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Tumor Microenvironment in Pancreatic Cancer Pathogenesis and Therapeutic Resistance

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Abstract

Pancreatic ductal adenocarcinoma (PDAC) features a prominent stromal microenvironment with remarkable cellular and spatial heterogeneity that meaningfully impacts disease biology and treatment resistance. Recent advances in tissue imaging capabilities, single-cell analytics, and disease modeling have shed light on organizing principles that shape the stromal complexity of PDAC tumors. These insights into the functional and spatial dependencies that coordinate cancer cell biology and the relationships that exist between cells and extracellular matrix components present in tumors are expected to unveil therapeutic vulnerabilities. We review recent advances in the field and discuss current understandings of mechanisms by which the tumor microenvironment shapes PDAC pathogenesis and therapy resistance.

1. INTRODUCTION

Pancreatic ductal adenocarcinoma (PDAC) is the most common malignancy of the pancreas and portends a dismal 5-year survival rate of 11% (1). For patients diagnosed with localized surgically resectable disease, outcomes have steadily improved over the past 10 years (1). However, most patients present with regional or distant metastasis, and for these patients, disease progression even with treatment intervention is the norm. Chemotherapy is the mainstay of treatment for PDAC, and while some patients derive significant clinical benefit, ultimately therapeutic resistance prevails. This resistance underscores the resilience of pancreatic cancer and reflects its remarkable capacity to orchestrate a microenvironment that is tumor protective.

The microenvironment that surrounds cancer cells is a key determinant of tumor growth and metastatic potential as well as treatment resistance, an unfortunate hallmark of PDAC (2). The PDAC microenvironment is involved in both primary and acquired resistance to therapies and extends beyond cytotoxic chemotherapies to targeted therapies and immune-modulating treatments (2). As such, there is much to gain from a more thorough understanding of the components of the microenvironment in PDAC and how they impact cancer cell biology. Many of the microenvironmental features that support PDAC pathogenesis are reminiscent of those that direct the development of endoderm-derived tissues. For example, endoderm-derived tissues are shaped by interactions between the epithelium and surrounding mesenchyme (3, 4). In the pancreas, mesenchymal cells control the proliferative capacity and cell fate specification of the developing epithelium by secreting instructive cues that regulate morphogenesis and, in doing so, engage reciprocal signaling pathways. In the context of pancreatic cancer, mesenchymal cells play similar roles as regulators of paracrine signaling and epithelial cell fate but without the exquisite regulation observed in the embryo. Further, PDAC has a particularly prominent mesenchymal compartment within its stroma compared with other solid tumors, often comprising most of the tumor volume. This stromal compartment includes fibroblasts, extracellular matrix (ECM) components, immune cells, nerves, and endothelial cells, which together profoundly impact neoplastic cell phenotypes.

The complexity of the PDAC microenvironment has presented a challenge to efforts aimed at better understanding the diverse cell types and acellular features therein and how they impact tumor progression. This complexity encompasses the mechanisms and consequences of heterotypic cell–cell interactions as well as spatial heterogeneity that impacts cell function and may contribute to PDAC pathogenesis. Spatially heterogeneous features of the PDAC landscape include cellular communities surrounding tumor-infiltrating nerves, tertiary lymphoid structures (TLSs), and distinct stromal versus peritumoral regions. Each of these features may contribute to tumor progression and the prospect for tumor evolution in the context of treatment (**Figure 1**). However, despite this underlying appreciation for the complexity of PDAC and its surrounding tumor microenvironment, the determinants that direct heterogeneity, adaptability, and dynamics across disease states remain largely ill defined. Addressing these gaps in knowledge forms the basis for ongoing investigations to unravel key pathways and molecules that sustain critical cell–cell interactions in the heterocellular milieu of tumors and to discover spatial relationships among diverse cell types that dictate the fate and function of cancer cells. Herein, we discuss recent insights into the cellular contexture and structure of the tumor microenvironment in PDAC and how the microenvironment that surrounds cancer cells shapes tumor pathogenesis and treatment resistance.

# 2. THE CELLULAR CONTEXTURE AND STRUCTURE OF THE TUMOR MICROENVIRONMENT

At first glance, the microenvironment that surrounds PDAC appears highly disorganized with a scattered arrangement of cancer cell nests. These nests are bordered by infiltrating cells that
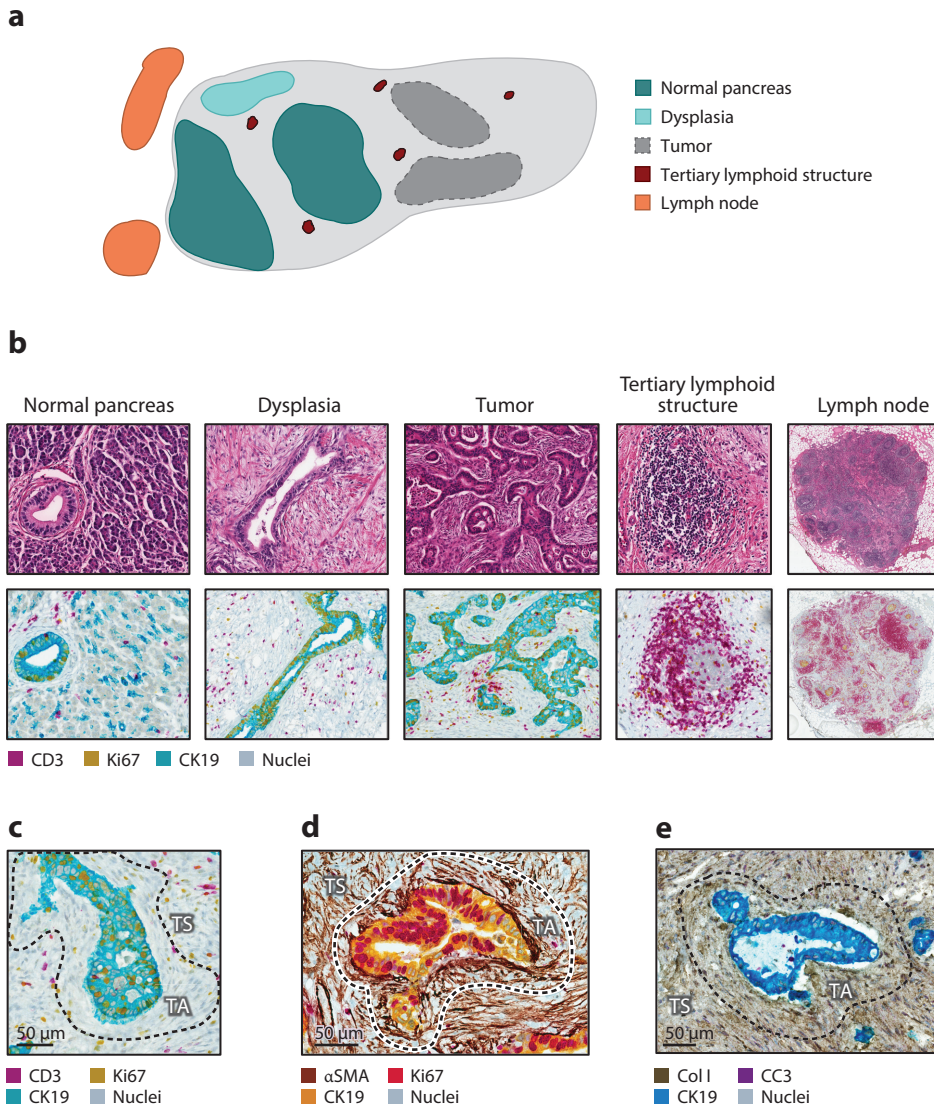
Figure 1

The PDAC microenvironment. (*a*) Schematic of PDAC lesion showing discrete cellular communities and their relationship to tumor regions. (*b*) Representative images showing hematoxylin and eosin staining (*top row*) and mIHC (*bottom row*) of cellular communities depicted in panel *a*. (*c*)–(*e*) Representative mIHC images showing the spatial relationship of cells and matrix elements with cancer cells (CK19). Regions of TA and TS stroma are depicted and separated with a dashed black line. Abbreviations: CC3, cleaved caspase 3; Col I, type I collagen; mIHC, multiplex immunohistochemistry; PDAC, pancreatic ductal adenocarcinoma; SMA, smooth muscle actin; TA, tumor-adjacent; TS, tumor-associated.

