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New insights in the development of pancreatic cancer

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Abstract

Purpose of review—The review intends to describe recent studies on the development of pancreatic cancer from a genetic, molecular, and microenvironment perspective.

Recent findings—Pancreatic cancer has been discovered to have distinct molecular subtypes based on transcriptome analyses that may have implications for treatment. Recent studies are also mapping the complex molecular biology of this cancer as it relates to the core signaling abnormalities inherent to this disease. There have been discoveries of novel modes of regulation of pancreatic cancer development, including alterations in key transcription factors, epigenetic modifiers, and metabolic pathways. Studies of the tumor-associated microenvironment continue to reveal its complex role in tumor development.

Summary—Pancreatic cancer development appears to depend on a multifaceted network of signals that are dynamic, involve multiple cell types, and are linked to spatiotemporal factors in tumor evolution. Understanding the development of pancreatic cancer in this context is key to identifying novel and effective targets for treatment.

Keywords

ductal cells; *KRAS*; pancreatic cancer; stroma

INTRODUCTION

Pancreatic ductal adenocarcinoma (PDAC) is an aggressive disease with a median survival of 6 months, a paradigm that has not shifted significantly in the era of modern medicine. Unfortunately, by 2020, PDAC is predicted to become the second leading cause of cancer-related death in the United States [1]. Recent research into the development of PDAC and its complex biology is providing us with a novel view into the multidimensional nature of this disease. The purpose of this review is to highlight the recent key discoveries in the field of

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PDAC development – from insights into its intrinsic genetic and molecular programming to the understanding of its external interactions with the microenvironment – and how they can potentially identify innovative and ‘out-of-the-box’ targets for treatment.

MOLECULAR CLASSIFICATION OF TUMORS

Major advances have been made in the past few years in defining the molecular aberrations in pancreatic cancer using increasingly sophisticated integrated genetic tools to map the mutational and gene expression landscape of PDAC. Whole-genome and whole-exome sequencing have been utilized to sequence tumors with greater attention paid to specifically enriching for the epithelial compartment. Based on multiple genetic studies, it is now well established that activating mutations of *KRAS* are near ubiquitous and inactivation of *TP53*, *SMAD4*, and *CDKN2A* occur at rates of more than 50%. Other less prevalent, recurrently mutated genes (about 10%) can be analyzed as functional gene groups and cluster into unique core molecular pathways, including DNA damage repair, chromatin modification, and cell cycle regulation [2]. More recently, transcriptome analyses of large cohorts of tumor samples have been able to further classify PDAC tumors into molecular subtypes that correlate with differential clinical outcomes (Table 1) [3,4[■]]. The first group is associated with gene networks involved in the C2-squamous-like class of tumors (defined in the Cancer Genome Atlas pan-cancer studies), the second with networks characteristic of committed endocrine and exocrine differentiation, and the third with transcriptional networks involved in early pancreas development [4[■]]. Bailey *et al.* [4[■]] used an integrative approach to investigate the mutational landscape as well as gene expression profiles of a large sample of tumors. In addition to mutations in known core molecular pathways, they identified mutations in several RNA processing genes, such as splicing factor 3B subunit 1 (SF3B1) and u2 small nuclear RNA auxiliary factor 1 (U2AF1) in a significant 16% of samples. Thus, altered RNA splicing mechanisms in PDAC may identify novel therapeutic targets. Their transcriptome analysis corroborated the prior classification of tumors and also identified a new subgroup characterized by a high immune infiltrate and expression profiles mostly related to infiltrating B and T cells. Within this ‘immunogenic’ subtype, gene programs associated with a macrophage signature and T-cell coinhibition were associated with a worse survival.

Adding to this body of work, Moffitt *et al.* [5[■]] utilized virtual microdissection and deconvolution of data to distinguish between normal and tumor compartments in their samples. Their tumor subtyping into two major groups also generally corroborated the aforementioned classifications, although there were some discrepancies that could possibly be explained by contamination with normal tissues in the prior studies. Importantly, they classified two stromal subtypes, a ‘normal’ stroma that was associated with the expression profile of stellate cells and an ‘activated’ and more aggressive stroma with strong signatures of macrophages as well as activated fibroblasts. The tumor-associated stroma is a complex microenvironment with great influences on tumorigenesis that will be discussed. Interestingly, the stroma subtypes were associated with both tumor subtypes, suggesting a more complex relationship between the two compartments.

