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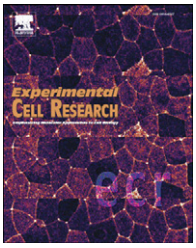

Review

Hallmarks of cancer: Interactions with the tumor stroma

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ABSTRACT

Ten years ago, Hanahan and Weinberg delineated six "Hallmarks of cancer" which summarize several decades of intense cancer research. However, tumor cells do not act in isolation, but rather subsist in a rich microenvironment provided by resident fibroblasts, endothelial cells, pericytes, leukocytes, and extra-cellular matrix. It is increasingly appreciated that the tumor stroma is an integral part of cancer initiation, growth and progression. The stromal elements of tumors hold prognostic, as well as response-predictive, information, and abundant targeting opportunities within the tumor microenvironment are continually identified. Herein we review the current understanding of tumor cell interactions with the tumor stroma with a particular focus on cancer-associated fibroblasts and pericytes. Moreover, we discuss emerging fields of research which need to be further explored in order to fulfil the promise of stroma-targeted therapies for cancer.

Contents

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Introduction

Tumors arise from normal cells through genetic alterations affecting the tightly controlled systems for growth control. Ten years ago, Hanahan and Weinberg enumerated six hallmarks of cancer that are essential for a cell to acquire on its way to any of the more than one hundred different types of human malignancies [1]. The specification of the traits of a tumor cell is the distillate of several decades of research dedicated to the malignant cell. However, the tumor cell-centric view of cancer does not take into account the context in which malignant cells subsist. As the cancer progresses, the surrounding microenvironment co-evolves into an activated state through continuous paracrine communication, thus creating a dynamic signaling circuitry that promotes cancer initiation and growth, and ultimately leads to a fatal disease. Indeed, many of the hallmarks of cancer delineated by Hanahan and Weinberg are provided by various stromal components, including endothelial cells, pericytes, fibroblasts, various classes of leukocytes, and extracellular matrix (Fig. 1). Herein, we review the pro-tumorigenic actions of tumor-associated mesenchymal cell types, *i.e.* cancer-associated fibroblasts (CAFs) and pericytes, in the context of the original hallmarks of cancer. Additionally, we discuss potential targeting opportunities for the development of drugs aimed at the tumor stroma, as well as delineate emerging areas of research.

Cancer-associated fibroblasts

The cancer-associated fibroblast is the most prominent cell type within the tumor stroma of many cancers, most notably breast and pancreatic carcinoma (Fig. 1) [2,3]. Recent studies highlight several different subpopulations of stromal fibroblasts within tumors designated by only partly overlapping marker expression, including α-smooth muscle actin (SMA), platelet-derived growth factor (PDGF) receptors, and fibroblast specific protein (FSP)-1 [4,5]. The heterogeneity in marker expression may in part be explained by a diverse origin of CAFs, which are variously reported to stem from resident local fibroblasts, bone marrow-derived progenitor cells or trans-differentiating epithelial cells [6]. Co-injection studies of tumor cells mixed with mesenchymal cells from different sources have conclusively demonstrated the importance of stromal fibroblasts for initiation, growth and metastatic spread of tumors [5,7,8].

Pericytes

Pericytes are contractile cells in close physical contact with endothelial cells in capillaries and venules (Fig. 1). In quiescent tissues, pericytes readily express markers such as PDGF receptor-β, NG2 and desmin, while lacking expression of α-SMA. However, tumor pericytes are characterized by a more loosely attached phenotype with a disparate pattern of marker expression, including α-SMA [9]. A range of signaling pathways, including PDGF, transforming growth factor (TGF)-β, angiopoietin and Notch family members, are implicated in pericyte recruitment, differentiation and function [10]. Recruitment of pericytes into tumors is crucially dependent on PDGF receptor-β expression, as well as on production of the PDGF-B ligand by endothelial cells [11]. In line with this notion, pericyte progenitor cells recruited into tumors from remote sources are denoted by expression of PDGF receptor-β [12].