compose a stromal reaction that weaves in between. However, histological analyses of PDAC tissues have unveiled spatially distinct tumor subregions that represent unique ecosystems within tumors (5). Subregions enriched in cellular infiltrates (termed reactive) are characterized by transcriptional and proteomic signatures that are distinct from subregions wherein cellular infiltrates
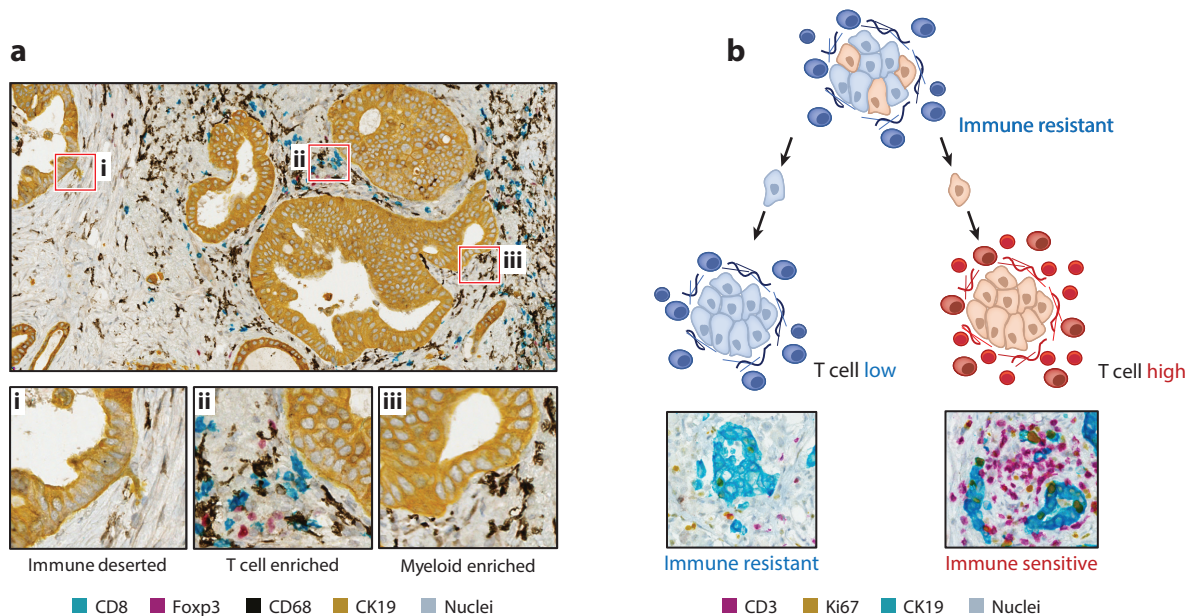
Figure 2

Spatially defined immune heterogeneity in PDAC. (*a*) Representative images showing mIHC staining of human PDAC to detect cellular communities that are immune deserted, T cell enriched, and myeloid enriched. (*b*) Conceptual model depicting a nest of clonally heterogeneous cancer cells that upon migration into the stroma give rise to either T cell low or T cell high tumor nests. Shown are representative mIHC images of T cell low and T cell high regions detected in human PDAC. Abbreviations: mIHC, multiplex immunohistochemistry; PDAC, pancreatic ductal adenocarcinoma.

are sparse (termed deserted). Reactive subregions are associated with cellular stress responses, whereas deserted subregions are enriched in ECM signaling. Notably, cancer-associated fibroblasts (CAFs) isolated from these distinct subregions are phenotypically and functionally distinct. Specifically, CAFs isolated from reactive subregions appear more motile and are more supportive of tumor proliferation. Together, these findings suggest that the phenotype of the stromal reaction that surrounds cancer cells contributes to tumor cell phenotypes and varies spatially within tumor lesions. In line with this idea, deserted subregions tend to harbor more well-differentiated tumors, whereas reactive subregions are associated with tumors defined by moderate and poor differentiation.

The stromal response to PDAC produces multiple distinct histopathologic regions that culminate in communities defined by unique biological processes. These regions include the immediate tissue adjacent to tumor cells (tumor-associated stroma), the prominent stromal compartment that separates cancer cell nests (tumor-adjacent stroma), TLSs, regions of dysplasia, adjacent normal pancreas tissue, and peritumoral and peripancreatic lymph nodes (**Figure 1**). Within each of these tissue subregions, the contexture and spatial organization of cells are distinct. For example, quantitation of the leukocyte infiltrate in PDAC further separates tumors into hypoinflamed, myeloid enriched, and lymphoid enriched (**Figure 2**) (6). Patients with surgically resected tumors characterized as lymphoid enriched tend to have a more favorable prognosis consistent with findings showing that the quality and quantity of T cell infiltrates seen in surgically resected PDAC tumors correlate with survival (7). However, the leukocyte density varies significantly by histopathologic subregion. For instance, adjacent normal tissue often contains a strong leukocyte infiltrate that is not seen in normal healthy pancreatic tissue (6). The predominance of tumor-adjacent stroma also

contributes to the significant spatial heterogeneity that is a hallmark of PDAC. To this end, this spatial heterogeneity represents a challenge to the interpretation of the cellular infiltrate on the basis of biopsies or tissue microarrays, which only sample a finite portion of a tumor lesion. Thus, PDAC is defined by multiple discrete cellular communities that coalesce around cancer cell nests and, in doing so, form a neoorgan that intrudes on the normal biology of adjacent healthy tissues.

Despite its well-recognized classification as an immunologically cold tumor, PDAC is commonly associated with a prominent leukocyte infiltrate (**Figure 2**). Notably, tumors often contain TLSs that are reminiscent of lymph node follicles (**Figure 1**). TLSs are lymphocyte aggregates that arise in peripheral tissues and when present within tumors, including PDAC (8–10), usually associate with a more favorable outcome (11). The maturation of TLSs is a continuum with some tumors containing early TLSs that primarily consist of T and B cells without the high-order architecture that is characteristic of mature TLSs. Overall, tumors that contain TLSs show an increased presence of lymphocytes in the tumor-adjacent stroma (8). However, tumors with mature TLSs, as compared with tumors with early TLSs, associate with more favorable survival outcomes after surgical resection (9). Studies in mouse models have revealed that TLS formation can be induced by chemokines including CXCL13 and CCL21 (10). Further, vaccines induce the formation of TLSs in resectable PDAC that associate with improved survival (12). Nonetheless, the precise mechanisms by which TLSs influence survival outcomes and the stromal response to PDAC remain poorly understood.

PDAC displays a high proclivity for metastasis to distant tissues (e.g., liver, lung, peritoneum, and lymph nodes, among other tissues). Yet, most of our understanding of the pathophysiology of human PDAC is based on findings from primary resection of tumors arising in the pancreas. Historically, primary and metastatic lesions in PDAC were viewed as biologically distinct. However, genetic studies suggest remarkable similarity between matched primary and metastatic lesions, although metastasis may require drivers beyond those involved in primary tumor formation (13, 14). Further, matrix components of the stromal compartment that surround cancer cells appear relatively similar in primary and metastatic PDAC lesions (15). Notably, metastatic lesions acquire and sustain a dense desmoplastic reaction through the recruitment of fibroblasts and leukocytes that is typical of primary tumors (15, 16). However, the distant organ microenvironments (e.g., liver and lung) within which these metastatic lesions most commonly arise differ substantially from the pancreas. Disseminated tumor cells (DTCs) are also subjected to a Darwinian selection process as part of the metastatic cascade (17). Cancer cell–extrinsic features associated with the matrix and cellular contexture of the distant organ microenvironment are known to influence the metastatic behavior of DTCs (18). Thus, despite the apparent genetic and stromal similarities seen between primary and metastatic lesions, it is likely that individual cancer cell clones or clusters of cancer cells give rise to metastatic lesions with unique biological phenotypes that ultimately render them distinct from primary lesions. Together, these observations underscore the biological complexity of PDAC. However, further studies will be needed to better understand the cellular and matrix contexture of both primary and metastatic PDAC lesions in patients and to inform determinants that coordinate the spatial arrangement of cells and proteins within distinct tumor subregions. Addressing these gaps in knowledge is expected to provide novel insights into PDAC pathogenesis, unique therapeutic targets, and biomarkers associated with disease subsets.