Overall, it is promising that in these independent studies, the tumor classifications showed general concordance, associated with clinical outcomes, and clustered with molecular subtyping of other solid malignancies in external cohorts (Table 1). It remains to be seen whether patients with these subtypes will have differential responses to treatment prospectively.

CELLS OF ORIGIN

The classification of PDAC tumors based on trans-cryptome profiles begs the question of whether the different tumor subtypes potentially represent origins from different pancreatic cellular compartments. Pancreatic intraepithelial neoplasia (PanIN), intraductal papillary mucinous neoplasm (IPMN), and mucinous cystic neoplasm are thought to represent precursor stages for PDAC and appear to have distinct cells of origin [6]. PanINs are thought to be the most common precursor lesions of human PDAC. Genetically engineered mouse models (GEMMs) of PDAC in which mutant *Kras* is targeted to specific cell types using an inducible *Cre* have shown that the PanINs can arise from adult acinar and endocrine cells. Often these models require concomitant loss of tumor suppressors such as *p53* and/or induction of chronic pancreatitis to promote PanIN formation and progression to PDAC. The current model of tumorigenesis proposes that in response to stress such as pancreatitis, acinar cells dedifferentiate into a progenitor-like state and acquire features of ductal cells. In the setting of the aberrant signaling associated with oncogenic *Kras*, this process of acinar-to-ductal metaplasia (ADM) goes unchecked and progresses to PanIN and PDAC. Remarkably, targeting oncogenic *Kras* to adult ductal cells via *Sox9-Cre* produces almost no PanINs, even with pancreatitis [7]. Recently, however, Bailey *et al.* [8[■]] showed that expression of oncogenic *Kras* in adult ductal cells via *Hnf1b-Cre* in combination with biallelic expression of the gain-of-function *p53*^{R172H}, a prevalent mutant allele in human PDAC, resulted in PDAC formation. Unlike acinar cells in which one allele of mutant *p53* along with oncogenic *Kras* is sufficient to drive PDAC in GEMMs, ductal cells require two *p53*^{R172H} alleles. Furthermore, the *Hnf1b-Cre* GEMM did not have precursor PanIN lesions, which are typical of acinar lineage targeted models. Taken together, these data suggest that ductal cells may have a different threshold as well as transformation sequence leading to PDAC development when compared with acinar cells. Interestingly, acinar, endocrine, and ductal compartments have been clearly shown to undergo dedifferentiation as part of pancreas regeneration in the setting of injury, a pathway that is mimicked by and goes unchecked in mutant *Kras*-mediated oncogenesis.

Ductal cells have been established as the cells of origin for IPMN, another precursor lesion of PDAC. Loss of Brahma-related gene 1 (*Brg1*), the catalytic unit of the SWItch/Sucrose Non-fermentable (SWI/SNF) transcription complex, cooperates with mutant *Kras* in adult ductal cells to promote PDAC formation. Similar to the finding that ductal cells may be resistant to transformation, biallelic loss of *Brg1* is required to transform ductal cells into neoplastic cells. Also, similar to acinar dedifferentiation that results in acinar-to-ductal reprogramming and formation of PanINs, loss of *Brg1* results in dedifferentiation of ducts with upregulation of embryonic markers such as *Pdx1* and *Hnf4a* [9]. This dedifferentiation leading to ductal atypia and IPMN formation can be blocked by constitutive activation of *Sox9*, a transcription factor that maintains mature duct identity. However, *Brg1* expression in

PDAC cells worsens their tumorigenic potential. Furthermore, as opposed to overexpression in ductal cells, suppression of Sox9 in acinar cells is protective against Kras-driven oncogenesis. Therefore, the role of different key players in Kras-mediated oncogenesis is time as well as context dependent.

