tumor vessels upon ganciclovir treatment, thus causing primary tumor growth inhibition in transgenic and orthotopic breast cancer mouse models [59]. However, tumor growth inhibition was followed by a significant increase in hypoxia and metastatic dissemination. Supporting these observations in preclinical models, analysis of

samples from patients with invasive ductal carcinoma showed that low PC^{NG2} coverage in tumor vessels strongly correlated with invasive disease and the presence of distant metastasis. Results from these studies show that targeting PC^{NG2} affects primary tumor growth, and that this subset might play an important role in controlling tumor cell dissemination. Moreover, the data suggests that analysis of PC^{NG2} coverage in clinical material might serve as a useful prognostic biomarker.

Platelet derived growth factor receptor- β

As stated earlier, members of the PDGF family play an important role in the recruitment and function of mesenchymal cells. Targeted deletion of PDGFR- β or its ligand, PDGF-BB, during embryonic development, results in failure in the early recruitment of PC^{PDGFR- β} into the developing vasculature [60, 61]. Moreover, this recruitment process has been shown to be essentially dependent on the expression of PDGF-BB by EC [62]. Studies using the RIP1-Tag2 mouse model for neuroendocrine pancreatic tumorigenesis demonstrate that PDGFR- β expression denotes a progenitor subpopulation that holds the ability to differentiate into PC^{NG2}, PC^{desmin} and PC ^{α -SMA} [58]. This proposition is analogous to the notion that CAF^{PDGFR- α} represent an immature subset of CAF [15]. As for CAF^{PDGFR- β} , PC^{PDGFR- β} have also been functionally implicated in the increased interstitial fluid pressure of tumors [50]. Moreover, recent reports indicate that PC^{PDGFR- β} provide EC with survival signals. Accordingly, we have shown that expression of the anti-apoptotic gene *Bcl2l2* was significantly decreased in tumor EC devoid of contact with PC^{PDGFR- β} [63]. Also, pharmacological depletion of PC^{PDGFR- β} from immature tumor blood vessels has been confirmed to sensitize EC to anti-angiogenic therapy [64-66]. Taken together, these results underline the functional importance of PC^{PDGFR- β} in stabilizing the tumor microvasculature during stress conditions.

α -smooth muscle actin

α -SMA is a contractile protein expressed by CAF, most PC and VSMC during tumor development and at sites of inflammation. Using the RIP1-Tag2 mouse model we have demonstrated that mice harboring tumors refractory to therapy following long-term treatment with a VEGFR-2 blocking antibody presented with increased number of PC ^{α -SMA} [67]. We suggest that the presence of blood vessels covered by PC ^{α -SMA} results from vessel co-option, a mechanism by which tumors use neighboring pre-existent vessels for oxygen supply. In support of this proposition, in a study using a mouse model of melanoma, it was shown that blood vessels covered by PC ^{α -SMA} are a particular feature of tumors that acquire vascularization through a non-angiogenic process [68]. In the same study, the authors show that metastasis from melanoma patients resistant to bevacizumab, a monoclonal antibody neutralizing VEGF-A, presented with increased coverage of vessels by PC ^{α -SMA} compared to untreated patients. Together, these results support the premise that PC promote resistance to anti-VEGF therapy [69], and that expression of α -SMA denotes a phenotype of PC from adjacent non-malignant tissues. Additionally, this highlights the potential benefit of using PC ^{α -SMA} coverage in tumor vessels from clinical material to establish correlations with response to therapy and overall clinical outcome.