3. HOW THE TUMOR MICROENVIRONMENT SHAPES TUMOR PATHOGENESIS

A local wounding environment renders a tissue permissive to tumor formation (19). Consistent with this concept, functional roles for the fibroinflammatory microenvironment in tumor

Table 1 Tumor-restraining and -promoting features of the PDAC microenvironment

Microenvironmental element	Tumor-restraining features	Tumor-promoting features
CAF	CAFs restrain tumor progression by depositing matrix proteins (41, 42, 48–50).	CAFs secrete metabolites that support cancer cell proliferation (53–58). CAFs can also suppress antitumor immunity (59).
Extracellular matrix	Type I collagen deposition suppresses tumor growth and metastasis (74, 75).	Matrix proteins engage cancer cells through integrins and support cancer cell proliferation and migration (72–74).
Myeloid cells (including dendritic cells, macrophages, granulocytes, and early myeloid progenitor cells)	Myeloid cells can be redirected therapeutically with antitumor (114) and antistromal (113, 114) properties. Myeloid cells can be engendered with immune stimulatory properties required for productive antitumor T cell immunity (117, 118).	CD11b+ myeloid cells facilitate PanIN development and PDAC progression (32–34). Myeloid cells also enable many of the hallmarks of cancer (114–116).
Treg cells	Treg cells suppress the protumor potential of CAFs (45).	Treg cells can inhibit the efficacy of immunotherapy by suppressing cytotoxic T cells (107).
T cells	Tumor-infiltrating cytotoxic T cells can survey and eliminate cancer cells (136, 144).	CD4 T cells secrete IL-17, which supports cancer progression (108), and IL-27, which suppresses effector activity of tumor-infiltrating T cells (20).
B cells	There is insufficient evidence to support a restraining role for B cells.	B cells stimulate cancer cell proliferation via IL-35 (110) and activate tumor-promoting macrophages (111).
Vasculature	High expression of genes involved in vascular stability associate with a better prognosis (83).	Increased cancer cell autophagic flux is a consequence of low vascularity due to limited nutrients and supports cancer survival, immune evasion, and therapeutic resistance (53, 127, 128).
Peripheral nerves	There is insufficient evidence to support a restraining role for peripheral nerves.	Innervation increases during pancreatic tumorigenesis (96) and supports tumor progression (101, 102).

Abbreviations: CAF, cancer-associated fibroblast; IL, interleukin; PDAC, pancreatic ductal adenocarcinoma; PanIN, pancreatic intraepithelial neoplasm; Treg, T regulatory.

progression have been well established in numerous cancer types, including PDAC. However, the functional roles of stromal cell types are complex and often exhibit context dependency with respect to stage of tumor evolution as well as anatomical site. These complex interactions between evolving tumors and their microenvironment impel careful investigation of cell types and mechanisms that shape PDAC progression, in hopes of parsing those that restrain from those that support tumor growth (**Table 1**). In the following sections, we discuss the composition of the PDAC microenvironment, the heterogeneity of cellular components therein, and the developing understanding of spatial relationships among these diverse cell types.

3.1. Tumor Microenvironment Development and Evolution

Reciprocal interactions between transformed epithelial cells and their surrounding microenvironment influence the development and evolution of PDAC. Insights into mechanisms that coordinate this biology have been informed by genetically engineered mouse models that faithfully

recapitulate the early stages of pancreatic tumorigenesis. For example, genetic mouse models incorporating epithelial-targeted Kras mutations, which are present in more than 90% of human PDAC (21), form the basis for modeling stepwise PDAC progression (22–24). Moreover, early hints that alterations in the tissue microenvironment functionally contribute to early-stage PDAC progression came from mice harboring Kras^{G12V}, using elastase-driven Cre to target this mutation to acinar and centroacinar cells. While expression of this driver mutation in the pancreatic epithelium throughout development yielded invasive PDAC, induction of Kras^{G12V} in the pancreas of adult mice was insufficient to drive the formation of premalignant lesions and invasive cancer. However, pancreatic inflammation combined with Kras^{G12V} expression effectively triggered premalignant pancreatic intraepithelial neoplasia (PanINs) formation and subsequent invasive PDAC at a high penetrance (24). The inflammatory microenvironment associated with PDAC was later found to suppress oncogene-induced senescence in Kras^{G12V}-expressing epithelial cells, thereby eliminating an important barrier to tumor progression (25). Notably, inflammatory cues induce expression of Kras and its effectors in the adult pancreas, while such expression is only marginal in noninflamed pancreatic tissue. Further, Kras activation drives oncogenic levels of mitogen–activated protein kinase (MAPK) signaling and enables tumor initiation (26). However, inflammation in the absence of an existing Kras mutation also appears to sensitize pancreatic epithelial cells to subsequent transformation by Kras (27). Specifically, pancreatic epithelial cells undergo transcriptional and epigenetic reprogramming during recovery from acute inflammation. While this adaptive response limits tissue damage from subsequent inflammatory events, it also primes epithelial cells for transformation by Kras. Thus, pancreatic tumorigenesis is an evolutionary process that is influenced by cell autonomous and paracrine factors.

Kras-transformed epithelial cells instruct and shape the stromal response in PDAC. The importance of Kras signaling in coordinating the microenvironment has been demonstrated in mouse models featuring inducible and reversible expression of Kras^{G12D} (28, 29). Here, the early response to Kras^{G12D} induction is associated with upregulation of Hedgehog signaling, leukocyte infiltration, and accumulation of a collagen-rich stroma. Formation of this fibroinflammatory stroma and subsequent PanIN development are accelerated when Kras^{G12D} is induced in the context of pancreatitis. However, extinguishing mutant Kras expression in established lesions results in rapid remodeling of the tumor stroma, including reduced abundance of activated fibroblasts and resolution of inflammation (28, 29). This finding illustrates the reciprocal relationship between oncogenic signaling in the pancreatic epithelium and the fibroinflammatory microenvironment.

Oncogenic mutations in Kras promote tumorigenesis via the activation of diverse effector molecules. Kras also cooperates with Myc, a pleiotropic transcription factor involved in regulating a variety of genetic programs that support cellular proliferation (30). Genetic activation of Myc in low-grade Kras^{G12D}-induced PanIN lesions leads to rapid accumulation of immune cells and activated stromal components that together facilitate the development of high-grade PanINs and invasive PDAC (31). Remarkably, inactivation of Myc in the epithelial compartment of established PDAC results in rapid disassembly of the tumor stroma and subsequent tumor cell death, as seen with inactivation of Kras. Thus, these findings demonstrate the dependence of PDAC on both KRAS and MYC signaling, which in tandem support a dynamic relationship between cancer cells and their surrounding microenvironment.

Kras signaling by cancer cells enforces an inflammatory reaction characterized by a robust infiltration of myeloid cells. Tumor-infiltrating myeloid cells are fundamental to establishing an immunosuppressive and protumor microenvironment. In mice, CD11b$^+$ myeloid cells are required for Kras-driven PanIN formation. In addition, CD11b$^+$ myeloid cells support tumor growth (32). Kras activation promotes the infiltration and polarization of myeloid cells to an immunosuppressive phenotype in part via induction of the cytokine GM-CSF (33, 34). However, multiple

signals likely converge to establish the inflammatory microenvironment that initiates the early development and progression of PDAC. For example, obesity can promote inflammation and fibrosis in the pancreas, contributing to PanIN development (35, 36). This process is reversible with caloric restriction and is driven by obesity-induced adaptations in the endocrine pancreas that support early-stage PDAC. Specifically, pancreatic islets respond to obesity by upregulating cholecystokinin, which acts in a paracrine manner to promote PDAC development (36). Obesity may also alter the proinflammatory properties of adipocytes and pancreatic stellate cells within tumors that support a desmoplastic reaction (37). Further, diet-induced obesity may promote PDAC tumorigenesis via supporting COX2-dependent inflammation and fibrosis (38) or metabolic aberrations (39). Thus, cancer cell–extrinsic factors (e.g., inflammation and obesity) contribute to the formation of a microenvironment that is pliable and fundamental to pancreatic tumorigenesis.

