

Can Targeting Stroma Pave the Way to Enhanced Antitumor Immunity and Immunotherapy of Solid Tumors? CME

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Abstract

Solid tumors are complex organ-like structures. The potential of normal neighboring cells to contribute to the initiation, progression, and metastasis of epithelial-derived carcinomas has long been appreciated. However, the role of host cells has proven complex. Through multiple local and systemic mechanisms, nontransformed host cells can promote transition from a tumor-resistant to tumor-permissive environment, drive neoplastic transformation of epithelial cells, promote tumor growth, progression, and metastasis, but also constrain tumorigenesis. This complexity reflects the spatially and temporally dynamic involvement of multiple cell types and processes, including the development and recruitment of inflammatory, immune, endothelial, and mesenchymal stromal cells, and the remodeling of extracellular matrix. Our mechanistic understanding, as well as our ability to translate advances in our understanding of these mechanisms for therapeutic benefit, is

rapidly advancing. Further insights will depend on delineating pathways that mediate the communication networks between inflammatory and immune cells with tumor and mesenchymal stromal cells and extracellular matrix. Here, we discuss the diversity of mesenchymal stromal cell populations and how context can dictate either their promotion or constraint of tumorigenesis. We review evidence for plasticity that allows for reprogramming of stromal cells and how tumor immunogenicity and desmoplasia influence the balance of immune-independent and immune-dependent regulation of tumor growth. The pivotal roles of matrix and mesenchymal stromal cells in modulating inflammation, antitumor immunity, and the efficacy of immune-based therapies are discussed. These concepts have emerged from data obtained from tumors of multiple organs, but we focus mostly on studies of pancreatic ductal adenocarcinomas. *Cancer Immunol Res*; 4(4); 269–78. ©2016 AACR.

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Learning Objectives

Cross-talk between multiple types of nontransformed host cells and tumor cells determines, in a spatially and temporally dynamic fashion, the relative resistance versus permissiveness of tissues to tumor initiation. It also determines the propensity toward tumor latency and dormancy versus progression and the emergence of metastatic disease. Through completion of this activity, the participant will gain a fundamental understanding of the communication networks between heterogeneous cancer-associated stromal cells and immune cells in the context of solid tumors. The potential for extracellular matrix remodeling and stromal cell targeting to modulate antitumor immunity and the efficacy of immune-based therapies will also become evident.

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Background and Rationale

The initiation and evolution of epithelial-derived solid tumors (i.e., carcinomas) involves extensive communication between epithelial cells and various nontransformed host cells such as mesenchymal stromal cells (MSC), fibroblasts, endothelial cells, and inflammatory and immune infiltrates (1–5). As reviewed in a prior Masters in Immunology article that also focused on carcinoma-associated fibroblasts (CAF; ref.6), tumor-associated

stromagenesis shares many aspects of wound healing, in which fibroblasts play critical roles in orchestrating inflammation, angiogenesis, and extracellular matrix (ECM) remodeling required for tissue repair. Many human carcinomas are characterized by CAFs that deposit and organize a matrix rich in collagens, hyaluronic acid (HA), and fibronectin, much like that found in wounds. Unlike in wounds, however, this pathophysiological response fails to resolve, resulting in the desmoplastic microenvironment common to many carcinomas (7, 8). However, the degree of desmoplasia in tumors can vary dramatically.

The conventional paradigm regarding the role of cancer-associated stromal cells (CASC) is that, regardless of invoking individual molecular mechanisms that can have opposing effects that either promote or constrain tumor growth and metastasis, their net effect is protumorigenic. But in fact, their net effect reflects the balance between protumorigenic and antitumorigenic activities that can vary depending on tumor type, regionally within tumors, and the ecology of the host milieu. Another important factor is the stage in evolution of a particular tumor. Stroma is an important factor in the tumor nonpermissive state of normal tissue that transitions to a tumor permissive state with perturbation and tumor progression (Fig. 1A and B). Thus, as is the case in the immune and vascular systems, tumor progression is associated with a stromagenic switch. Transition of all three compartments is integrally related with progression from immune editing to a state of immune equilibrium, to immune escape (9) and angiogenesis occurring in concert with the reprogramming of stromal cells and matrix remodeling in an interdependent manner. Indeed, stromal cells have emerged as important drivers of immune modulation through their release of cytokines, chemokines, and other soluble factors such as prostaglandin E₂, and their expression of ligands for inhibitory receptors such as CTLA-4 and PD-1 (Fig. 1B). In addition, stromal cells deposit the ECM, which can present a physical barrier to immune cell infiltration but also provides the substratum essential to the interstitial migration of immune cells (10).