Desmin

Desmin is a class-III intermediate filament found in muscle cells and PC, both in normal and tumor capillaries. In tumors, PC^{desmin} appear more closely associated with vessels whereas PC ^{α -SMA} or PC^{PDGFR- β} are normally partly detached [70]. Together with expression of α -SMA and NG2, desmin positivity has also been suggested to represent a more mature population of PC [71]. Given the proposed role of PC in protecting EC against anti-VEGF therapy, different strategies have been designed to attempt to target both cell types in order to achieve improved

therapeutic benefit. In one such study, the authors treated B16 mouse melanoma tumors ectopically expressing PDGF-BB with a combination regimen targeting VEGFR-2 in EC and PDGFR- β in PC. Analyses of the PC population revealed that the total number of PC $^{\alpha\text{-SMA}}$ or PC $^{\text{PDGFR-}\beta}$ were significantly reduced, whereas PC $^{\text{desmin}}$ did not respond to combination therapy [72]. An important observation is that, apart from desmin, this PC subset also expressed other PC markers, which demonstrates the existence of overlapping PC subpopulations adding increased complexity to the conclusions drawn from this analysis. Moreover, from the same study the authors reasoned that communication between PC $^{\text{desmin}}$ and EC in tumors might require a different nature of signals or that their recruitment and differentiation is dependent on a distinctive signaling pathway compared to other PC subsets. A second study strengthens the proposition that different PC subsets respond differently to pharmacological targeting. While treatment with the PDGF-B targeting aptamer AX102 eventually led to a significant reduction in total PC coverage in LLC tumors, the kinetics of the loss of different subtypes of PC were distinct, with PC $^{\alpha\text{-SMA}}$ showing the quickest reduction and PC $^{\text{PDGFR-}\beta}$ the slowest [73]. Although this particular subset might represent an interesting target, more studies using different tumor models and a further characterization of PC $^{\text{desmin}}$ is still needed.

Regulator of G-protein signaling 5

Regulator of G-protein signaling 5 (RGS5), is a PC marker in the tumor vasculature with a largely unknown function [74]. Using the RIP1-Tag5 mouse model, it was observed that loss of RGS5 results in vascular normalization characterized by a blunted angiogenic response coupled with PC maturation and increased expression of NG2 and $\alpha\text{-SMA}$ [75]. Furthermore, it was shown that the normalized vessels improved the influx of tumor-specific immune cells. Although

this marker needs further characterization, these preliminary observations indicate that PC^{RGS5} may serve as a barrier to efficient anti-tumor immunity.

Others

Other markers are also commonly used to identify PC such as endosialin [76], CD13 and promoter trap transgene XlacZ4 [77]. However, more extensive studies in preclinical mouse models and human tissues are necessary to infer about the functional relevance of such subsets in tumor development.

Perspective

The detailed elucidation of distinct subsets of various stromal cell types has recently been recognized as an important challenge in our understanding of the paracrine circuitry within the tumor microenvironment. Cell type diversity within the widely defined CAF and PC population most likely highlights different aspects of biology and encompasses both cell-of-origin and activation state of different signaling pathways that impinge on mesenchymal cells, such as PDGF, transforming growth factor- β , and hedgehog signaling. Moreover, increasing evidence points to that there may be a hierarchy of cell type subsets, as demonstrated by differentiation and diversification of marker expression by CAF and PC [15, 71]. Intensified efforts to demonstrate the functional significance of such subsets is likely to follow over the next decade, and should be greatly facilitated by new technologies that allow for *in situ* characterization of activation states of signaling pathways, and single cell methodologies for genomic, transcriptomic and proteomic analyses. Also, a thorough characterization is crucial to identify key molecular differences between normal and tumor-associated mesenchymal cells and the different existent subsets in

order to better design strategies to efficiently target these cells in the tumor compartment. An emerging controversial field is that of transdifferentiation of pericytes and fibroblasts during various fibrotic conditions. Pericytes expressing ADAM12 or GLAST have recently been found to contribute to the formation of scar tissue after injury in the skin or spinal cord, respectively [78, 79]. In contrast, lineage tracing studies in mice demonstrate that the pericyte contribution of fibroblasts responsible for kidney fibrosis is negligible [80]. Similar studies investigating the relationship between CAF and PC in the tumor stroma are highly warranted.