While inflammation has been shown to be a reproducible proponent of PDAC development and progression, several other prominent stromal components have been implicated in restraining PDAC initiation. Activated fibroblasts adjacent to neoplastic lesions, or CAFs, secrete diverse cytokines, growth factors, and ECM components. CAFs were long thought to be tumor promoting in the context of PDAC. However, landmark studies published in 2014 revealed this to be an oversimplification. Epithelium-derived Hedgehog signaling was initially found to promote pancreatic fibroblast activation and the development of a desmoplastic stroma (40), prompting the expectation that Hedgehog inhibition would suppress desmoplasia and, in turn, tumor formation. Indeed, genetic or pharmacologic inhibition of Hedgehog prior to tumor formation suppresses fibroblast activation and formation of the desmoplastic reaction (41, 42). However, these interventions surprisingly accelerated PanIN formation and PDAC development. Consequently, these studies provided strong evidence for the tumor-restraining potential of CAFs, at least in the early stages of PDAC. While the precise tumor-suppressive mechanisms engaged by PDAC CAFs still remain unclear, type I collagen deletion in fibroblasts can accelerate the emergence of PanIN lesions in the context of mutant Kras and deleted p53 in the epithelial compartment (43). Several immune cell populations in addition to cytotoxic T lymphocytes can also restrain PDAC initiation, including natural killer (NK) T cells (44) and, unexpectedly, T regulatory (Treg) cells (45). The tumor-restraining potential of these immune cells stems from cross talk with other cell populations in the microenvironment, including macrophages in the case of NK T cells and fibroblasts in the case of Treg cells. Altogether, these observations illustrate functionally significant and heterotypic interactions that likely vary in consequence over the course of tumor evolution.

3.2. Organization and Heterogeneity of the Tumor Microenvironment

Interactions among cellular and acellular features of the PDAC microenvironment profoundly impact tumor phenotypes. The diverse nonneoplastic cells that constitute most of the tumor volume in PDAC exhibit inter- and intratumor heterogeneity with respect to their functions and spatial relationships. Our understanding of these microenvironmental components continues to expand, as discussed in the subsections below.

3.2.1. Cancer-associated fibroblasts.
Fibroblasts and CAFs play critical roles in development, tissue homeostasis, and tumor progression (46). However, considerable transcriptional overlap between CAFs and other stromal cell types, together with their extensive heterogeneity (47), has made CAFs challenging to study in specific and robust ways. As a result, the precise contributions of distinct CAF subsets to stepwise tumorigenesis have remained somewhat elusive. In recent years, our understanding of CAF functions in pancreatic cancer has improved dramatically. CAFs may restrain tumor growth by depositing matrix proteins that biophysically contribute to enhanced tissue stiffness and increased interstitial pressures that impede the vasculature and,

in doing so, limit accessibility to serum nutrients (41, 42, 48–50). As a result, the nutrient-poor microenvironment that surrounds PDAC may act to restrain cancer cell proliferation (51, 52). However, CAFs also secrete diverse metabolites that are consumed by cancer cells and, in turn, support their proliferation within the nutrient-poor milieu (53–58). Activated pancreatic fibroblasts also release high levels of CXCL12 (59), which signals through its receptor CXCR4 to impact additional cell types in the microenvironment. To this end, CXCR4 signaling in pancreatic epithelial cells supports PanIN initiation and progression (60). However, invasive PDAC eventually develops in mice lacking CXCR4 in the epithelium, and these tumors are larger and less differentiated than CXCR4-expressing tumors. Collectively, these findings illustrate the context-dependent functional consequences of CAFs on PDAC pathogenesis.

CAF features within PDAC are spatially heterogeneous, suggesting distinct functional roles for CAFs depending on their proximity to cancer cells. For instance, CAFs located adjacent to cancer cells exhibit a classically activated, myofibroblast-like phenotype, whereas CAFs present in the surrounding stromal tissue exhibit an inflammatory phenotype characterized in part by cytokine expression (61). In this regard, single-cell genomics have provided new insights into PDAC CAF heterogeneity and revealed the existence of several subtypes including myofibroblastic and inflammatory CAFs (62–64). However, the precise functions of these CAF subtypes and how their transcriptional programs influence cancer cell fate remain unclear.

Cytokines, including interleukin 1 (IL-1) and transforming growth factor beta (TGFβ), differentially shape CAF heterogeneity (63, 65). For instance, IL-1 supports JAK/STAT (Janus kinase/signal transducer and activator of transcription) signaling and differentiation to inflammatory CAFs. In contrast, TGFβ is antagonistic to this pathway and promotes differentiation to myofibroblasts. Cancer cells also shape CAF phenotype by secreting Hedgehog ligands, which have profound, dosage-dependent effects (41, 42, 48, 66, 67). For example, reduced Hedgehog signaling in CAFs correlates with increased tumor-associated vascularity. Cancer cells with a gain-of-function mutant p53 also differentially influence CAFs and promote their capacity to establish a prometastatic environment (68). In addition to paracrine factors derived from cancer cells that may influence CAF biology, CAF heterogeneity also arises due to distinct cells of origin (69, 70). Though PDAC CAFs were previously thought to arise from a common cell of origin—the pancreatic stellate cell (PSC)—a recent fate mapping study demonstrated that PSCs give rise to a numerically minor subset of PDAC CAFs, with unique functional significance (71). Taken together, cytokines, cancer cell–derived factors, and the progenitor cell of origin are determinants of CAF heterogeneity in PDAC.

3.2.2. Extracellular matrix. The ECM that surrounds cancer cells has an active role in PDAC pathogenesis. CAFs are major producers of components of the ECM, which may suppress or support tumor progression. The ECM directly engages cancer cells through integrins and, in doing so, facilitates cancer cell proliferation and migration (72). The ECM also establishes a reservoir of proteins including cytokines and growth factors that influence the metastatic potential of cancer cells. Among the most abundant ECM proteins are fibrillar collagens (e.g., type I and III collagen) (73). Fibrillar collagens are mainly deposited in the ECM by CAFs, although cancer cells also contribute to 2–3% of fibrillar collagen deposition. However, with PDAC progression, there is an enrichment of fibrillar collagens with retained C-prodomains. Notably, cleavage of procollagen I by cancer cell–derived bone morphogenesis protein 1 (BMP1) stimulates collagen I deposition and suppression of tumor growth and metastasis (74). Consistent with this finding, type I collagen deletion in alpha smooth muscle actin (α-SMA$^+$) cells, including CAFs, accelerates PDAC initiation and growth in mice, leading to shortened overall survival (43). In this case, type I collagen deletion causes an upregulation of *Cxcl5* in cancer cells, promoting recruitment of myeloid cells,

which then facilitate tumor progression (43). In the metastatic microenvironment, deletion of type I collagen in the CAF compartment also accelerates tumor progression with shortened survival (75). However, these findings contrast with the effects of CAF deletion, which results in a decrease in metastatic tumor growth, suggesting that type I collagen opposes the tumor-promoting effects of CAFs. Taken together, these observations motivate careful consideration of collagen fibrillogenesis along with its abundance as a determinant of PDAC pathogenesis.

In addition to producing fibrillar collagens, cancer cells contribute to the ECM by producing matrisome proteins that promote tumor progression at various stages of the metastatic cascade. These proteins include agrin, which supports epithelial-mesenchymal transition, and cystatin B and serine protease inhibitor B5, which enhance invadopodia formation and extravasation (76). Further, matrisome proteins derived from cancer cells correlate with poor outcomes (73). Notably, this contrasts with the favorable prognosis associated with individual matrisome proteins derived from stromal cells (73). This observation supports a tumor-suppressive role for the ECM in PDAC but also emphasizes the complexity of the ECM and the distinct contribution of components contributed by cancer cells, which act to support tumor pathogenesis.