CASCs are phenotypically and functionally heterogeneous, raising the possibility that some of the opposing effects on tumor and immune cell behavior might be propagated by distinct subpopulations, or by cells at different stages as they co-evolve with their neighbors. Evidence indeed has emerged to support this latter concept in models of pancreatic cancer, as discussed below. CASCs can affect tumorigenesis via mechanisms defined broadly as immune independent and immune dependent (Fig. 1A, D, and E). In both cases, myriad mechanisms, some with potentially opposing effects on tumor cell behavior, appear to be involved. In addition, the stroma can play major roles in resistance to therapy. Thus, a systematic understanding of the role of the distinct subpopulations involved and the complex networks of stromal cell-dependent intracellular, cell-matrix, and soluble factor-mediated biochemical signaling pathways and biomechanical forces underlying tumorigenesis and the immune response to cancer has important implications for the advancement of cancer prevention, diagnostics, prognostics, and development of novel targeted therapies. In particular, understanding the stromagenic response in cancer has important implications for the development of immune-based cancer therapies that, rather than being effective in relatively small subpopulations of patients, might be effective in the vast majority of patients afflicted with a broad array of solid tumor types.

Heterogeneity of CAFs

CAFs are found in virtually all human carcinomas, but their prevalence varies dramatically. They are typically prevalent in pancreatic, non-small cell lung, colorectal, breast, and prostate cancers but relatively sparse in ovarian, thyroid, renal, brain, and head and neck cancers (11, 12). CAFs are often identified morphologically by their elongated spindle-like fibroblastic appearance. To date there is no consensus on the molecular definition of CAFs, but several markers have been used to identify them, including fibroblast-specific protein 1 (FSP-1), vimentin, desmin, neuron-gial antigen-2 (NG2), platelet-derived growth factor receptor- β (PDGFR β), podoplanin, fibroblast-associated antigen, prolyl 4-hydroxylase (4, 13–16), and the two that we focus on herein, alpha-smooth muscle actin (α SMA) and the plasma membrane serine protease fibroblast activation protein (FAP). Alpha-SMA is a well-established marker for myofibroblasts (MF) and MF-like cells in the tumor microenvironment (17), but it is also expressed in visceral smooth muscle cells, perivascular smooth muscle cells, and pericytes (18). FAP is a robust CAF marker originally found highly expressed in various solid tumors but not in most quiescent stromal cells (12, 19). However, as discussed in detail in Dr. Fearon's Masters of Immunology article (6), it is also expressed in mesodermal cells in multiple tissue types under homeostatic conditions and in tissues that undergo active remodeling (20–24).

FAP⁺ and α SMA⁺ cells represent overlapping, but distinct populations of CASCs with the degree of overlap ranging from several percent (e.g., in breast or pancreatic cancer) to virtually a hundred percent (e.g., lung squamous cell carcinoma) depending on the tumor type (25–28). FAP is also expressed on a subset of M2-like (CD11b⁺F4/80⁺CD206⁺) tumor-associated macrophages (25, 26, 29), which must be taken into account when interpreting studies aimed at defining the role of FAP⁺ cells that include CAFs and this subset of macrophages versus other populations such as α SMA⁺ cells. Gastric tumors from reporter mice expressing red fluorescent protein driven by the SMA promoter contain multiple phenotypically distinct subsets of tumor stromal cells, including SMA⁻ MSCs (30), whereas others showed that MSCs express FAP (24). Therefore, MSCs likely account for at least a portion of the FAP⁺SMA⁻ cells observed in the microenvironment of many human and murine carcinomas. Interestingly, these cells were shown to have the capacity to both self-renew, as well as differentiate into SMA⁺ myofibroblasts, both in wound healing and the tumor microenvironment (30, 31), suggesting that a lineage relationship exists between some terminally differentiated SMA⁺ myofibroblasts and mesenchymal progenitor cells that at some stage may express both SMA and FAP, but at least some of which may later downregulate or lose FAP expression, but retain SMA. Similar analyses of other markers provide further evidence for the heterogeneity of CAFs.

One source of heterogeneity of CAFs is the multiple cells from which they can be generated (5), including resident fibroblasts, stellate cells, and bone marrow-derived and local mesenchymal stem cells (MSC) or by trans-differentiation of epithelial or endothelial cells (Fig. 1; refs.30, 32–38). CAFs can also originate from adipocytes or adipose tissue-associated stromal cells (39–41). Additionally, bone marrow-derived fibrocytes or MSCs can be recruited into tumors and differentiate into CAFs (30, 39, 42, 43).

Regardless of the source, the phenotypic heterogeneity of CAFs raises the interesting question as to their potential to exert distinct

effects on tumorigenesis, which has important implications for developing stromal cell-targeted therapies. Indeed, taken together, the results of three recent independent studies, discussed in detail below, indicate that although FAP⁺ CASCs promote tumorigenesis, α SMA-expressing MFs may constrain the aggressiveness of tumors in related models of pancreatic cancer (27, 28, 44).