Contributions of CAFs and pericytes to the "Hallmarks of cancer"

Self-sufficiency in growth signals

CAFs directly stimulate tumor cell proliferation through provision of various growth factors, hormones and cytokines in a context-dependent manner. Prototypical epithelial mitogens, such as hepatocyte growth factor (HGF), and members of the epidermal growth factor, fibroblast growth factor (FGF) and Wnt families, as well as cytokines such as stromal-derived factor (SDF)-1α (CXCL12) and IL-6, are all highly expressed by CAFs in different tumor types [13]. Intriguingly, many of these factors acting in isolation are sufficient to induce transformation of epithelial cells, indicative of a tumor-initiating capability of CAFs. One illustrative example comes from studies in which the gene for Notch1 was deleted in epidermal keratinocytes in mice [14]. Consequential to an impaired barrier function of the skin, a chronic wound healing response ensued and ultimately led to the formation of papillomas and subsequent overt invasive carcinomas, demonstrating that prolonged activation of a normal stroma may be a causal factor in the development of cancer. Direct evidence for the cancer-initiating capacity of CAFs is provided by a study by Bhowmick and colleagues, in which the TGFβ type II receptor was selectively ablated in fibroblasts by expression of the Cre recombinase from the FSP-1 promoter in genetically modified mice [15]. Upon loss of TGFβ responsiveness in fibroblasts, the mice spontaneously developed intraepithelial neoplasia of the prostate and invasive carcinoma of the forestomach. The induction of malignant progression was accompanied by stromal expansion and an increased expression of HGF, leading to augmented signaling by c-Met in epithelial cells. Whether genetic alterations in stromal fibroblasts leading to transformation of adjacent epithelia are frequent events in the initiation of cancer is still unclear. Strikingly, isolated skin fibroblasts from patients predisposed to develop basal cell carcinoma because of germline mutations in one allele of the gene encoding *PTCH1* (Gorlin syndrome) exhibit features of CAFs, including the secretion of keratinocyte growth factor and SDF-1α [16]. Moreover, several studies demonstrate tumor-promoting effects of *Trp53* inactivation in the stromal compartment, and genetic inactivation of *Pten* in fibroblasts accelerates initiation and progression of mammary tumors [17–19].

Evasion of apoptosis

In addition to providing cues permissive for proliferation, oncogenic signaling invariably also triggers apoptotic pathways