Cancer cells can also regulate ECM composition and stiffness. For instance, genetic inhibition of TGFβ signaling in the epithelial compartment causes elevated mechanosignaling evidenced by activation of STAT3, MLC2, and YAP1 as well as increased matrix stiffness (77). In patients with PDAC, epithelial TGFβ signaling (assessed by SMAD4 mutation status) also associates with elevated mechanosignaling and tissue stiffness, which together correlate with a worse prognosis (77). Further, increased collagen fiber thickness, rather than abundance, correlates with a worse prognosis (77). These findings may indicate a role for β1-integrin signaling in regulation of epithelial STAT3 activation and ECM stiffness. Thus, components of the ECM are influenced by signaling pathways activated in cancer cells.

Proteoglycans and hyaluronan are additional prominent components of the ECM and contribute to water retention and increased interstitial fluid pressures. In particular, hyaluronic acid (HA) promotes a high-pressure PDAC microenvironment and impedes the vasculature, such that enzymatic digestion of HA with a PEGylated hyaluronidase (PEGPH20) reduces fluid pressures in PDAC mouse models. PEGPH20 also increases vascular perfusion and increases delivery of chemotherapeutic agents, specifically gemcitabine, to the tumor microenvironment in mice (49, 50). Consistent with these preclinical findings, PEGPH20 combined with chemotherapy in patients with PDAC increased tumor response rates. However, progression and overall survival were not improved (78). HA may also serve as a fuel source for pancreatic cancer (79). In addition, HA degradation may enhance the efficacy of immunotherapy, suggesting a role for the ECM in coordinating immune resistance in PDAC (80). Taken together, these studies highlight the complexity of the ECM and its role in shaping cancer cell, CAF, and immune biology.

3.2.3. Endothelial cells.
Solid tumors commonly feature abnormal vasculature, including blood and lymphatic vessels. Poor vascularity contributes to a microenvironment characterized by low pH, hypoxia, altered metabolism, and immune evasion. In response to hypoxia, tumors usually invoke mechanisms to stimulate angiogenesis that then support tumor growth and metastasis. In this regard, leaky vessels formed during angiogenesis promote cancer cell aggressiveness at the primary site and serve as a route for hematogenous dissemination (81).

Therapeutic agents that inhibit angiogenesis and/or normalize the tumor vasculature are widely used in the treatment of patients with cancer. For example, bevacizumab is an antiangiogenic agent that binds to and inhibits vascular endothelial growth factor (VEGF). However, despite elevated levels of VEGF detected in PDAC, bevacizumab failed to improve outcomes for patients with PDAC (82). More recently, analysis of PDAC transcriptional profiles has revealed

that high expression of the endothelial cell marker CD31 associates with improved overall survival (83). In a multivariate analysis, low CD31 expression and residual tumor after surgery were the only independent prognostic factors associated with worse survival in PDAC. In contrast, high expression of genes involved in vascular stability including *TIE1*, *TIE2*, *ANGPT1*, *S1PR1*, and *CDH5* (VE-cadherin) associate with a better prognosis. CD31 expression also correlates with immune-related genes including *CD4*, *CD8A*, *GZMB*, and several Toll-like receptors, suggesting that increased vessel density may facilitate antitumor immunity. Thus, the considerable heterogeneity observed in vascular density suggests that a subset of patients may in fact benefit from an antiangiogenic agent. Consistent with this idea, mice with highly vascular PDAC showed modest but significant extension of overall survival with VEGF receptor inhibition (41).

Poor vasculature limits nutrient and oxygen delivery to tumors and restricts leukocyte recruitment. As a result, hypovascularity associates with hypoxia in PDAC. Consistent with this, hypoxia-inducible factor 1α (HIF1α), a key mediator of hypoxia, is stabilized in poorly vascularized PDAC tumors in mice (84). Stabilized HIF1α transactivates an array of genes fundamental to metabolism, angiogenesis, cell survival, and inflammation. Elevated HIF1α also associates with a poor prognosis for many malignancies (85). However, in PDAC, deletion of HIF1α accelerates tumor development in mouse models. In the absence of HIF1α, increased pancreatic tumorigenesis is facilitated by infiltrating B cells (86). This finding illustrates the resilience of PDAC and its intricate redundancies that serve to support disease progression.

In addition to the blood vasculature, lymphatics are fundamental to PDAC pathogenesis. Lymphatics not only are a major conduit for leukocytes to transport tumor antigens to draining lymph nodes for presentation to and priming of tumor-reactive T cells but also are a major route of dissemination in PDAC with cancer cells commonly detected in peritumoral lymph nodes, a finding that correlates with worse survival outcomes (87, 88). The determinants of lymphatic vasculature in PDAC and how they contribute to PDAC pathogenesis, though, are poorly understood and yet likely complex. Mechanistically, chemokines are known to contribute to lymphangiogenesis and cell migration. For example, lymphatic endothelial cells secrete CCL21, which lures dendritic cells via lymphatics to lymph nodes. Tumor cells expressing CCR7 may also co-opt this mechanism to disseminate to lymph nodes (89–91). Similarly, CXCL12 produced in lymph nodes may attract CXCR4-expressing cancer cells or leukocytes (92, 93). Thus, the lymphatic vasculature is fundamental to PDAC metastasis and may be a determinant of impaired immune surveillance.

3.2.4. Peripheral nerves.

The normal pancreas tissue is highly innervated. Autonomic innervation critically regulates development and hormone secretion by the endocrine pancreas (94, 95). Notably, innervation increases rather dramatically during pancreatic tumorigenesis. This is first observed in premalignant lesions (96). PDAC cell invasion of intrapancreatic nerves, known as perineural invasion (PNI), can promote both pain and tumor spread. While PNI is somewhat common in some tumor types, the incidence of PNI in PDAC is particularly high (>80%) (97). In a recent analysis of long-term survivors after upfront PDAC resection, absence of PNI was the only favorable independent predictor of survival greater than 5 years (98), highlighting a detrimental role for peripheral nerves in tumor progression.

Genetically engineered mouse models of PDAC also show neuroplastic changes. These neuroplastic changes are evident even at the PanIN stage, with focal areas of hyperinnervation coincident with fibrosis (96). In premalignant lesions, tumor-promoting epithelial-neuronal cross talk promotes STAT3 activation and cancer cell proliferation. This observation is attributable, at least in part, to a subpopulation of neuroendocrine PanIN cells that produce the neuropeptide substance P receptor neurokinin 1-R. Interestingly, sensory denervation in mice impairs PanIN progression to PDAC (99), suggesting a role for innervation in directing disease progression.

In invasive PDAC, large intrapancreatic nerve bundles are observed in proximity to tumor cells and vasculature (96). These nerve bundles are likely supported by expression of proinflammatory neurotrophic growth factors, such as nerve growth factor (NGF), that are released by PDAC cells. Consistent with this notion, a neutralizing antibody targeting NGF reduced neural inflammation, neural invasion, and metastasis in PDAC-bearing mice (100). More recent work suggests a feed-forward signaling loop between PDAC cells and tumor-infiltrating sympathetic nerves. For instance, nerves produce catecholamines, which stimulate PDAC development as well as NGF secretion and, subsequently, increased pancreatic nerve density (101). This observation suggests the interdependence between sensory nerves and PDAC. In line with this idea, sensory neuron ablation slows PDAC initiation and progression and prolongs survival. Further, sensory neurons appear to convey inflammatory signals from Kras-driven neoplasia to the central nervous system (102).

While tumor-promoting functions of peripheral axons in the PDAC microenvironment remain poorly understood, the functions of neural components likely extend beyond PNI and metastasis, as evidenced by a recent study suggesting that nerves secrete serine to promote PDAC cell metabolism and growth (103). However, subdiaphragmatic vagotomy has been found to accelerate PDAC progression in mice, suggesting a role in tumor suppression. In addition, cholinergic signaling from parasympathetic nerves was found to be tumor inhibitory in this disease setting (104), in part by suppressing the expansion of cancer stem-like cells. Taken together, these studies highlight the complexity and functional heterogeneity of PDAC-infiltrating nerves and impel further investigation of this prevalent element of the tumor microenvironment.