SIGNALING PATHWAYS

Numerous studies have elucidated the key signaling pathways that cooperate with oncogenic Kras. Several central downstream signaling nodes have been identified that are critical to Kras-induced tumorigenesis, including canonical PI3K/Akt, Raf/Mek/Erk, RalGDS/p38 Mapk, Rac and Rho, and Plc- ϵ signaling. Other factors that influence these key nodes are emerging themselves as checkpoints in oncogenesis [10,11]. Progenitor genes that are upregulated in the setting of oncogenic Kras activation have been best characterized in acinar cells undergoing Kras-mediated acinar-to-ductal reprogramming. These embryonic markers can directly influence Kras downstream effector pathways [12]. Sox9, for example, has been shown to be required for PanIN formation from Kras-active acinar cells, and its exact influence on signaling pathways is unraveling [7]. Grimont *et al.* [13[■]] showed that Sox9 contributes to acinar neoplasia by stimulating the ErbB pathway resulting in epidermal growth factor receptor (Egfr) and Erk phosphorylation in Kras-induced ADM in the setting of pancreatitis. There also appears to be a feed-forward loop in the activation of Sox9 in transformed acinar cells where Egfr activation leads to upregulation of Sox9 expression through the transcription factor, nuclear factor of activated T cells c1 (Nfatc1) that complexes with signal transducer and activator of transcription 3 (Stat3) in the nucleus to activate target genes [14]. Both Nfatc1 and Stat3 have been shown to be critical in early PanIN tumorigenesis [15–17]. Taken together, these studies link Sox9 expression to pancreatitis related acinar dedifferentiation and provide evidence of influences of the inflammatory microenvironment on activation of oncogenic signaling early in PanIN development.

Another developmental pathway, Notch signaling which controls differentiation and is upregulated in acinar cells in the setting of pancreatitis, has an oncogenic as well as a tumor suppressor role in PDAC [6]. Notch influences on tumorigenesis appear to depend on the timing of Notch expression in the embryonic versus adult acinar cells and the subtypes of Notch receptors involved. Activation of the Notch3 receptor, indirectly by knocking down its modifying protein – lunatic fringe – at the embryonic stage in a GEMM, accelerates PanIN progression and worsens survival [18]. This increased Notch3 activity is associated with suppression of transforming growth factor beta (TGF- β) at early time points. The role of TGF- β in PDAC is also time and context dependent, but its significance in human PDAC is clearly emphasized by the very high frequency of inactivating mutations in the tumor suppressor SMAD4, which is a major downstream signal relay for the TGF- β family. The complex role of Notch signaling and its interplay with Kras-mediated oncogenesis need to be further characterized.

Recent studies have emphasized the constantly relevant role of PI3K in PDAC oncogenesis [19,20]. Expression of the constitutively active form of PI3K in acinar cells results in ADM, PanIN, and PDAC formation even in the absence of mutant Kras, thus establishing PI3K as a

key mediator of downstream Kras signaling [21]. Chalabi-Dchar *et al.* [22] showed that the somatostatin receptor subtype 2 (Sst2) that is normally expressed in acinar cells serves as an endogenous brake on Kras signaling and its mono-allelic loss amplifies PI3K signaling. Mechanistically, Sst2 inhibits PI3K activity by disrupting a preexisting complex comprising the Sst2 receptor and the p85 PI3K regulatory subunit. Interestingly, in human PDAC, there is loss of Sst2 expression because of epigenetic silencing.

EPIGENETIC REGULATION

Importantly, epigenetic modifiers are emerging as significant players in pancreatic tumorigenesis. The tumor suppressor gene in PDAC, CDKN1A, classically undergoes promoter methylation and gene silencing (10–15% of cases) [23]. A subset of genes that undergoes epigenetic modification in PDAC is also silenced by promoter methylation in PanIN, IPMN, and mucinous cystic neoplasm lesions, suggesting an early role of epigenetic changes in tumorigenesis [24,25]. Recent sequencing efforts have revealed frequent alterations in genes regulating chromatin remodeling and modification in human tumors, such as the SWI/SNF pathway (42% of cases) [26]. Mazur *et al.* [27[■]] examined the role of bromodomain and extraterminal (BET) family of proteins, which recognize acetylated lysines on histones through their bromodomains and control the transcription of oncogenic drivers, in PanIN and PDAC tumorigenesis. In their oncogenic Kras-driven GEMM, there is progressively increased expression of the BET proteins with PanIN progression. Prophylactic treatment with the BET inhibitor, JQ1, inhibits the development of PanINs through suppression of the Myc oncogene as well as downregulation of inflammatory mediators such as IL-6 and STAT3. Interestingly, the authors identified direct bromodomain-containing protein 4 association to the IL-6 promoter. This finding further emphasizes the key role BET proteins likely have on PanIN tumorigenesis, as IL-6 activation has been shown to be critical for Kras-induced oncogenesis in the GEMMs [16]. Furthermore, treatment with JQ1 in mice with established tumors also led to decreased tumor volume, suggesting an ongoing role of BET proteins even at the tumor stage. This pivotal study establishes the importance of epigenetic modifiers such as the BET proteins in early as well as late tumorigenesis and identifies epigenetic-based targeting approaches in PDAC as potential new avenues of treatment.