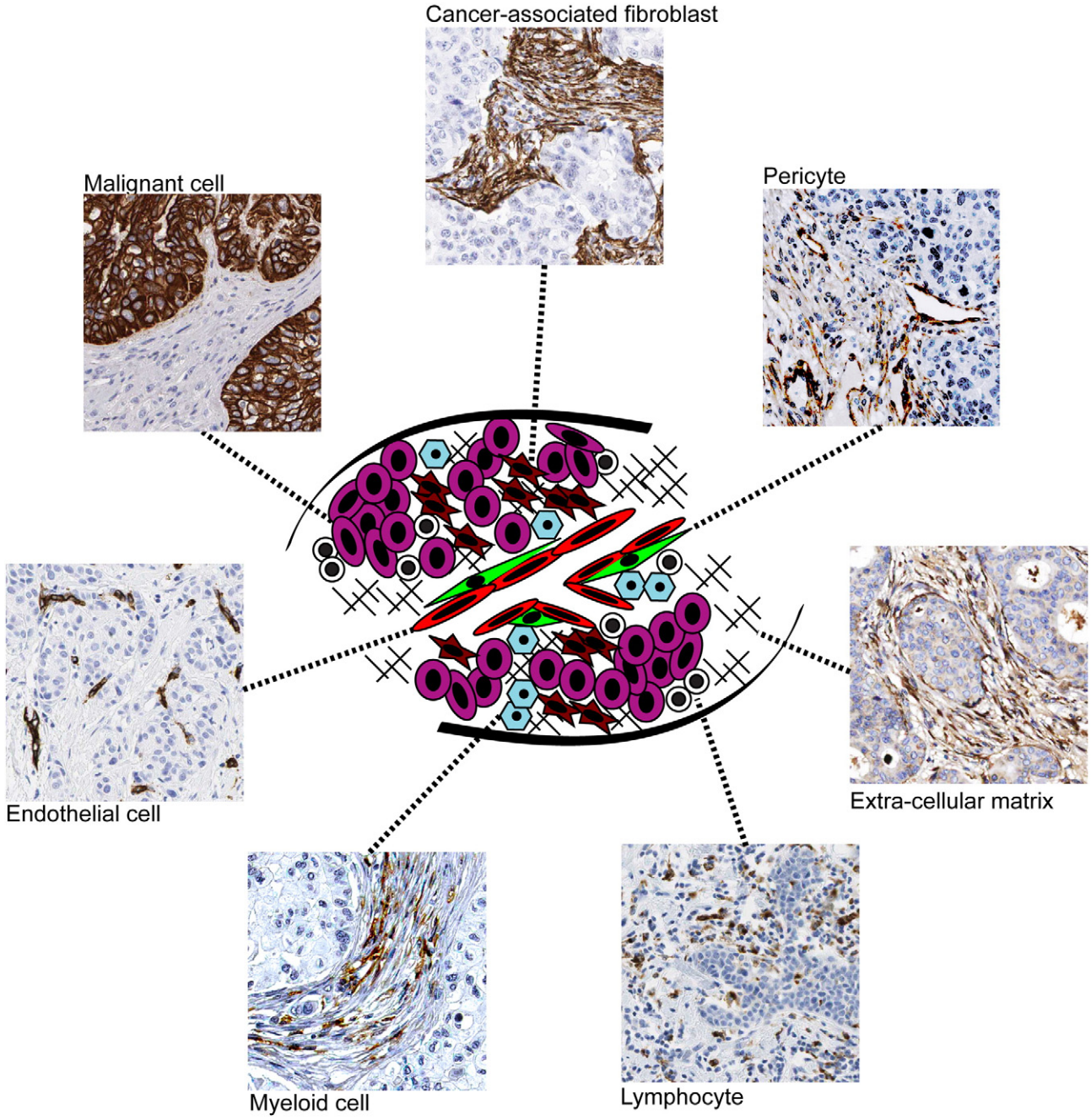

Fig. 1 – A schematic cartoon portraying the various constituent cell types within a tumor, surrounded by micrographs from breast carcinomas illustrating immunohistochemical staining of representative markers for each compartment. Markers depicted are: Malignant cells, cytokeratin 14; Cancer-associated fibroblasts, α-SMA; Pericytes, PDGF receptor-β; Extra-cellular matrix, collagen-1a1; Lymphocytes, CD45; Myeloid cells, CD11c; Endothelial cells, CD34. All images taken from the public database Human Protein Atlas ([72], http://www.proteinatlas.org).

within transformed cells. Part of the ability of tumor cells to evade programmed cell death is derived from survival signals supplied by the stromal compartment. CAFs are known to produce insulin-like growth factor-1 and -2 [20,21], the actions of which appear to predominantly impart tumor growth by conveying survival signals. Moreover, CAFs are abundant providers of extra-cellular matrix (ECM) components, such as various types of collagen. A conceptually novel study demonstrated the importance of ECM stiffening, *i.e.* collagen abundance and cross-linking, in the process of malignant growth and invasion [22]. In conjunction with an increasing ECM stiffening during the malignant progression of oncogene-expressing breast epithelium in transgenic mice, activation of the prototypical PI3 kinase and Akt survival signaling pathway ensued downstream of integrin ligation by collagen fibers.

Sustained angiogenesis

A role for CAFs as providers of pro-angiogenic factors is well documented. A survey of angiogenic inducers expressed within tumors showed that all factors, apart from VEGF-A, were produced in higher quantities by stromal cells, as compared to the overt tumor cells [23]. Also, using mice expressing green fluorescent protein under the VEGF promoter, Fukumura *et al.* demonstrated a dramatic induction of VEGF promoter activity in stromal cells in spontaneous mammary tumors or upon implantation of solid tumors [24]. In related studies, prostaglandin E_2 produced by cyclooxygenase-2, as well as signaling by the bradykinin B_2 receptor, was found to induce production of VEGF by CAFs [25–27]. Also, paracrine activation of PDGF receptor signaling, or autocrine signaling by the cytokine CXCL14 in CAFs induces the expression of FGF-2 [5,28,29]. Finally, secretion of SDF-1α from CAFs enhances the recruitment of endothelial progenitor cells into the tumor neo-vasculature, thereby promoting the angiogenic phenotype of xenograft tumors derived from the MCF-7-ras human breast carcinoma [30].