The recent development of targeted therapy for cancer has been introduced into clinical practice over the course of the past decade. High hopes were placed on targeting specific overactive signaling pathways within tumors following the preceding successful drug development in the preclinical setting. Indeed, the therapeutic efficacy observed in mouse models of cancer has in some cases been translated into clinical benefit for patients. Nevertheless, many targeted therapies have failed to provide substantial improvement in overall patient survival in large phase III clinical trials. The main reason for this shortfall is a lack of reliable biomarkers predictive of response to therapy. In light of the emerging realization that the tumor microenvironment encompasses a multitude of cell types, and subsets of cell types, it is likely that stromal biomarkers will hold complementary prognostic and/or predictive capabilities. It is our vision that a pre-treatment biopsy from each cancer patient will be screened, either transcriptionally or histologically, for the content of signatures derived from different subsets of stromal and/or malignant cells. Based on the composition of each tumor and abundance of individual subtypes of the constituent cell types, oncologists will be able to make informed choices about which therapy to prescribe in order for the patient to benefit the most.

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Conflict of interest statement

The authors declare that there are no conflicts of interest.

References

- [1] Ferlay J, Steliarova-Foucher E, Lortet-Tieulent J, Rosso S, Coebergh JW, Comber H et al. Cancer incidence and mortality patterns in europe: Estimates for 40 countries in 2012. *Eur J Cancer* 2013;49:1374-403.
- [2] Hanahan D, Weinberg RA. Hallmarks of cancer: The next generation. *Cell* 2011;144:646-74.
- [3] Pietras K, Östman A. Hallmarks of cancer: Interactions with the tumor stroma. *Exp Cell Res* 2010;316:1324-31.
- [4] Hanahan D, Coussens LM. Accessories to the crime: Functions of cells recruited to the tumor microenvironment. *Cancer Cell* 2012;21:309-22.
- [5] Nguyen LV, Vanner R, Dirks P, Eaves CJ. Cancer stem cells: An evolving concept. *Nat Rev Cancer* 2012;12:133-43.
- [6] Geudens I, Gerhardt H. Coordinating cell behaviour during blood vessel formation. *Development* 2011;138:4569-83.
- [7] De Palma M, Lewis CE. Macrophage regulation of tumor responses to anticancer therapies. *Cancer Cell* 2013;23:277-86.
- [8] Kalluri R, Zeisberg M. Fibroblasts in cancer. *Nat Rev Cancer* 2006;6:392-401.
- [9] Crawford Y, Kasman I, Yu L, Zhong C, Wu X, Modrusan Z et al. Pdgf-c mediates the angiogenic and tumorigenic properties of fibroblasts associated with tumors refractory to anti-vegf treatment. *Cancer Cell* 2009;15:21-34.
- [10] Tsujino T, Seshimo I, Yamamoto H, Ngan CY, Ezumi K, Takemasa I et al. Stromal myofibroblasts predict disease recurrence for colorectal cancer. *Clin Cancer Res* 2007;13:2082-90.
- [11] Fuyuhiko Y, Yashiro M, Noda S, Kashiwagi S, Matsuoka J, Doi Y et al. Myofibroblasts are associated with the progression of scirrhous gastric carcinoma. *Exp Ther Med* 2010;1:547-51.
- [12] Surowiak P, Murawa D, Materna V, Maciejczyk A, Pudenko M, Ciesla S et al. Occurrence of stromal myofibroblasts in the invasive ductal breast cancer tissue is an unfavourable prognostic factor. *Anticancer Res* 2007;27:2917-24.
- [13] Sugimoto H, Mundel TM, Kieran MW, Kalluri R. Identification of fibroblast heterogeneity in the tumor microenvironment. *Cancer Biol Ther* 2006;5:1640-6.
- [14] Anderberg C, Pietras K. On the origin of cancer-associated fibroblasts. *Cell Cycle* 2009;8:1461-2.
- [15] Anderberg C, Li H, Fredriksson L, Andrae J, Betsholtz C, Li X et al. Paracrine signaling by platelet-derived growth factor-cc promotes tumor growth by recruitment of cancer-associated fibroblasts. *Cancer Res* 2009;69:369-78.
- [16] Roswall P, Pietras K. Of mice and men: A comparative study of cancer-associated fibroblasts in mammary carcinoma. *Ups J Med Sci* 2012;117:196-201.
- [17] Karnoub AE, Dash AB, Vo AP, Sullivan A, Brooks MW, Bell GW et al. Mesenchymal stem cells within tumour stroma promote breast cancer metastasis. *Nature* 2007;449:557-63.
- [18] Qian H, Badaloni A, Chiara F, Stjernberg J, Poliseti N, Nihlberg K et al. Molecular characterization of prospectively isolated multipotent mesenchymal progenitors provides new insight into the cellular identity of mesenchymal stem cells in mouse bone marrow. *Mol Cell Biol* 2013;33:661-77.
- [19] Zhang H, Maric I, Diprima MJ, Khan J, Orentas RJ, Kaplan RN et al. Fibrocytes represent a novel mdsc subset circulating in patients with metastatic cancer. *Blood* 2013;