METABOLIC ABERRATIONS

Oncogenic Kras-driven signals activate an abnormal chain of metabolic changes – including enhanced glycolysis, diverted glutamine consumption, anomalous pentose phosphate pathway, and autophagy – which PDAC cells become ‘addicted’ to and which contribute extensively to tumorigenesis. Disruption of these metabolic pathways significantly inhibits cancer growth [28–30]. PDAC cells have been shown to be dependent on autophagy, a dynamically regulated catabolic pathway to degrade cellular organelles and macromolecules, for growth [31,32]. Important contributions have been made to understanding the regulatory circuits that activate autophagy in PDAC. Perera *et al.* [33[■]] found that human PDAC samples have elevated expression of autophagy-lysosome genes compared with normal pancreatic tissue. They discovered that the microphthalmia-associated transcription factor/TFE family of transcription factors coordinates the expression of a cohort of genes

that is responsible for high lysosomal catabolic activity necessary for PDAC growth [33[■]]. Selective knockdown of these transcription factors in cell lines and in orthotopic tumors greatly diminishes tumorigenesis, highlighting the dependency of PDAC cells on catabolic activity for their high energy needs. We are just beginning to understand the complex metabolic disturbances inherent to PDAC cells, and more studies are needed to understand how these pathways are influenced by the mutational landscape in which they arise. Furthermore, emerging data show that epithelial metabolism can be influenced by noncell autonomous mechanisms such as crosstalk with the tumor stroma [34].

STROMAL INTERACTIONS

PanIN progression is not only accompanied by accumulating genetic mutations and cellular atypia but also the development of a complex tumor-associated stroma. The emerging understanding of the stroma in PDAC is that it is not simply a bystander but promotes carcinogenesis, tumor progression, and therapeutic resistance. With the numerous studies in GEMMs, it is clear that the neoplastic epithelium elicits a desmoplastic reaction early in PanIN tumorigenesis which progresses with PanIN evolution [35]. Oncogenic Kras-driven epithelial signaling results in activation of pro-inflammatory pathways, such as IL-6/Stat3 and granulocyte macrophage colony-stimulating factor, which create a unique chronic inflammatory micro-environment [15,36,37]. Cancer-associated fibroblasts and activated pancreatic stellate cells (PSCs) produce an abundant extracellular matrix which harbors several secreted growth factors and cytokines. The environment becomes progressively immunosuppressive with a predominance of M2 macrophages, immunosuppressive T regulatory cells, and myeloid-derived suppressor cells. PSCs have an important role in regulating the stroma as a whole. Sherman *et al.* [38] demonstrated that activation of the vitamin D receptor in PSCs reduces their function and this helps reprogram the stroma to a more quiescent and noninflammatory state.

The tumor-associated macrophages are an important stromal immune cell and can promote a more immunosuppressive microenvironment that helps neoplastic cells increasingly evade the immune system and progress to tumor [16,39–41]. The epithelium and stroma crosstalk is dynamic between different cell populations and can even depend on external environmental factors [42]. Seifert *et al.* [43[■]] showed that under stress conditions such as radiation exposure, the damaged epithelium signals to M2 macrophages to expand in the stroma, which in turn, modulate the local T-cell population and ultimately accelerate the progression of dysplasia to invasive carcinoma. Similar to these findings, in the setting of epithelial necroptosis, a type of programmed cell death that can occur with exposure to toxic chemotherapeutic drugs, upregulation of necrosome-related proteins such as receptor-interacting protein and RIP3 results in secretion of the chemokine attractant chemokine (C-X-C motif) ligand 1 (CXCL1) that increases the population of immunosuppressive macrophages [44].