The Stromagenic Switch

Normal fibroblasts and MSCs, analogous to the immune system, have the capacity to protect against the emergence of neoplastic epithelial cells (Fig. 1B; refs.45, 46). The conditional inactivation of the TGF β type II receptor gene in mouse fibroblasts allowed epithelial tumors to develop, which indicated that normal fibroblasts can constrain epithelial tumorigenesis and that genetically modified fibroblasts can be sufficient to drive tumorigenesis (47). This concept is reinforced by the demonstration that PTEN, p53, and IKK β signaling pathways in fibroblasts also play critical roles in restraining the tumorigenic potential of multiple solid tumors (48–50). In addition, fibroblast-derived HIF1 α and asporin inhibit breast cancer tumorigenesis and progression, respectively (51, 52). Taken together, in addition to the immune-mediated tumor-suppressive mechanisms, stromal surveillance programs may also prevent tumorigenesis or impose a dormant state (53). Whether any such stromal-dependent pathways affect tumorigenesis, at least in part through immune-dependent mechanisms, has yet to be fully explored.

Both preneoplastic cells and established tumors evolve in part by overcoming stroma-mediated barriers and by driving transition of tumor-suppressive fibroblasts into tumor-promoting CAFs. Indeed, many studies have demonstrated that normal fibroblasts are functionally distinct from CAFs (Fig. 1C). Prostatic CAFs are more proliferative and less prone to contact inhibition than normal prostate fibroblasts (54). Moreover, prostatic CAFs are more dependent on anaerobic glycolysis and produce more lactate relative to normal prostatic fibroblasts (55). Colonic CAFs enhance the proliferation and migration of tumor cells to a greater extent than do normal colonic fibroblasts (56). Metabolic competition can contribute to immune suppression in the tumor microenvironment and thus may be another mechanism by which the stroma affects antitumor immunity (57–59). Normal fibroblasts can be reprogrammed into CAFs that drive tumor progression, invasion, and metastasis by modulating various aspects of the tumor microenvironment, including ECM remodeling, angiogenesis, and inflammatory and immune responses (60–67). For example, CAFs have a greater capacity to drive inflammation and angiogenesis relative to their normal counterparts (30, 68). These functional differences between CAFs and their normal counterparts reflect transcriptional regulation and genetic and epigenetic programs that drive evolution of the gene expression profiles in fibroblasts (67, 69–73). It should be noted that CAFs may retain some tumor-inhibitory mechanisms as well. For instance, some primary CAF lines derived from breast cancer secrete Slit ligands that bind Robo1 expressed by breast cancer cells. Robo1 signaling inhibits PI3K/Akt signaling and thereby restrains tumor growth (74).

An important question is whether the transition of fibroblasts to an activated and protumorigenic state is preventable or reversible, which might present an opportunity to reprogram stromal cells as a therapeutic approach. In wound repair, resolution is associated with apoptosis of activated fibroblasts. In contrast, in