3.2.5. Leukocytes.

Leukocytes are a prominent component of PDAC tumors. Their phenotype and function contribute to the formation of a microenvironment that is ultimately permissive of tumor progression. The spatial distribution of leukocytes within PDAC tumors is vastly heterogeneous. For example, while some PDAC tumors are associated with an increased frequency of subregions containing T cells, the infiltration of T cells is by no means diffuse; rather, spatial distribution is focal and confined to unique communities that are present adjacent to tumor cells, within the stroma, or as part of TLSs (6). That said, an increased frequency of CD8^{+} T cells does associate with improved survival for patients with surgically resected PDAC (7, 105). However, tumors with both the highest number of neoantigens—a marker of cancer cell immunogenicity—and a strong CD8^{+} T cell infiltrate strongly associate with long-term survivors compared with either metric alone (7). These findings illustrate the potential for the immune infiltrate in PDAC to suppress disease progression but also highlight its variable involvement.

Single-cell RNA-sequencing analyses of surgical or fine-needle biopsy specimens have unveiled the remarkable heterogeneity of the leukocyte infiltrate in human PDAC (62, 106). Tumor-infiltrating leukocytes are composed of multiple subsets of T cells, NK cells, B cells, macrophages, and granulocytes including eosinophils, mast cells, and neutrophils. Multiplexed immunohistochemical analyses have revealed significant inter- and intrapatient spatial heterogeneity in the leukocyte infiltrate (6). Multiple leukocyte subsets have been shown to impact tumor biology in PDAC. For instance, Treg cells limit the efficacy of vaccines in mouse models (107). In contrast, depletion of Treg cells results in the differentiation of inflammatory fibroblast subsets that in turn drive myeloid cells to infiltrate the pancreas and support disease progression (45). CD4^{+} T cells are also recruited during early pancreatic cancer pathogenesis and support disease progression by secreting IL-17 (108). Further, B cells infiltrate PDAC lesions albeit at much lower frequencies than T cells (109). To this end, tumor-infiltrating B cell subsets can stimulate cancer cell proliferation by releasing IL-35 (110). Tumor-infiltrating B cells also activate Bruton tyrosine

kinase signaling in tumor-associated macrophages, programming them with a tumor-promoting phenotype (111). Tumor-infiltrating myeloid cells are among the most abundant leukocyte subset detected in tumors. Most notably, myeloid cells are quite spatially and transcriptionally diverse (106, 112). The phenotype of myeloid cells in PDAC tumors is a determinant of treatment outcomes (113). Further, the myeloid cell phenotype is pliable such that infiltrating myeloid cells may be engendered with either tumor-promoting or tumor-suppressive features. Most often, myeloid cells support tumor progression by enabling many of the hallmarks of cancer, including tumor growth, tumor survival, invasion and metastasis, and immune evasion (114–116). However, myeloid cells can also be endowed with antitumor properties including the capacity to directly mediate tumor cell killing (114), remodel the stroma for enhanced chemotherapy efficacy (113, 114), and support productive T cell immunity (117, 118). Altogether, the leukocyte infiltrate in PDAC is dynamic and shaped by cancer cells as well as the stromal microenvironment.

3.3. Implications of the Tumor Microenvironment for Metastasis

The microenvironment that surrounds cancer cells is a critical determinant of the metastatic cascade. Stromal cells and the ECM contribute directly to metastasis by supporting cancer cell invasion and intravasation into the blood stream. In addition, the microenvironment at secondary sites influences the capacity of disseminated tumor cells to seed and form metastatic colonies (17, 119). Epithelial-to-mesenchymal transition (EMT) endows cancer cells with mesenchymal features including motility and invasiveness that are broadly associated with metastatic progression (120). EMT is triggered by a variety of factors present within the tumor microenvironment including TGFβ, leukemia inhibitory factor, hypoxia, and migration inhibitory factor (17). Remarkably, even preinvasive pancreatic lesions associate with cells undergoing EMT—notably, these cells have been shown to escape the pancreas and seed distant organs (121, 122). Induction of pancreatic inflammation dramatically increases EMT, local delamination, and mutant pancreatic cells detected in the circulation. In contrast, suppressing inflammation in mouse models of PDAC inhibits epithelial cell dissemination, thereby establishing a link between inflammation, the microenvironment, and PDAC metastasis.

Pancreatic cancer cells disseminate via multiple routes including blood and lymphatic vasculature and even by invading peripheral nerves. While the determinants that direct these distinct routes of dissemination remain elusive, it is known that hypoxia enforced by hypovascularity and defective vasculature can foster the metastatic potential of cancer cells. For instance, hypoxia induces the expression of transcription factor B lymphocyte-induced maturation protein-1 (Blimp1) in pancreatic cancer cells. Importantly, Blimp1 regulates prometastatic hypoxia-induced target genes and is required for metastatic potential (123). Microenvironmental factors are also critical at distant sites and contribute to the metastatic seeding and colonization of disseminated tumor cells (17). During cancer development, the cellular and biochemical contexture of distant organs is altered to support metastasis. This prometastatic niche that forms is characterized by the recruitment of myeloid cells and deposition of ECM proteins. In addition, disseminated tumor cells recruit and establish a desmoplastic stroma that progressively accumulates as metastatic colonies form (16). The importance of paracrine factors in supporting metastasis at distant organs is underscored by studies in which select cellular components are deleted. For instance, recent functional studies show that CAF ablation reduces the growth of PDAC liver metastases and prolongs overall survival in mice (75). Epithelial cells at secondary sites also contribute to the metastatic microenvironment and aid in formation of a prometastatic niche. In the liver, hepatocytes are activated via STAT3 signaling by IL-6 released from CAFs present in the primary PDAC tumor. Activated hepatocytes respond by producing serum amyloid proteins, which then

coordinate formation of a prometastatic niche that supports liver metastasis (124). However, the role of hepatocytes is exclusive in supporting liver metastasis. As such, distinct factors coordinate the metastatic permissiveness of other distant sites (125). Nonetheless, the microenvironment is a clear and fundamental determinant of metastasis and acts to enable metastatic potential of cancer cells.

4. HOW THE TUMOR MICROENVIRONMENT SUPPORTS THERAPEUTIC RESISTANCE

The environment that surrounds cancer cells in PDAC is dynamic and pliable. This vast and heterogeneous landscape poses many barriers to the success of treatments. Notably, cancer cells co-opt elements of the stromal microenvironment to withstand insults imposed by nutrient deprivation, immune surveillance, and cytotoxic therapies. This closely knitted relationship between cancer cells and its stroma is fundamental to therapeutic resistance. To this end, multiple redundancies in cellular networks and signaling pathways thwart the efficacy of intervening on single elements of the stroma. This resilience and the capacity of PDAC to endure remarkable stress underlie the nearly uniform fate of eventual progression. Here, we discuss several determinants of the microenvironment in PDAC that contribute to treatment resistance.

4.1. Hypovascularity

Tumor growth is reliant on vasculature that nourishes cancer cells with necessary nutrients and oxygen. To keep pace with cancer proliferation, small molecules, such as VEGF, promote the development of new blood vessels, or angiogenesis (81). However, PDAC is most often characterized by a low microvascular density (83). This reflects the dense fibrotic reaction that is characteristic of the PDAC microenvironment and may trap proangiogenic molecules in the stroma, thereby limiting their capacity to trigger angiogenesis. Consistent with this idea, fibrosis degradation in PDAC associates with increased vascularity (41). In a comprehensive proteogenomic analysis of PDAC, vascularity was also found to inversely correlate with cellular infiltration (126). Notably, endothelial cells and their expression of cell adhesion molecules regulate cell trafficking. PDAC tumors with decreased leukocyte infiltration show reduced endothelial cell adhesion molecules, and these tumors display activation of hypoxia pathways that may in turn influence endothelial cell biology. These observations highlight the interdependent relationships between vascularity, fibrosis, and cellular recruitment.