- [20] Garin-Chesa P, Old LJ, Rettig WJ. Cell surface glycoprotein of reactive stromal fibroblasts as a potential antibody target in human epithelial cancers. *Proc Natl Acad Sci U S A* 1990;87:7235-9.
- [21] Bae S, Park CW, Son HK, Ju HK, Paik D, Jeon CJ et al. Fibroblast activation protein alpha identifies mesenchymal stromal cells from human bone marrow. *Br J Haematol* 2008;142:827-30.
- [22] Scanlan MJ, Raj BK, Calvo B, Garin-Chesa P, Sanz-Moncasi MP, Healey JH et al. Molecular cloning of fibroblast activation protein alpha, a member of the serine protease family selectively expressed in stromal fibroblasts of epithelial cancers. *Proc Natl Acad Sci U S A* 1994;91:5657-61.
- [23] Pineiro-Sanchez ML, Goldstein LA, Dodt J, Howard L, Yeh Y, Tran H et al. Identification of the 170-kda melanoma membrane-bound gelatinase (seprase) as a serine integral membrane protease. *J Biol Chem* 1997;272:7595-601.
- [24] Christiansen VJ, Jackson KW, Lee KN, Downs TD, McKee PA. Targeting inhibition of fibroblast activation protein-alpha and prolyl oligopeptidase activities on cells common to metastatic tumor microenvironments. *Neoplasia* 2013;15:348-58.
- [25] Lee HO, Mullins SR, Franco-Barraza J, Valianou M, Cukierman E, Cheng JD. Fap-overexpressing fibroblasts produce an extracellular matrix that enhances invasive velocity and directionality of pancreatic cancer cells. *BMC Cancer* 2011;11:245.
- [26] Wikberg ML, Edin S, Lundberg IV, Van Guelpen B, Dahlin AM, Rutegard J et al. High intratumoral expression of fibroblast activation protein (fap) in colon cancer is associated with poorer patient prognosis. *Tumour Biol* 2013;34:1013-20.
- [27] Shi M, Yu DH, Chen Y, Zhao CY, Zhang J, Liu QH et al. Expression of fibroblast activation protein in human pancreatic adenocarcinoma and its clinicopathological significance. *World J Gastroenterol* 2012;18:840-6.
- [28] Saadi A, Shannon NB, Lao-Sirieix P, O'Donovan M, Walker E, Clemons NJ et al. Stromal genes discriminate preinvasive from invasive disease, predict outcome, and highlight inflammatory pathways in digestive cancers. *Proc Natl Acad Sci U S A* 2010;107:2177-82.
- [29] Kraman M, Bambrough PJ, Arnold JN, Roberts EW, Magiera L, Jones JO et al. Suppression of antitumor immunity by stromal cells expressing fibroblast activation protein-alpha. *Science* 2010;330:827-30.
- [30] Liao D, Luo Y, Markowitz D, Xiang R, Reisfeld RA. Cancer associated fibroblasts promote tumor growth and metastasis by modulating the tumor immune microenvironment in a 4t1 murine breast cancer model. *PLoS One* 2009;4:e7965.
- [31] Santos AM, Jung J, Aziz N, Kissil JL, Pure E. Targeting fibroblast activation protein inhibits tumor stromagenesis and growth in mice. *J Clin Invest* 2009;119:3613-25.
- [32] Cabezon T, Celis JE, Skibshoj I, Klingelhofer J, Grigorian M, Gromov P et al. Expression of s100a4 by a variety of cell types present in the tumor microenvironment of human breast cancer. *Int J Cancer* 2007;121:1433-44.
- [33] Grum-Schwensen B, Klingelhofer J, Berg CH, El-Naaman C, Grigorian M, Lukanidin E et al. Suppression of tumor development and metastasis formation in mice lacking the s100a4(mts1) gene. *Cancer Res* 2005;65:3772-80.
- [34] Bhowmick NA, Chytil A, Plieth D, Gorska AE, Dumont N, Shappell S et al. Tgf-beta signaling in fibroblasts modulates the oncogenic potential of adjacent epithelia. *Science* 2004;303:848-51.
- [35] O'Connell JT, Sugimoto H, Cooke VG, MacDonald BA, Mehta AI, LeBleu VS et al. Vegf-a and tenascin-c produced by s100a4+ stromal cells are important for metastatic colonization. *Proc Natl Acad Sci U S A* 2011;108:16002-7.