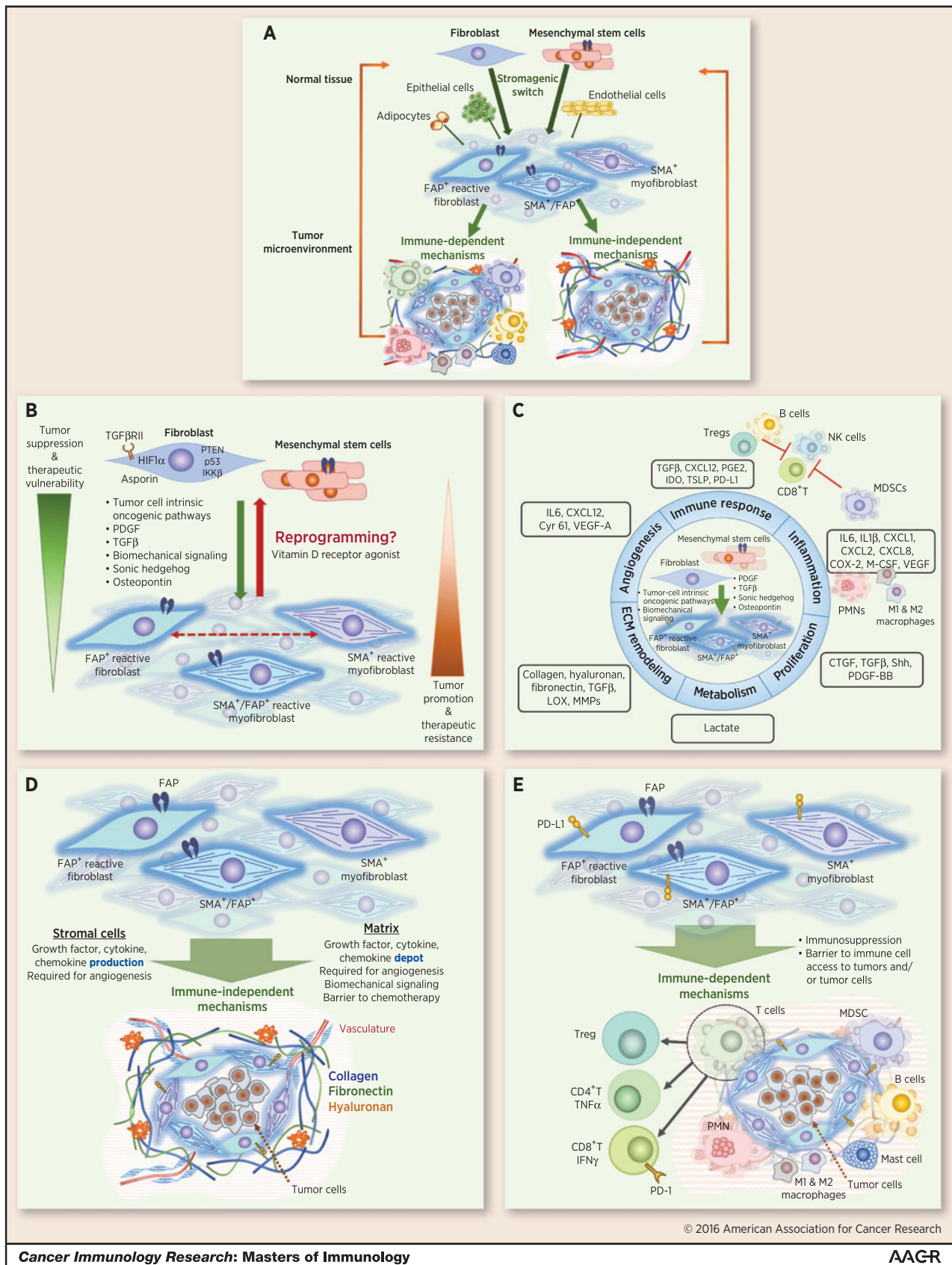
pathologic fibrosis and tumors, activated fibroblasts persist. However, the differentiation of fibroblasts to the fibrotic and protumorigenic state may indeed be reversible. Specifically, the vitamin D receptor agonist calcipotriol reversed the stellate cell response in hepatic fibrosis (75) and pancreatitis (76). Calcipotriol also reduced markers of inflammation and fibrosis and, when given in combination with gemcitabine, reduced tumor volume and increased survival in a model of pancreatic cancer (Fig. 1B; ref. 76).

Modulation of Inflammation and Immunity

Inflammation is increasingly appreciated as an important contributing factor in promoting the development of a wide range of malignancies, including colon, gastric, liver, lung, and pancreatic cancers (77–81). Studies indicate that cross-talk between inflammatory cells and tumor stroma regulates the desmoplastic response in tumors, and the desmoplastic response may limit infiltration and regulate the function of infiltrating leukocytes. CAFs orchestrate the inflammatory response by secreting cytokines such as IL6 and IL1 β (Fig. 1). Secreted chemokines such as CXCL1 and CXCL2 recruit tumor-associated neutrophils and macrophages (68, 82). Carcinomas, including breast, lung, and pancreatic cancers, exhibit CAF-mediated NF- κ B-dependent pro-inflammatory gene signatures (68, 83, 84). CAFs promote survival and polarization of tumor-associated myeloid cells to an M2 macrophage phenotype (85). This alternative activation of macrophages is likely due to the overexpression of IL6, TGF β , and CCL2 by CAFs (86–88).

Activated pancreatic stellate cells (PSC) can secrete SDF-1 α /CXCL12 to promote CD8⁺ T-cell chemotaxis toward the juxtatumoral stroma, thereby preventing CD8⁺ T cells from accessing tumor nests. Knockdown of SDF-1 α /CXCL12, or treatment of activated PSCs with all-*trans* retinoic acid, rendered PSCs quiescent, abrogated CD8⁺ T-cell chemotaxis toward PSCs, while enhancing CD8⁺ T-cell proximity to neoplastic cells in pancreatic cancer (89). In contrast, SDF-1 α /CXCL12 derived from FAP⁺ CAFs bound to pancreatic cancer cells and inhibited T-cell access to tumor cells. Inhibition of CXCR4 (a receptor for SDF-1 α /CXCL12) by AMD3100 reversed immune suppression and potentiated the efficacy of checkpoint blockade with anti-PD-L1 to restrain the growth of pancreatic tumors (44). Additionally, cancer-associated PSCs can promote the differentiation of peripheral blood mononuclear cells into myeloid-derived suppressor cells (MDSC) by secreting pro-inflammatory cytokines, including IL6, M-CSF, VEGF, and SDF-1 α /CXCL12. IL6 and STAT3 signaling was required for PSC-induced MDSC differentiation that in turn inhibited T-cell activation, thereby contributing to tumoral immune suppression (90). Depleting granulocytic MDSCs with anti-Ly-6G antibody treatment increased intratumoral accumulation of activated CD8⁺ T cells, enhanced tumor cell apoptosis, and remodeled the tumor stroma in pancreatic tumors (91). CAFs can also impair activation, cytokine production, and cytotoxicity of NK and T cells through secretion of prostaglandin E2 and indoleamine 2,3-dioxygenase (92, 93).