Hypovascularity is a determinant of therapeutic resistance. Most notably, hypovascularity hinders the penetration and distribution of drugs into the tumor microenvironment, thereby limiting their efficacy. However, poor vascularization also produces a deprived nutrient milieu that invokes cancer cell autophagy to support metabolic processes and tumor growth. Autophagy is a conserved self-degradation process and associates with increased therapeutic resistance to cytotoxic therapies (127). In addition, autophagy has been found to promote immune evasion by causing degradation of major histocompatibility complex (MHC) class I molecules in pancreatic cancer cells, which are necessary for recognition by $CD8^+$ cytotoxic T cells (128). Inhibiting autophagy reverses this process and, in mouse models, sensitizes PDAC to immunotherapy (128). Autophagy is also activated in stromal cells including PSCs. Notably, PSC autophagy is triggered by cancer cells and causes PSCs to release nonessential amino acids (e.g., alanine) that, in turn, are utilized by cancer cells as a fuel source (53). Thus, hypovascularity induces a concerted response by pancreatic cancer cells and their surrounding stromal microenvironment that acts to promote tumor survival and thwart the efficacy of cytotoxic- and immune-based therapies.

4.2. Fibrosis

Dense fibrosis is commonly referred to as a physical barrier to treatment efficacy in PDAC. Fibroblasts contribute to fibrosis by producing components of the ECM. CAFs also shape and remodel the stromal microenvironment by mediating collagen cross-linking and, in doing so, modulate tumor stiffness, which supports tumor progression and cancer cell migration. Single-cell analyses have revealed multiple CAF subsets present within PDAC tumors that are defined by unique transcriptional signatures (62–64). The spatial arrangement of CAF subsets is also distinct. For instance, CAFs appear to associate with cancer cells on the basis of their differentiation status. For instance, α-SMA$^+$ CAFs are found juxtaposed to well-differentiated cancer cell nests, whereas fibroblast activation protein (FAP$^+$) CAFs tend to localize adjacent to poorly differentiated regions of tumor (61). Further, these markers of CAF subsets have been used to define the stromal microenvironment in PDAC as enriched in collagen, FAP, or α-SMA. Notably, these unique microenvironments associate with distinct survival and molecular features (61).

CAFs may be engendered with tumor permissive or restrictive functions. For instance, CD105$^+$ CAFs. including both myofibroblastic and inflammatory CAFs, are tumor permissive, whereas CD105^{neg} CAFs restrict tumor growth (129). CD105^{neg} CAFs show reduced FAP expression and uniquely express MHC class II antigen machinery corresponding to antigen-presenting CAFs (62). This CAF subset has been implicated in supporting the tumor-suppressive potential of adaptive immunity, whereas other CAF populations restrict antitumor immune responses.

CAFs and their associated fibrosis may be barriers to treatment efficacy. Mouse modeling has shown the potential benefit of disrupting elements of stromal fibrosis for enhancing the efficacy of cytotoxic chemotherapy (49). For instance, depletion of HA facilitates enhanced efficacy of gemcitabine. This finding reflects improved vascular perfusion in tumors leading to a shorter diffusion time for gemcitabine, which has a short half-life and is readily metabolized in tumors (130). However, for other chemotherapeutics with longer half-lives, the benefit of fibrosis degradation may not be as readily apparent.

4.3. Immune Evasion

A hallmark of PDAC is its remarkable resistance to immunotherapy. Primary immune resistance in cancer can involve multiple mechanisms (131). For instance, cancer cells may evade immune recognition because of a lack of potent immunogenic tumor antigens (antigenicity). Importantly, neoantigens resulting from gene mutations are less abundant in PDAC compared with many other immune-sensitive cancers (132). Poorly immunogenic tumors may also escape immune recognition due to poor antigen presentation required for effective priming of tumor-reactive T cells (immunogenicity). Together, the poor antigenicity and immunogenicity of PDAC form the basis for ongoing studies investigating vaccines and drugs targeting molecules (e.g., CD40 and CTLA4) that regulate T cell priming (133–135). However, the microenvironment that surrounds PDAC is also fundamental to productive immune surveillance (microenvironment). Exclusion of leukocyte populations (e.g., T cells and dendritic cells) that are necessary for immune recognition of cancer is a central theme in PDAC (136, 137). In contrast, PDAC is known for its recruitment of myeloid and fibroblast populations that possess immune suppressive and tumor-promoting features. Finally, the functional competency of leukocyte populations in patients may be compromised (immune health). For instance, PDAC is commonly associated with systemic inflammation (138), a deficiency of circulating dendritic cells (117, 118), and peripheral T cells that are terminally differentiated (139). Notably, systemic inflammation marked by an elevated neutrophil-to-lymphocyte ratio in the peripheral blood associates with reduced efficacy of cytotoxic chemotherapy (138,

140). Altogether, the immune evasiveness of PDAC is multifactorial and influenced by cancer cell–intrinsic and –extrinsic mechanisms.

Leukocytes are a predominant component of the PDAC microenvironment and a determinant of therapeutic outcomes. Within tumors, the leukocyte infiltrate is remarkably heterogeneous. This variability in the localization and density of leukocytes is particularly pronounced for T cells, which tend to congregate in the stroma and less commonly interact directly with cancer cells. In this regard, T cells appear to be drawn to some cancer cell nests while being excluded from others (**Figure 2**). T cell infiltration correlates with improved outcomes in patients with surgically resected PDAC, and in mouse models T cells suppress metastasis (7, 141). However, the precise determinants that direct T cell intratumoral heterogeneity are unclear. Genetic mouse models suggest that pancreatic tumors may be composed of cancer cell clones with vastly distinct immunogenicity and responsiveness to immunotherapy (141). In these studies, clonal cell lines generated from tumors arising spontaneously in KPC mice were found to produce tumors with either a strong or weak T cell infiltrate when transplanted into syngeneic mice. In addition, these clones responded differentially to immunotherapy, with strong responses observed for T cell–enriched tumors. However, upon mixing of the cancer cell clones, the phenotype of T cell–poor tumors was shown to dominate. Taken together, these observations suggest that immune heterogeneity in PDAC is directed by cancer cell–intrinsic features, with individual clones cooperating to fine-tune elements of the tumor microenvironment that support a fitness advantage for tumor progression and immune evasion (**Figure 2**).

4.4. Remodeling of the Tumor Microenvironment

The tumor microenvironment is a well-recognized therapeutic barrier in PDAC. However, the microenvironment that surrounds cancer cells is pliable, and this suggests the potential to shift its polarity from treatment resistant to treatment sensitive. Disrupting specific elements of the microenvironment, such as myeloid cells, fibroblasts, or ECM components, can enhance the sensitivity of cytotoxic- and immune-based therapies. For instance, depletion of $CSF1R^+$ myeloid cells in mouse models and in some patients with PDAC improves responses to cytotoxic chemotherapy by augmenting the infiltration of T cells and their antitumor activity (142). Similarly, inhibiting focal adhesion kinase (FAK) causes a reduction in immunosuppressive leukocyte populations including myeloid cells and Treg cells and, in doing so, sensitizes PDAC to both chemotherapy and immunotherapy in mice (143). Together, these findings suggest that multiple mechanisms underlie therapeutic resistance in PDAC.

An alternative strategy to remodeling tumors for enhanced treatment sensitivity involves harnessing the therapeutic potential of tumor-infiltrating leukocytes. For instance, activation of the CD40 pathway using an agonistic antibody redirects tumor-infiltrating inflammatory monocytes to remodel the ECM of tumors and sensitize PDAC to chemotherapy (113, 114). Engineered T cells recognizing mesothelin also trigger remodeling of the ECM in PDAC, suggesting that immune activation may be a generalized mechanism for altering the contexture of the microenvironment in PDAC (144). Another approach to remodeling the tumor microenvironment involves restoring deficits in immune health associated with PDAC. For example, Fms-like tyrosine kinase 3 ligand triggers dendritic cell mobilization, survival, and infiltration into PDAC tumors and combines with a CD40 agonist to promote T cell infiltration and antitumor activity in mouse models (117, 118). Partial activation of CD11b using a small molecule agonist can also redirect tumor-infiltrating myeloid cells and reduce resistance to immunotherapy in PDAC models (145). Thus, treatment strategies designed to induce tumor-suppressive mechanisms, as opposed to removing tumor-promoting elements, offer an approach for leveraging the tumor microenvironment for therapeutic benefit.