- [36] Zhang J, Chen L, Xiao M, Wang C, Qin Z. Fsp1+ fibroblasts promote skin carcinogenesis by maintaining mcp-1-mediated macrophage infiltration and chronic inflammation. *Am J Pathol* 2011;178:382-90.
- [37] Zhang J, Chen L, Liu X, Kammertoens T, Blankenstein T, Qin Z. Fibroblast-specific protein 1/s100a4-positive cells prevent carcinoma through collagen production and encapsulation of carcinogens. *Cancer Res* 2013;73:2770-81.
- [38] Andrae J, Gallini R, Betsholtz C. Role of platelet-derived growth factors in physiology and medicine. *Genes Dev* 2008;22:1276-312.
- [39] Pietras K, Sjöblom T, Rubin K, Heldin CH, Ostman A. Pdgf receptors as cancer drug targets. *Cancer Cell* 2003;3:439-43.
- [40] Shao Z-M, Nguyen M, Barsky SH. Human breast carcinoma desmoplasia is pdgf initiated. *Oncogene* 2000;19:4337-45.
- [41] Pietras K, Pahler J, Bergers G, Hanahan D. Functions of paracrine pdgf signaling in the proangiogenic tumor stroma revealed by pharmacological targeting. *PLoS Med* 2008;5:e19.
- [42] Ehnman M, Missiaglia E, Folestad E, Selfe J, Strell C, Thway K et al. Distinct effects of ligand-induced pdgfralpha and pdgfrbeta signaling in the human rhabdomyosarcoma tumor cell and stroma cell compartments. *Cancer Res* 2013;73:2139-49.
- [43] Erez N, Truitt M, Olson P, Arron ST, Hanahan D. Cancer-associated fibroblasts are activated in incipient neoplasia to orchestrate tumor-promoting inflammation in an nf-kappab-dependent manner. *Cancer Cell* 2010;17:135-47.
- [44] Forsberg K, Valyi-Nagy I, Heldin C-H, Herlyn M, Westermark B. Platelet-derived growth factor (pdgf) in oncogenesis: Development of a vascular connective tissue stroma in xenotransplanted human melanoma producing pdgf-bb. *Proc. Natl. Acad. Sci. USA* 1993;90:393-7.
- [45] Skobe M, Fusenig NE. Tumorigenic conversion of immortal human keratinocytes through stromal cell activation. *Proc. Natl. Acad. Sci. USA* 1998;95:1050-5.
- [46] Pena C, Cespedes MV, Lindh MB, Kiflemariam S, Mezheyeuski A, Edqvist PH et al. Stc1 expression by cancer-associated fibroblasts drives metastasis of colorectal cancer. *Cancer Res* 2013;73:1287-97.
- [47] Shinagawa K, Kitadai Y, Tanaka M, Sumida T, Onoyama M, Ohnishi M et al. Stroma-directed imatinib therapy impairs the tumor-promoting effect of bone marrow-derived mesenchymal stem cells in an orthotopic transplantation model of colon cancer. *Int J Cancer* 2013;132:813-23.
- [48] Pietras K, Ostman A, Sjöquist M, Buchdunger E, Reed RK, Heldin CH et al. Inhibition of platelet-derived growth factor receptors reduces interstitial hypertension and increases transcapillary transport in tumors. *Cancer Res* 2001;61:2929-34.
- [49] Pietras K, Rubin K, Sjöblom T, Buchdunger E, Sjöquist M, Heldin C-H et al. Inhibition of pdgf receptor signaling in tumor stroma enhances antitumor effect of chemotherapy. *Cancer Res* 2002;62:5476-84.
- [50] Falcon BL, Pietras K, Chou J, Chen D, Sennino B, Hanahan D et al. Increased vascular delivery and efficacy of chemotherapy after inhibition of platelet-derived growth factor-b. *Am J Pathol* 2011;178:2920-30.
- [51] Paulsson J, Sjöblom T, Micke P, Ponten F, Landberg G, Heldin CH et al. Prognostic significance of stromal platelet-derived growth factor beta-receptor expression in human breast cancer. *Am J Pathol* 2009;175:334-41.