Conversely, inflammatory myeloid cells can promote CAF activation and desmoplasia. The IL6 or SDF-1 α /CXCL12 secreted by M2 macrophages can promote human prostatic fibroblast activation, as measured by α SMA expression (88). Treatment with the anti-inflammatory drug dexamethasone reduces the recruitment of Gr-1⁺CD11b⁺ cells, decreases CAF accumulation, and decreases collagen deposition, thereby attenuating squamous carcinoma



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progression (85). CAFs and M2 macrophages also act in concert to activate endothelial cells to enhance angiogenesis (88).

Finally, desmoplastic stroma can represent a physical barrier for naturally occurring antitumor immunity and T-cell-based cancer immunotherapy. Tumor stroma precludes T-cell infiltration and migration into tumor cell nests in human lung cancers *ex vivo*. Collagenase treatment augments T-cell mobilization and increases the accessibility of T cells to tumor cell nests (94). Expression of heparanase, which degrades heparan sulfate proteoglycans in the ECM, can potentiate tumor infiltration of CAR-redirectioned T cells and augments the antitumor activity (95).

Immune-Dependent and Immune-Independent Mechanisms

The role of stromal cell populations in tumorigenesis has primarily been determined by depleting them in established tumors. Early studies used FAP vaccines and antibody-based targeting. A dendritic cell-based FAP vaccine inhibited the growth of multiple tumor types (96). Depletion of CD8⁺ T cells partially abrogated the antitumor effects of this vaccine. However, it was not determined whether this was due to the elimination of the subpopulation of CD8⁺ T cells specific for FAP⁺ stromal cells (induced by the vaccine) or the loss of antitumor CD8⁺ T cells that had been unleashed by reducing the number of FAP⁺ stromal cells. An oral FAP vaccine suppressed primary tumor growth and metastasis in mouse models of colon and breast carcinoma (97). FAP⁺ stromal cells played an important role in matrix remodeling as evidenced by a significant reduction in collagen in tumor tissues of FAP-vaccinated mice. Although the mechanisms involved were not determined, this study also provided early evidence that targeting tumor stroma could enhance uptake of chemotherapeutic drugs. Tumor growth could also be inhibited by other approaches, such as immunoconjugates based on antibodies to FAP (98), and using a FAP-activated prodrug to target carcinoma-associated stromal cells (99). No overt toxicity was observed in any of these studies other than a modest delay in wound healing in the first vaccine study.

Two other direct approaches can delete stromal cell subpopulations. Genetic approaches have been used to conditionally ablate either SMA⁺ cells in mouse models of pancreatic cancer (27) or FAP⁺ stromal cells in related models of pancreatic cancer as well as a syngeneic transplant model of lung cancer (100). The former used transgenic mice expressing a thymidine kinase gene under the control of the α SMA promoter. Upon administration of ganciclovir, a subset of α SMA⁺ CASC were depleted. These investigators posited that proliferating myofibroblasts were preferentially deleted, whereas α SMA⁺ vascular smooth muscle cells, pericytes, and myoepithelial cells were

spared. This approach led to negligible loss of FAP⁺ stromal cells, consistent with evidence for minimal overlap between FAP⁺ and α SMA⁺ stromal cell subsets in pancreatic ductile adenocarcinoma (PDA). Contrary to expectations based on evidence that stromal cells in established tumors are on balance protumorigenic, conditional ablation of this subset of α SMA⁺ CASC was associated with a more aggressive tumor phenotype and reduced animal survival. Tumors were invasive and undifferentiated, with enhanced hypoxia and epithelial-to-mesenchymal transition, and had more cancer stem cells. In retrospect, this result is perhaps not surprising, given that fewer myofibroblasts in tumors correlate with reduced survival in PDA patients. The impact on tumorigenesis was associated with suppressed immune surveillance and increased CD4⁺Foxp3⁺ regulatory T cells and a reduction in collagen, but not HA. Although tumors in α SMA⁺ cell-depleted mice did not respond to gemcitabine, which may require loss of HA (101, 102), anti-CTLA-4 immunotherapy reversed the disease progression and prolonged survival.