Pericytes are crucial regulators of endothelial cell function. However, the ultimate outcome of pericyte-derived signals appears to be highly context-dependent. The recruitment of pericytes into tumor blood vessels is dependent on signaling by PDGF receptor-β. Thus, genetic perturbation of PDGF-B expression has been used to elucidate the role of pericytes in tumor angiogenesis and growth. Ectopic expression of PDGF-B in mouse B16 melanoma cells accelerates tumor growth consequential to an increased recruitment of pericytes and stabilization of the neo-vasculature [31,32]. In sharp contrast, transfection of PDGF-B into colorectal or pancreatic carcinoma cell lines results in growth inhibition through pericyte-mediated angiostatic effects [33]. Additional complexity is brought about by diverse effects on pericyte recruitment depending on the cellular source of PDGF-B. Tumors grown in mice engineered to express a deletion variant of PDGF-B that is not retained in close proximity to the producing cell display a hemorrhagic and disordered vasculature, with pericytes partly detached from the endothelium [11]. Surprisingly, the functional and morphological vessel phenotype does not result in an altered tumor growth rate. Taken together, it may be that a certain proportion of different subsets of pericytes needs to be maintained in order to achieve an optimal balance between blood vessel growth and function during angiogenesis.

Tissue invasion and metastasis

As cancers progress into a malignant state they attain the capability of invading surrounding tissue and seeding metastases through lymphatic or blood vessels. In order for this to occur, tumor cells need to acquire a migratory phenotype and bring about extensive remodeling of the surrounding ECM. CAFs contribute to the invasive and metastatic process by inducing epithelial-to-mesenchymal transition of tumor cells through secretion of TGFβ and HGF [13]. Moreover, CAFs represent a source for various types of protease activity, including matrix metalloproteases, cathepsins and plasminogen activators [34]. Remodeling of the ECM by proteases conceivably enhances tumor invasion and metastasis by severing the adhesion between tumor cells and adjacent cells or matrix, by paving a path through degradation of the ECM, and by making different growth factors with ECM-adhering properties bio-available. In addition, a recent study suggests that epithelial cells undergo permanent pro-invasive changes upon exposure to stromal cells [35].

A role for pericytes in limiting metastatic spread of tumor cells has also been described [36]. Mice deficient for neural cell adhesion molecule (NCAM) are more prone to develop metastatic disease when challenged with tumors. In conjunction, the vasculature of tumors devoid of NCAM is leaky and characterized by dysfunctional endothelial cell and pericyte interactions. Moreover, an increased rate of metastasis from spontaneously arising pancreatic islet tumors is observed in RIP1-Tag2 mice genetically engineered to be pericyte-poor [36]. However, it is still unclear whether pericytes are active participants in the metastatic process, or if they merely represent a physical barrier to extravasation.

Therapeutic implications

CAF- and pericyte-mediated resistance to therapy

Given the wealth of stroma-derived factors aiding in cancer initiation, growth and progression, the stromal compartment is likely to influence therapeutic outcome, as well as provide ample opportunities for targeting. Indeed, a stroma-derived gene expression signature prognostic of clinical outcome was described for breast carcinoma [37]. Further evidence for the notion that CAFs determine therapeutic outcome in breast cancer patients comes from elucidation of a CAF gene signature predictive for response to neoadjuvant chemotherapy [38]. Moreover, recent pre-clinical studies indicate that CAFs also mediate resistance to anti-angiogenic therapy and targeted therapy using tyrosine kinase inhibitors [39,40]. In addition to direct modulation of the sensitivity of tumor cells to anti-cancer agents, CAFs harbor the capacity to influence trans-capillary transport of drugs through PDGF receptor-mediated regulation of the interstitial fluid pressure in tumors [41,42].

Novel targeting opportunities

Various strategies have been employed in pre-clinical studies in order to eliminate or disarm CAFs. Notably, targeting of PDGF receptors, hedgehog signaling or fibroblast activation protein through pharmacological inhibition or vaccine-mediated approaches holds the promise of generating beneficial effects [28,43–45].