- [52] Podhajcer OL, Benedetti LG, Girotti MR, Prada F, Salvatierra E, Llera AS. The role of the matricellular protein sparc in the dynamic interaction between the tumor and the host. *Cancer Metastasis Rev* 2008;27:691-705.
- [53] Nolan-Stevaux O, Lau J, Truitt ML, Chu GC, Hebrok M, Fernandez-Zapico ME et al. Gli1 is regulated through smoothened-independent mechanisms in neoplastic pancreatic ducts and mediates pdac cell survival and transformation. *Genes Dev* 2009;23:24-36.
- [54] Calon A, Espinet E, Palomo-Ponce S, Tauriello DV, Iglesias M, Cespedes MV et al. Dependency of colorectal cancer on a tgf-beta-driven program in stromal cells for metastasis initiation. *Cancer Cell* 2012;22:571-84.
- [55] Lindskog H, Athley E, Larsson E, Lundin S, Hellstrom M, Lindahl P. New insights to vascular smooth muscle cell and pericyte differentiation of mouse embryonic stem cells in vitro. *Arterioscler Thromb Vasc Biol* 2006;26:1457-64.
- [56] Armulik A, Abramsson A, Betsholtz C. Endothelial/pericyte interactions. *Circulation Research* 2005;97:512-23.
- [57] Ozerdem U, Grako KA, Dahlin-Huppe K, Monosov E, Stallcup WB. Ng2 proteoglycan is expressed exclusively by mural cells during vascular morphogenesis. *Dev Dyn* 2001;222:218-27.
- [58] Song S, Ewald AJ, Stallcup W, Werb Z, Bergers G. Pdgfr beta(+) perivascular progenitor cells in tumours regulate pericyte differentiation and vascular survival. *Nature Cell Biology* 2005;7:870-U16.
- [59] Cooke VG, LeBleu VS, Keskin D, Khan Z, O'Connell JT, Teng Y et al. Pericyte depletion results in hypoxia-associated epithelial-to-mesenchymal transition and metastasis mediated by met signaling pathway. *Cancer Cell* 2012;21:66-81.
- [60] Lindahl P, Johansson BR, Leveen P, Betsholtz C. Pericyte loss and microaneurysm formation in pdgf-b-deficient mice. *Science* 1997;277:242-5.
- [61] Hellstrom M, Kalen M, Lindahl P, Abramsson A, Betsholtz C. Role of pdgf-b and pdgfr-beta in recruitment of vascular smooth muscle cells and pericytes during embryonic blood vessel formation in the mouse. *Development* 1999;126:3047-55.
- [62] Lindblom P, Gerhardt H, Liebner S, Abramsson A, Enge M, Hellstrom M et al. Endothelial pdgf-b retention is required for proper investment of pericytes in the microvessel wall. *Genes Dev* 2003;17:1835-40.
- [63] Franco M, Roswall P, Cortez E, Hanahan D, Pietras K. Pericytes promote endothelial cell survival through induction of autocrine vegf-a signaling and bcl-w expression. *Blood* 2011;118:2906-17.
- [64] Bergers G, Song S, Meyer-Morse N, Bergsland E, Hanahan D. Benefits of targeting both pericytes and endothelial cells in the tumor vasculature with kinase inhibitors. *J Clin Invest* 2003;111:1287-95.
- [65] Pietras K, Hanahan D. A multitargeted, metronomic, and maximum-tolerated dose "chemo-switch" regimen is antiangiogenic, producing objective responses and survival benefit in a mouse model of cancer. *J Clin Oncol* 2005;23:939-52.
- [66] Erber R, Thurnher A, Katsen AD, Groth G, Kerger H, Hammes HP et al. Combined inhibition of vegf and pdgf signaling enforces tumor vessel regression by interfering with pericyte-mediated endothelial cell survival mechanisms. *Faseb J* 2004;18:338-40.
- [67] Franco M, Paez-Ribes M, Cortez E, Casanovas O, Pietras K. Use of a mouse model of pancreatic neuroendocrine tumors to find pericyte biomarkers of resistance to anti-angiogenic therapy. *Horm Metab Res* 2011;43:884-9.