In marked contrast, conditional ablation of FAP⁺ stromal cells inhibited tumor growth in both a lung and a pancreatic tumor model. BAC transgenic mice were constructed that express the human diphtheria toxin receptor (DTR) under control of a FAP promoter (FAP.DTR mice). Upon administration of diphtheria toxin to tumor-bearing FAP.DTR mice, FAP⁺ stromal cells were ablated. Depletion of FAP⁺ cells inhibited tumor growth of established tumors derived from Lewis lung cancer cells expressing ovalbumin (OVA) as a nominal antigen. Inhibition of tumor growth was achieved in spite of FAP⁺ stromal cells representing a very small minority of total tumor cells in this model. Depletion of FAP⁺ cells using this approach also inhibited the growth of established tumors in a transplant model of PDA (100). Although the role of immune-independent mechanisms was not investigated in this study, immune-mediated mechanisms were implicated. Specifically, although intratumoral T-cell populations did not change, the effect of FAP⁺ stromal cell depletion in the lung tumor model was negated by neutralizing antibodies to TNF α and IFN γ . FAP⁺ stromal cell depletion had no effect on PDA tumors transplanted into immune incompetent RAG-deficient mice, in apparent contradiction to results from other studies in which immune-based FAP⁺ stromal cell depletion inhibited the growth of transplanted PDA tumors to a similar extent in immune competent and immune incompetent NSG mice (28). Subsequent studies from the same group implicated FAP⁺ hemoxygenase-expressing macrophages in both the lung and PDA transplant models (29). Furthermore, SDF-1 α /CXCL12 derived from FAP⁺ fibroblasts was implicated as a mediator of immune suppression in the KPC autochthonous model of PDA. Consistent with a role of stromal cell-derived SDF-1 α /CXCL12, blockade of SDF-1 α /CXCL12 synergized with

Figure 1.

Evolution of tumor stroma and its immune-dependent and immune-independent control of tumor growth. A, overview. B, multiple stromal cell types found in normal tissues, including fibroblasts, mesenchymal stem cells, adipocytes, endothelial cells, and epithelial cells, can (trans)differentiate into CAFs/stromal cells. This stromagenic switch that transitions a tumor-suppressive environment to a tumor-promoting environment is driven by multiple factors, including those indicated, and may be reversible based on recent evidence of reprogramming following treatment with the vitamin D receptor agonist calcitriol. Two prominent subclasses are myofibroblasts, characterized by the expression of proteins of the contractile apparatus such as α SMA, and FAP⁺ reactive fibroblasts. These two subpopulations are distinct but overlap to varying degrees in different tumor types and can have opposing effects on tumorigenesis, at least in pancreatic cancer. C, the products regulated in CAFs relative to normal fibroblasts that promote cell proliferation: a shift from oxidative phosphorylation to glycolysis, matrix remodeling, angiogenesis, inflammation, and immune suppression in the tumor microenvironment are shown. The indicated pathways regulate tumor growth through immune-independent (D) and immune-dependent (E) mechanisms.

immune checkpoint inhibitors (anti-PD-L1) in controlling tumor growth following conditional ablation of FAP⁺ cells (44).

Finally, several groups have used adoptive transfer of FAP-specific redirected T cells to deplete FAP⁺ stromal cells in a variety of tumor models (25, 28, 103–106). The majority of these studies utilized adoptive transfer of FAP-specific chimeric antigen receptor expressing (FAP-CAR) T cells. FAP-CAR have been generated using single-chain Fv regions based on the sequence of three different monoclonal antibodies (mAb) to FAP, two of which, 73.3 (25) and MO36 (105), inhibited tumor growth in multiple tumor models and exhibited little if any toxicity unless administered repeatedly or expressed in hyperfunctional T cells (25, 28, 104). The loss of antitumor activity of 73.3 FAP-CAR T cells in FAP-deficient mice established the specificity of these CAR-T cells and demonstrated that the antitumor activity was dependent on expression of FAP by host stromal cells. The third, based on FAP5, showed no significant antitumor activity and at the same time caused significant toxicity (107). The toxicity of this particular CAR appears to be attributable to recognition of a distinct epitope on FAP that resulted in depletion of any cell expressing FAP, regardless of amount, whereas 73.3FAP-CAR selectively depletes FAP^{high} cells (25, 28). Consistent with this possibility, toxicity was also noted after extended periods of time in naïve or tumor-bearing FAP.DTR mice that had been treated with diphtheria toxin and therefore lacked all FAP⁺ cells (108). Depletion mediated by 73.3FAP-CAR was not only partial, but also transient, which again may limit toxicity. Collectively, with the one exception, these studies of FAP-CAR T cell-mediated depletion implicate FAP⁺ stromal cells as potential targets across a broad spectrum of solid tumors.

The studies described above show that inhibition of tumor growth after depleting FAP⁺ cells can occur through both immune-dependent and immune-independent mechanisms (Fig. 1A, D, and E). Deletion of FAP⁺ cells enhanced both spontaneous and vaccine-induced endogenous immunity (25, 100) and enhanced the antitumor effect of checkpoint inhibitors (44). The degree to which immune-dependent versus immune-independent mechanisms contributed relates to the immunogenicity and degree of desmoplasia, with the latter directly related to the prevalence of FAP⁺ stromal cells (28). Thus, the elimination of stromal cell-mediated immune suppression resulted in enhanced endogenous and vaccine-induced antitumor immunity that contributed to inhibition of tumor growth in immunogenic tumors. On the other hand, the inhibition of growth of highly desmoplastic nonimmunogenic tumors in immune-competent mice and moderately and highly desmoplastic human xenografts in immune-incompetent mice was attributed to immune-independent mechanisms. The immune-independent mechanisms involved stromal cell depletion and the disruption of matrix that led to reduced angiogenesis, stromagenesis, and tumor cell proliferation and increased tumor cell apoptosis.