4.5. Pliability

PDAC shows remarkable resilience in response to therapeutic intervention. Determinants that contribute to this resilience are multifactorial and include the cellular and spatial heterogeneity of stromal cell populations as well as cancer cell clonal heterogeneity, which supports tumor evolution. Pancreatic cancer cells adapt to interventions by invoking compensatory signaling pathways. For instance, inhibiting autophagy in pancreatic cancer cells prompts their upregulation of alternative pathways, such as macropinocytosis, for extracting nutrients from the microenvironment (146). However, blocking both autophagy and macropinocytosis deprives cancer cells of essential metabolic needs and induces cell death. Similarly, pancreatic cancer cells respond to suppression of pathways downstream of KRAS, including ERK/MAPK and RAF/MEK/ERK, by increasing autophagic flux (147, 148). This establishes a vulnerability in cancer cells such that intervening on these downstream KRAS signaling pathways in combination with autophagy inhibition causes synergistic antiproliferative effects.

Cancer cells coevolve with their surrounding microenvironment such that insults sustained by cancer cells provoke reciprocal responses in the microenvironment, and vice versa. For instance, cancer cells respond to cytotoxic stress by inducing the recruitment of myeloid cell populations that support neovascularization and tumor survival (149). Dying cancer cells also release ATP into the extracellular milieu, acting as a potent danger signal for the recruitment of inflammatory cells, which then degrade ATP by converting it to adenosine, which, in turn, is immunosuppressive (150). Intervening on these compensatory inflammatory responses by blocking chemokines/chemokine receptors (e.g., CCL2, CCR2, and CXCR2) or inhibiting adenosine formation (e.g., CD73) has been found to improve survival outcomes in mouse models (149, 151). However, intervening on a single determinant of inflammation provoked by cytotoxic therapy triggers compensatory responses. For instance, combining chemotherapy with CCR2-blocking antibodies to prevent monocyte recruitment to tumors triggers a compensatory increase in neutrophil recruitment (152). Conversely, blocking neutrophil recruitment using CXCR2-blocking antibodies induces the recruitment of monocytes to tumors in the setting of chemotherapy. To this end, combined blockade of both CCR2 and CXCR2 improves survival in mouse models of PDAC. Similarly, inhibition of FAK remodels PDAC to be more sensitive to cytotoxic chemotherapy and immunotherapy, but ultimately resistance is observed (153). This compensatory survival pathway reflects a remodeling of the tumor microenvironment. Specifically, FAK inhibition reduces the antagonistic effects of TGFβ on tumor-intrinsic STAT3 signaling such that combined inhibition of FAK and STAT3 synergizes to suppress PDAC growth in mouse models (153). Thus, multiple nonredundant and compensatory pathways may need to be targeted to prevent cancer cells from undermining the efficacy of cytotoxic and targeted therapies.

While PDAC cell biology is fundamentally defined by oncogenic drivers (e.g., KRAS) and other mutations (e.g., TP53, INK4A, and SMAD4, among others), cancer cells are continuously influenced by their surrounding microenvironment. To this end, cancer cells must adapt to immune pressure or succumb to elimination. Secretion of cytokines (e.g., interferons) by infiltrating T cells triggers upregulation of antigen presentation machinery in cancer cells and, in doing so, sensitizes them to recognition and elimination by cytotoxic T cells (131). However, this pressure imposed by the immune system invokes a Darwinian selection process whereby cancer cells with increased fitness ultimately emerge. For instance, adoptive cell therapy with T cells engineered to recognize mesothelin provokes the emergence of cancer cells that have lost mesothelin expression (144). In addition, tumor-infiltrating T cells are ultimately coaxed into an exhausted state by resident myeloid cells. These findings illustrate the adaptability of PDAC and emphasize that a multipronged approach that addresses both primary and acquired resistance mechanisms to treatment intervention will need to be considered.

5. CHALLENGES AND OPPORTUNITIES FOR STUDYING AND INTERVENING ON THE TUMOR MICROENVIRONMENT

Genomic studies have revealed unique patient subsets in PDAC that derive benefit from cytotoxic-, targeted-, and immune-based therapies. For instance, PARP inhibitors show activity in patients with germline BRCA mutations who have not progressed on platinum-based chemotherapy (154, 155). In addition, patients with mismatch repair deficient PDAC have an increased likelihood of responding to immune checkpoint inhibitors (156). Given the remarkable inter- and intrapatient heterogeneity seen in the tumor microenvironment of PDAC, similar approaches to patient selection may be needed to realize the benefit of strategies that intervene on elements of the tumor microenvironment. One approach to patient selection involves the use of genetic determinants (e.g., mutations in Kras, p53, SMAD4, and BRCA) that represent significant patient subsets. Another approach is to consider elements of immune health that may influence the therapeutic potential of a treatment strategy. For example, patients with a low neutrophil-to-lymphocyte ratio show improved outcomes to treatment with gemcitabine and a CD40 agonist (138). Lastly, baseline and on-treatment tumor analyses may inform patient selection. For example, patients might be selected for treatment on the basis of the phenotype of the tumor, such as (*a*) deserted or reactive (5), (*b*) SMA, FAP, or collagen enriched (61), (*c*) T cell or myeloid cell enriched or poor (6), or (*d*) classical or basal (157, 158). On-treatment biopsies may also be needed to assess whether treatments focally or diffusely alter the tumor microenvironment (159). However, with these approaches, stringent criteria will be necessary given the potential for tissue sampling bias. Specifically, interpretation of the tumor microenvironment using biopsies is challenged by the remarkable cellular and spatial heterogeneity of PDAC. Thus, results from biopsies could be misleading if this heterogeneity is not appropriately considered.

Determinants of spatial and cellular heterogeneity in PDAC are poorly understood. Current tissue analyses offer only a snapshot of this biology. Given the impact that the intra- and extratumoral elements can have on tumor pathogenesis, it is expected that the microenvironment is dynamic and is continuously shaping the evolution of cancer cells. Future efforts will need to identify determinants of this heterogeneity and better understand how cancer cell–intrinsic signaling pathways of mutational landscapes instruct the contexture and arrangement of cells within the microenvironment. Such analyses have the potential to unveil novel therapeutic targets and approaches to dismantle the complex PDAC ecosystem.

The microenvironment that surrounds PDAC holds potentially key insights into treatment vulnerabilities. Preclinical models will be fundamental to complementing observations made from human tissue analyses. Organoid and mouse models have demonstrated value in defining functional relationships between cell subsets within tumors and compensatory mechanisms of resistance to treatment interventions. These model systems hold promise for addressing fundamental questions in PDAC. For instance, how do genomic alterations (e.g., mutations in p53, BRCA, etc.) affect the cellular contexture of the microenvironment in PDAC? Are intercellular relationships involving cancer cells and stromal cells similarly observed in primary and metastatic lesions? How does the microenvironment regulate the transition from low-grade dysplasia to invasive cancer? These questions and more will begin to unravel unique patient subsets with therapeutic implications and may reveal strategies for early disease intervention in high-risk patients.

6. CONCLUDING REMARKS

Over the past two decades, great strides have been made in understanding the complex stromal microenvironment of PDAC and its impact on disease biology and treatment resistance. Although seemingly disorganized, the tumor microenvironment in PDAC is intricate and interwoven with

many dynamic elements that govern disease progression, immune evasion, metastasis, and therapeutic resistance. Reciprocal interactions between cancer cell–intrinsic and –extrinsic mechanisms shape the tumor microenvironment and form the basis of PDAC's remarkable resilience. Emerging and existing state-of-the-art technologies offer unprecedented opportunities to discover fundamental biology underlying the contexture, functional status, and spatial organization of the tumor microenvironment in PDAC. To this end, many key gaps in knowledge exist including understudied components of the microenvironment (e.g., peripheral nerves, adipocytes, and endothelial cells) and the dynamics of intercellular relationships in tumors. The conceptual advances described herein serve to provide a framework for future investigations. Ultimately, it is expected that this knowledge will inform novel strategies and therapeutic targets for improving patient outcomes and advancing a personalized medicine approach to PDAC.

DISCLOSURE STATEMENT

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