Little is known about the signals interchanged by pericytes and endothelial cells, but *in vitro* studies suggest that pericytes provide endothelial cells with survival signals through cell-to-cell contacts. Indeed, targeting of pericytes by the use of PDGF receptor inhibitors concurrently induces pericyte detachment and an enhanced sensitivity of endothelial cells to various anti-angiogenic regimens, including inhibition of VEGF signaling and metronomic chemotherapy [46–48]. Interestingly, a number of clinically approved targeted agents, including sunitinib and sorafenib, incorporate both PDGF and VEGF receptor-inhibitory action.

Emerging areas in CAF and pericyte research

Towards an improved CAF and pericyte taxonomy

It is becoming well recognized that CAFs within individual tumors display heterogeneity, and that the dominating CAF phenotypes

also differ within and between histological tumor types. Clinical significance of these variations is suggested by many recent studies demonstrating prognostic or response-predictive significance of CAF-derived gene-expression profiles or markers, such as the PDGF receptor-β, SPARC or α-SMA [37,38,49–51]. Identification of biologically relevant CAF subsets is therefore highly warranted. It is likely that the functional variations of CAFs are derived from their activation by different growth factors, such as PDGF, TGFβ or hedgehog. The cell of origin is another likely source for this variation. Tissue-type differences in fibroblast profiles have been described, and it can also be assumed that CAF progeny derived from bone-marrow-derived precursors will differ from those derived from local precursors.

A series of approaches can thus be envisioned for identification of biologically relevant CAF subsets. The activation status of different growth factor pathways should be possible to identify in gene expressions profiles from *in situ* CAFs by comparisons with profiles obtained from tissue culture studies with selected growth factors. Novel methods for *in situ* description of growth factor signaling status should also facilitate these efforts [52]. Another approach would be the identification of master transcription factors with global effects on CAF phenotypes. One example of this type of studies is the recent identification of FoxF1 as a CAF phenotype inducer, of particular significance for some subsets of non-small cell lung cancer [53]. FoxF1 expression was strongly correlated with hedgehog activation, highlighting the connection to developmental pathways, since a hedgehog-FoxF1 pathway in mesenchymal cells has been defined during lung development.

It is less clear whether there are also discrete subpopulations of pericytes within tumors. However, judging from differential expression of markers, such as PDGF receptor-β, NG2, desmin and α-SMA, it appears that not all pericytes are alike [12]. Studies utilizing bone-marrow transplantation suggest that a subset of tumor pericytes is derived from Sca-1^+ progenitor cells recruited from the bone-marrow [12]. The pericyte progenitor cells are denoted by high expression of PDGF receptor-β, and subsequently undergo differentiation into mature pericytes expressing NG2 and desmin. No information on the potential functional significance of different pericyte phenotypes is available as of yet. However, a recent study indicates that various subsets of pericytes respond differently to combined blockade of VEGF and PDGF [54]. Given the proposed role for pericytes in the development of resistance to anti-angiogenic therapy [55], a better understanding of pericyte subpopulations may enable the prediction of response in patients to this new class of agents. Towards that goal, preliminary studies demonstrate differential pericyte marker expression in tumors that are responsive or resistant to anti-VEGF receptor therapy (KP, unpublished observation).

Multi-potency of peri-vascular cells

Apart from being an integral part of the vasculature, pericytes have been unexpectedly attributed with mesenchymal stem cell properties. Isolated pericytes from a range of normal tissues exhibit expression of hallmark proteins for mesenchymal stem cells and harbor the potential to differentiate into myogenic, osteogenic, chondrogenic, and adipogenic lineages [56]. Confirmation and a functional significance for the multipotency of pericytes is provided by the demonstration that pericytes secrete the extra-cellular matrix component laminin-511/521, thereby promoting epidermal tissue renewal [57]. Intriguingly, laminin-511 is a substrate that supports the proliferation of various stem cell populations, and cancer stem cells in different malignancies have been demonstrated to preferentially reside in peri-vascular niches [58,59]. It is tempting to speculate that pericytes in the tumor vasculature also holds mesenchymal stem cell-properties and influence cancer cell and stem cell propagation, although experimental evidence for this proposition is still lacking.