In the one study where it was analyzed, depletion of FAP⁺ also led to depletion of α SMA⁺ cells, even in models of PDA where only a small proportion of the stromal cells coexpress α SMA and FAP (28). This indicates that either α SMA⁺ CASCs are derived from FAP⁺ progenitors and/or that FAP⁺ cells are required for the generation or recruitment of α SMA⁺ cells to the tumor microenvironment. In either case, loss of FAP⁺ cells overcame the protumorigenic effect of depleting α SMA⁺ stromal cells. Tumor growth was inhibited with no evidence of more aggressive tumor phenotypes. It will, of course, be of interest to define the relationship between various stromal subsets in future studies.

Potential Risks and Benefits

Tumor cells exhibit intratumoral heterogeneity and genomic instability, in many cases rendering tumors resistant to therapeutic intervention and ultimately causing treatment failure. In contrast, nontransformed CASCs are genetically stable, making them appealing targets for developing therapeutic strategies. Stromal cells in many solid tumor types share properties, and therapies targeting them can potentially synergize with other tumor cell and immune-targeted therapies.

In addition to revealing the role of specific subpopulations and the mechanisms by which they modulate tumorigenesis, preclinical models of stromal cell depletion can guide the development of selective depletion therapies in patients. However, preclinical studies have provided reason to proceed with caution, given two associated risks. First, some subpopulations of stromal cells are protective. It will be necessary to identify them, keeping in mind that these may differ in the context of various tumor types or in different stages of disease. This concern is highlighted by the evidence that whereas targeting FAP⁺ stromal cells in PDA may prove therapeutic, targeting SMA⁺ cells in this tumor type might in fact be detrimental (27, 28, 44). In any case, because α SMA is localized to the cytoplasm, it is not readily apparent how current technologies could be used to efficiently target SMA⁺ cells. This practical concern, however, does not apply in the case of FAP that localizes to the surface of stromal cells. Deletion of FAP⁺ cells has now been analyzed in preclinical models of multiple tumor types and resulted in inhibition of tumor growth in every case save one, with no evidence that this approach enhances tumor aggressiveness. In the one exception, deletion had neither a beneficial nor a detrimental impact on tumor progression. Longer-term studies, however, will be required to evaluate the impact on metastatic disease.

The second major potential concern is of on-target/off-tumor effects. In the case of α SMA, this may be a moot point in the face of the evidence that targeting α SMA⁺ CASCs may be contraindicated, at least in the one tumor type studied thus far. However, this concern does apply to targeting FAP⁺ cells. FAP⁺ stromal cells reside in many tissues of the adult mouse, including skin, bone marrow, skeletal muscle, pancreas, adipose, and lymph node. They can serve homeostatic functions as well as important positive roles in reparative responses, such as tissue remodeling in wound healing. Indeed, genetic ablation (108) or immune-targeting studies using mAb FAP5-based FAP-CAR T cells (107) have shown that depletion of FAP⁺ stromal cells induces bone marrow hypoplasia, cachexia, and anemia. FAP⁺ stromal cells from skeletal muscle are also a major source of follistatin, a protein that can promote muscle growth. FAP⁺ stromal cells in the bone marrow produce SDF-1 α and KitL, which are essential in regulating B lymphopoiesis and erythropoiesis (108). Fibroblastic reticular cells in lymph nodes also express FAP, and experimental ablation of these cells in mice disrupts lymph node homeostasis and can impair the launch of an effective immune response to clear influenza virus infection (109).

Thus, FAP⁺ stromal cells are important in the maintenance of normal muscle mass, lymph node homeostasis, and hematopoiesis. Nonetheless, although caution is warranted, the data suggest that partial and/or transient depletion of FAP⁺ stromal cells may provide a therapeutic window in which tumor growth can be inhibited with little to no toxicity. Although this approach may be limited in efficacy when used as a monotherapy, intriguing early

evidence indicates that this approach can synergize in combination with chemotherapies or other immune-based therapies such as tumor vaccines and immune checkpoint inhibitors. Multiple approaches are also under development to spatially and temporally control CAR T-cell activity as a means to circumvent off-tumor activity (110–114).