Recent work has also identified B cells in the stroma to be another immune cell type with proneoplastic functions [45,46]. Pylayeva-Gupta *et al.* [47[■]] found that CXCL13 expression by fibroblasts in the stroma likely recruits B cells into the microenvironment. Of these B cells, the IL-35-producing subgroup is required to support growth of early pancreatic

neoplasia. Interestingly, the IL-35 receptor is expressed by multiple immune cell types, so the mechanism by which B cells may exert their influence likely involves a complex network of interactions with tumor and stromal cells. For example, in advanced tumors, B cells crosstalk with macrophages, which results in their M2 reprogramming and further promotes an immunosuppressive microenvironment [45].

These studies highlight the complex network of communication and regulation among the different cell types of the stroma. The crosstalk between the neoplastic epithelium and myriad components of the stromal microenvironment is also likely to depend on spatiotemporal factors. For example, not all elements of the stroma support tumor growth, as early loss of α -smooth muscle actin-positive myofibroblasts in a GEMM not only decreases the stroma but also results in highly undifferentiated, invasive, and necrotic carcinomas [48,49]. It is unclear, however, what effects the loss of smooth muscle actin-positive fibroblasts have on established tumors in the GEMMs. Interestingly, long-term chemical inhibition of a key stromal signaling pathway in a GEMM model with established tumors results in worse outcomes. More studies are definitely needed to understand the function of the desmoplastic stroma in PDAC, with its heterogeneous cellular and noncellular constituents, which likely evolve during cancer progression.

CONCLUSION

Only when we understand the genetic and molecular evolution of PDAC, its complex signaling pathways and its interactions with its dynamic microenvironment can we hope to tackle this devastating disease. The last few years of intensive research in these areas have led to significant advances in the understanding of the development of PDAC. Multiple independent studies have unveiled the novel transcriptome-based subtypes of PDAC that will certainly broaden our understanding of this disease and hopefully have positive implications for treatment. The GEMMs have been pivotal in understanding the pancreatic cells of origin for PDAC and the molecular progression leading to tumorigenesis. The dissection of the complex signaling pathways downstream of oncogenic Kras, and how they interact with the stromal microenvironment, has identified novel and potentially targetable key mediators of tumorigenesis. It is possible that successful treatment of PDAC will involve multimodal targeting of the different aspects of its biology. We are confident that rapidly evolving research will convert pancreatic cancer to an entirely treatable disease.

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- of special interest
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KEY POINTS

- Pancreatic cancer can now be classified into distinct molecular subtypes based on transcriptome analysis.
- Novel key players in regulating oncogenic signaling in pancreatic cancer include transcription factors, epigenetic modifiers, regulators of metabolism, and stromal elements.
- Influences on pancreatic cancer development depend on the timing and context in which they occur in tumor progression and offer opportunities for therapeutic intervention.

Table 1

Transcriptome-based molecular subtypes of human pancreatic cancer and stroma and their effects on survival

Classification	Molecular subtype	Effect on survival
Collisson <i>et al.</i> [3]	Quasimesenchymal	↓ ¹
	Exocrine-like	
	Classical	
Bailey <i>et al.</i> [4 [■]]	Squamous	↓ ¹
	ADEX ²	
	Pancreatic progenitor	↓ ³
	Immunogenic	↓ ⁴
Moffitt <i>et al.</i> [5]	Basal-like ⁵	↓ ¹
	Classical ⁶	
	'Normal' stroma	
	'Activated' stroma	↓ ⁷

¹This group has the worst survival in the respective classification.

²Aberrantly differentiated endocrine and exocrine.

³Within the progenitor group, patients with a higher expression of genes in this gene program have a worse prognosis.

⁴Within the immunogenic group, high macrophage and T-cell coinhibition signatures are associated with a worse prognosis.

⁵The basal group only partially overlaps with the quasimesenchymal group.

⁶The classical group only partially overlaps with the Collisson classical group.

⁷'Activated' stroma was associated with a worse prognosis when compared with 'normal' stroma. Stromal types are not tumor subtype specific.

The different classification schemes by the authors are shown. The matching colors correspond to overlapping groups under the different classification schemes. The Moffitt tumor subtypes only have partial overlap with the other corresponding groups.