- [68] Helfrich I, Scheffrahn I, Bartling S, Weis J, von Felbert V, Middleton M et al. Resistance to antiangiogenic therapy is directed by vascular phenotype, vessel stabilization, and maturation in malignant melanoma. *J Exp Med* 2010;207:491-503.
- [69] Bergers G, Hanahan D. Modes of resistance to anti-angiogenic therapy. *Nature Reviews Cancer* 2008;8:592-603.
- [70] Morikawa S, Baluk P, Kaidoh T, Haskell A, Jain RK, McDonald DM. Abnormalities in pericytes on blood vessels and endothelial sprouts in tumors. *Am J Pathol* 2002;160:985-1000.
- [71] Song S, Ewald AJ, Stallcup W, Werb Z, Bergers G. Pdgfrbeta+ perivascular progenitor cells in tumours regulate pericyte differentiation and vascular survival. *Nat Cell Biol* 2005;7:870-9.
- [72] Hasumi Y, Klosowska-Wardega A, Furuhashi M, Ostman A, Heldin CH, Hellberg C. Identification of a subset of pericytes that respond to combination therapy targeting pdgf and vegf signaling. *Int J Cancer* 2007;121:2606-14.
- [73] Sennino B, Falcon BL, McCauley D, Le T, McCauley T, Kurz JC et al. Sequential loss of tumor vessel pericytes and endothelial cells after inhibition of platelet-derived growth factor b by selective aptamer ax102. *Cancer Res* 2007;67:7358-67.
- [74] Nisancioglu MH, Mahoney WM, Jr., Kimmel DD, Schwartz SM, Betsholtz C, Genove G. Generation and characterization of rgs5 mutant mice. *Mol Cell Biol* 2008;28:2324-31.
- [75] Hamzah J, Jugold M, Kiessling F, Rigby P, Manzur M, Marti HH et al. Vascular normalization in rgs5-deficient tumours promotes immune destruction. *Nature* 2008;453:410-4.
- [76] Simonavicius N, Robertson D, Bax DA, Jones C, Huijbers IJ, Isacke CM. Endosialin (cd248) is a marker of tumor-associated pericytes in high-grade glioma. *Mod Pathol* 2008;21:308-15.
- [77] Tidhar A, Reichenstein M, Cohen D, Faerman A, Copeland NG, Gilbert DJ et al. A novel transgenic marker for migrating limb muscle precursors and for vascular smooth muscle cells. *Dev Dyn* 2001;220:60-73.
- [78] Goritz C, Dias DO, Tomilin N, Barbacid M, Shupliakov O, Frisen J. A pericyte origin of spinal cord scar tissue. *Science* 2011;333:238-42.
- [79] Dulauroy S, Di Carlo SE, Langa F, Eberl G, Peduto L. Lineage tracing and genetic ablation of adam12(+) perivascular cells identify a major source of profibrotic cells during acute tissue injury. *Nat Med* 2012;18:1262-70.
- [80] LeBleu VS, Taduri G, O'Connell J, Teng Y, Cooke VG, Woda C et al. Origin and function of myofibroblasts in kidney fibrosis. *Nat Med* 2013;19:1047-53.