Defining Molecular Pathways

Although a detailed analysis is beyond the scope of this article, it is important to address an alternative approach to targeting stromal cells for depletion. An intense focus is under way to define stroma-dependent molecular pathways amenable to targeting, which would inhibit protumorigenic mechanisms and to overcome therapeutic resistance. In this regard, FAP itself, which functions as a cell-surface protease involved in matrix remodeling, has been explored as a potential therapeutic target. Genetic deletion of FAP and pharmacologic inhibition of its protease activity inhibits primary growth in syngeneic transplant models of lung, and colon cancer, and a KRAS-driven autochthonous model of lung cancer (115). Its role in metastasis is actively being investigated. FAP is a type II transmembrane cell-surface proteinase belonging to the prolyl dipeptidyl aminopeptidase (DPP) family, which cleaves amino-terminal dipeptides from polypeptides with proline or alanine in the penultimate position (P₁Pro or P₁Ala; ref.116). FAP also exhibits endopeptidase activity that preferentially cleaves after the Gly-Pro sequence motif (P₂GlyP₁Pro; ref.117). Although FAP was initially discovered in membrane-bound form, low concentrations of circulating soluble FAP, also known as α 2-antiplasmin-cleaving enzyme (APCE), have been reported in human and mouse serum (118–120). FAP protease activity *in vitro* has been studied extensively, but its substrate repertoire *in vivo* is not fully defined. *In vitro* screening identified neuropeptide Y, B-type natriuretic peptide, substance P, and peptide YY as potential substrates. Moreover, CCL22/MDC, CXCL2/Gro β , and CXCL12/SDF-1 α can be cleaved by FAP, albeit less efficiently (121). FAP's endopeptidase activity is capable of modifying gelatin, type I and type III collagens, FGF21, and α 2-antiplasmin (118–120, 122–124). Many of these substrates have been implicated in tumor progression; for instance, CXCL12/SDF-1 α is crucial for promoting tumor cell invasion, angiogenesis, and T-cell exclusion (44, 89, 125, 126); collagen is important in enhancing tumor cell proliferation, invasion, and metastasis (127–129). Nonetheless, the functional consequences of FAP-dependent proteolytic processing in the context of the tumor microenvironment, other than its direct role in matrix remodeling, remain to be explored.

Another pathway that has drawn attention is Sonic hedgehog (Shh), a soluble ligand critical for driving the formation of desmoplastic stroma that is overexpressed by pancreatic tumor cells. Deletion and pharmacologic inhibition of Shh reduces stromal contents in autochthonous pancreatic tumors. However, Shh-deficient tumors developed earlier and were more aggressive, exhibiting undifferentiated histology and heightened levels of angiogenesis and proliferation. Administration of VEGFR-blocking antibody improved survival of Shh-deficient tumor-bearing mice, indicating that Hedgehog-driven stromagenesis suppresses tumor growth in part by restraining tumor angiogenesis (130). Together, these studies raise substantial concerns as to whether targeting Shh is in fact a promising direction for pancreatic cancer.

The SDF1 α /CXCL12–CXCR axis is yet another pathway of interest as discussed previously in this venue (6). Finally,

Box 1. Future Directions

1. Do changes in stroma contribute to the increase in cancer risk associated with aging?
2. Do stromal-dependent mechanisms play a role in obesity-associated increased cancer risk?
3. Does stroma provide common therapeutic targets across tumor types?
4. Does stroma provide therapeutic targets common to primary tumors and metastatic disease?
5. Can targeting stroma overcome resistance to chemotherapy and immunotherapy for cancer?

promising data have been obtained using a pegylated hyaluronidase (PEGPH20) to disrupt the HA-rich matrix in pancreatic cancer. All told, although explorations of targeting stromal-dependent molecular pathways are in their infancy, they hold great potential. Studies suggest that the evolution of stromal cells to a protumorigenic state may be reversible (Fig. 1B). Future efforts will therefore undoubtedly include attempts to reprogram CASCs to shut down their protumorigenic functions and activate or reactivate antitumorigenic functions at primary tumor sites and maintain or restore a tumor nonpermissive environment in target organs of metastases.

All of these are promising avenues to pursue in the quest to enhance the antitumor activity of conventional tumor-targeted therapies, whether they are dependent on antitumor immunity or act through immune-independent mechanisms, or both, and to improve the response rate to, and efficacy of, rapidly emerging immunotherapies for cancer.

Future Directions

Over the past several decades, the identification of malignant cell-intrinsic oncogenic and tumor suppressive pathways has been extremely successful. This has led to the birth of targeted therapies for cancer. Here, we have tried to convey a sense of our burgeoning understanding of the significance of extrinsic factors in tumorigenesis and the potential wealth of new therapeutic targets they present. Future studies will be required to test the assumption that targeting stromal pathways will synergize with malignant cell-targeted chemotherapies, radiation therapy, and immunotherapies in solid tumors. In addition, the concept that stromal targets may be shared between tumor types and between primary and metastatic disease will need to be tested. The appreciation of the role of stroma-dependent pathways also raises the question as to how they interface with risk factors such as aging, obesity, and smoking. Elucidating the molecular pathways that mediate the impact of stroma on tumorigenesis will lay the groundwork required to understand and manage cancer risk (Box 1).

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