Communication between mesenchymal cells and leukocytes

Studies on the tumor stroma have traditionally focused on how the different stromal cell types interact with the malignant cells. Evidently, a network of communication also occurs between the different stromal cell types. Some examples which have been highlighted above are the interactions between pericyes and endothelial cells, and the CAF-endothelial cell cross-talk. It is likely that these studies will be extended by analyses of many other pairs of stromal cells. To this end, development of *in vitro* assays which include multiple different stromal cell types is highly warranted.

It is increasingly appreciated that inflammation is a hallmark of cancer in its own right, and that the inflammatory process triggers tumor initiation, growth and progression [60]. Strikingly, already at early stages of tumor progression, CAFs harbor a pro-inflammatory signature which promotes macrophage infiltration, angiogenesis and tumor growth [61,62]. In addition, CAFs participate in the inflammatory process by modulating the activity and polarization of different immune cells, thus improving angiogenesis and bringing about immune evasion. A study by Liao *et al.* showed that elimination of CAFs by a DNA vaccination approach dramatically shifted the immune microenvironment in experimental 4T1 breast cancer in a manner that involved suppressed recruitment of tumor-associated macrophages, and a shift from a Th2 to a Th1 polarization [63]. Immunosuppresive effects of CAFs were also demonstrated by analyses of *in vitro*-cultured melanoma-derived CAFs, which interfered with NK-cell cytotoxicity and cytokine production [64]. Finally, reduced T-cell infiltration was implied as one of the mechanisms underlying the anti-metastatic effects of depletion of S100A4 in the tumor microenvironment [65].

Tumors that develop in mice deficient for the pericyte-specific gene regulator of G-protein coupled signaling 5 display improved vessel function, as witnessed by relieved tumor hypoxia, and the vasculature adopts a permissive state for trafficking of immune effector cells [66]. Thus, pericytes evidently aid in tumor evasion of immune rejection, possibly by orchestrating both autonomous and endothelial cell changes in phenotype.

Systemic effects of CAFs

A series of experimental studies have demonstrated that tumor-derived signals exert long-distance pro-metastatic systemic effects, *e.g.* by activation of bone marrow-derived cells that contribute to the formation of a pre-metastatic niche in distant organs [67,68]. Similarly, growing subcutaneous tumors have been shown to act as instigators for tumor growth at secondary injection sites [69]. Some of the molecules involved in these effects have been identified and include members of the VEGF family, osteopontin, and lysyl oxidases. CAFs are known producers of these factors and it can thus be envisioned that CAFs, in general, act as an important source

of such systemically acting pro-metastatic factors. Experimental strategies to further test this could include comparisons of the "instigator-potency" of tumors that differ in their mesenchymal composition. Such notions could also be explored by analyses of clinical material. It would, for example, be most interesting to investigate if some of the elevated secreted components included in CAF-derived poor prognosis profiles can be detected at increased levels also in corresponding blood samples.

Enforcing the barrier function of normal fibroblasts

In spite of the past and ongoing development of novel molecularly targeted therapies it is likely that the biggest impact of cancer research on public health will be derived from improved strategies for cancer prevention. Research performed from a microenvironment perspective will hopefully contribute to this process. It has been well argued that the normal microenvironment acts as a potent barrier for tumor growth through multiple mechanisms. A better understanding of the molecular details of this microenvironmental barrier could possibly uncover new strategies for prevention.

In this context it is noteworthy that co-culture experiments with various cancer cell lines have consistently shown that normal fibroblasts exert anti-growth effects on cancer [70]. To what extent the microenvironment can affect the recently promoted phenomena of oncogene-induced senescence or DNA damage response also appears as most interesting topics for future studies. Some support for environmental regulation of these processes was obtained with the recent demonstration of hedgehog-induced modification of the DNA damage response in mouse embryonic fibroblasts [71].

Concluding remarks

Taken together, CAFs and pericytes are increasingly recognized as integral parts of the tumorigenic process. Conceivably, studies in this area will be most productive if they manage to integrate tissue culture studies, analyses in animal models and characterization of human tissue material representing early stages of cancer. Given the multitude of cellular interactions already described, the prospects for development of novel anti-cancer therapies or preventive strategies based on an understanding of the communication within the tumor stroma should be excellent.

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