Figure legends

Figure 1 Functional Subsets of Cancer Associated Fibroblasts (CAF).

Several studies show the existence of different subpopulations of CAF in the tumor microenvironment based on marker expression and functional analysis using different mouse models of cancer and human tumor material. The importance of CAF for tumor development and metastatic dissemination has been widely investigated. Here we summarize the current understanding on CAF subsets and their role in tumor development.

Figure 2. Functional Subsets of tumor pericytes (PC) Here we summarize current data on PC subsets defined based on expression of the most well characterized PC markers in studies using different mouse models of cancer and human tumor material. Tumor vascular targeting has been expanded to include PC that provide survival signals and structural support to EC. However, the exact functional significance of PC in tumor development is not fully understood. Importantly also, it should be taken into account that expression of these markers in PC seems to be dynamic during progression of the disease and not exclusive to the PC compartment.

CAF^{FAP}

Activated/reactive CAF
Modulation of the ECM
Invasion
Immunomodulatory functions

CAF^{PDGFR-α}

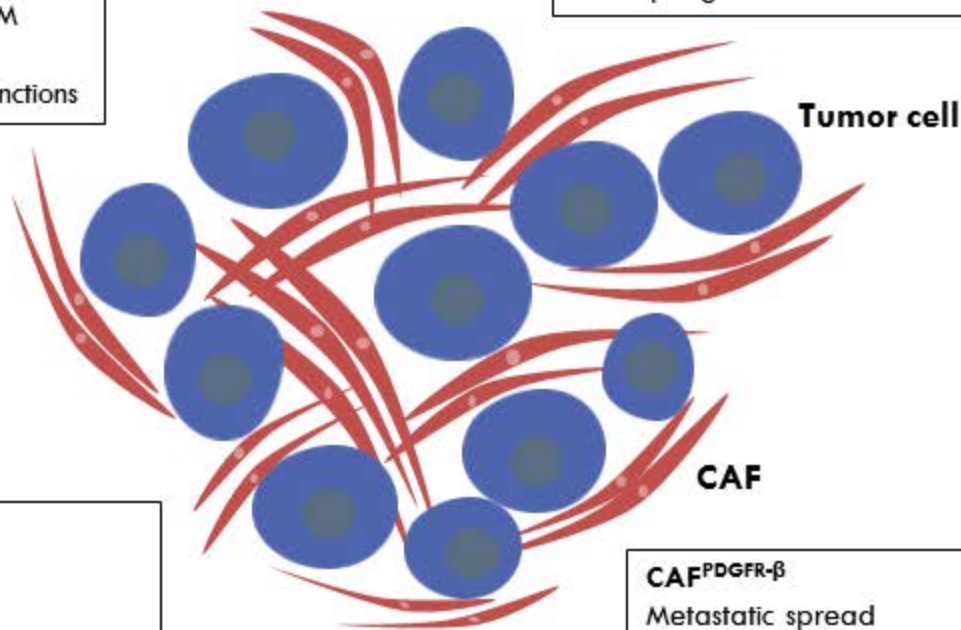
Paracrine signaling - tumor cell growth
and angiogenesis
Macrophage recruitment

CAF^{FSP1}

Metastatic colonization
Macrophage infiltration
Protection of epithelial cells from carcinogen

CAF^{PDGFR-β}

Metastatic spread
High interstitial fluid pressure



PC^{PDGFR-β}

Immature PC
EC survival
High interstitial fluid pressure

Tumor cell

PC^{NG2}

Mature PC
Tumor cell invasion/metastasis

Blood vessel

PC^{α-SMA}

Mature PC
Tumors refractory to anti-VEGF

PC^{RG55}

Barrier to anti-tumor immunity

Pericyte

PC^{desmin}

Mature PC
Closely attached to EC
Resistant to anti-PDGFR-β

