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Review

Drivers of Gene Expression Dysregulation in Pancreatic Cancer

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Pancreatic ductal adenocarcinoma (PDAC) remains a devastating disease with a poor prognosis. The functional consequences of common genetic aberrations and their roles in treatment strategies have been extensively reviewed. In addition to these genomic aberrations, consideration of non-genetic drivers of altered oncogene expression is essential to account for the diversity in PDAC phenotypes. In this review we seek to assess our current understanding of mechanisms of gene expression dysregulation. We focus on four drivers of gene expression dysregulation, including mutations, transcription factors, epigenetic regulators, and RNA stability/isoform regulation, in the context of PDAC pathogenesis. Recent studies provide much-needed insight into the role of gene expression dysregulation in dissecting tumor heterogeneity and stratifying patients for the development of personalized treatment strategies.

Highlights

Non-genetic mechanisms of dysregulated gene expression changes are integral drivers of the pancreatic ductal adenocarcinoma (PDAC) phenotype.

Mutations, transcription factors, epigenetic modifications, and RNA regulation are key drivers of PDAC gene expression programs. Mechanistic dissection and pharmacological targeting of aberrant gene expression changes may represent an important strategy in PDAC treatment.

Recent PDAC metastatic analyses highlight the possible effectiveness of driver mutation-specific therapies in uniformly treating distant and local metastases. Prioritizing the study of non-genetic drivers may help to develop a multipronged treatment approach.

Inter- and intratumoral transcriptomic heterogeneity delineates the classical and basal PDAC subtypes. The identification of epigenetic regulators and transcription factors that drive these differences may improve the clinical relevance of this classification system.

Gene Expression Dysregulation Is Integral to the PDAC Phenotype

PDAC remains a deadly disease and is projected to become the second leading cause of cancer deaths in the coming decade [1]. It is an aggressive malignancy characterized by a heterogeneous stromal microenvironment resulting in poor tumor vascularization and a complex signaling landscape that governs tumor initiation, progression, and maintenance [2,3]. Prognosis remains poor owing to the lack of early-stage symptoms, leading to detection only at advanced stages, accompanied by modestly effective treatment strategies. PDAC etiology has been extensively characterized in terms of key coding genetic drivers and transcriptomic subtypes in a bid to advance patient-specific treatments [4,5]. However, little focus has been placed on mechanistic dissection of the molecular drivers of dysregulated gene expression, and how these gene expression changes correlate with the PDAC phenotype. Such efforts seek to reconfigure the aberrant transcriptomic landscape via precision therapy [6–10]. Pharmacological targeting of aberrant gene expression changes is an emerging strategy in PDAC treatment (Box 1).

Although genome-wide studies have extensively characterized the mutational landscape, studies on the mechanisms underlying downstream expression changes that drive the PDAC phenotype are scarce. Mutational events in candidate oncogenes (*KRAS*) and tumor suppressors (*SMAD4*, *CDKN2A*, *TP53*) occur at high frequency and promote aberrant downstream signaling events that characterize PDAC initiation [11]. Non-coding regions constitute a majority of the mammalian genome; regulatory mutations in these regions significantly impact on the expression of PDAC genes [12]. Deep sequencing has identified recurrently altered genes that mediate chromatin remodeling (*KDM6A*, *ARID1A*, *MLL2*), DNA damage repair (*BRCA2*, *BRCA1*, *ATM*), and other key PDAC pathways (*MYC*, *GATA6*, *MET*, *ROBO1*) [11,13]. These efforts have spawned clinical trials in patients with *BRCA* mutations, with moderate effects on patient survival [14,15].

Transcriptomic studies have used gene expression signatures and non-coding RNA (ncRNA) profiles to define two major PDAC subtypes – basal/quasimesenchymal and classical – with implications for patient prognosis and treatment [5,16]. This work spurred a series of recent

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Box 1. Advances in Targeting Mechanistic Drivers of Gene Expression in PDAC

Mutations

Although advances have been made in targeting mutant $KRAS^{G12C}$, this only accounts for 1% of all *KRAS* mutations in PDAC. The most common mutation in PDAC is $KRAS^{G12D}$ which remains undruggable [93–95]. Although direct inhibitors of $KRAS^{G12D}$ are not currently available, new approaches to blocking KRAS signaling have emerged. For example, $KRAS^{G12D}$ siRNA delivered by mesenchymal stromal cell-derived exosomes is under investigation in pancreatic cancer and nearing clinical trials (NCT03608631). Targeting downstream KRAS effectors has been used as an alternative approach. For instance, the combination of trametinib (MEK inhibitor) plus hydroxychloroquine resulted in PDAC tumor regression in preclinical animal studies and is now in a Phase I clinical trial (NCT03825289) [96]. This opens the possibility of inhibiting mutant KRAS by alternatively targeting upstream and downstream effectors.

Transcription Factors

Owing to difficulties in inhibiting transcription factors, targeting upstream and downstream processes have shown promising results. For example, statins have been shown to disrupt YAP nuclear localization and delay the progression of PanIN to PDAC in GEMMs [97]. *FOSL1* is highly expressed and is associated with an unfavorable prognosis in PDAC [98,99]. Targeting of AURKA, a downstream target of FOSL1, synergizes with a MEK inhibitor and impairs cell proliferation of mutant *KRAS* cells, suggesting a potential combinatorial strategy to treat tumors harboring *KRAS* mutations [49].

Epigenetic Regulators

Because epigenetic regulators play key roles in gene expression, targeting these factors presents an opportunity for new therapeutic approaches. For example, the histone deacetylase (HDAC) inhibitor entinostat alters the expression of genes involved in myeloid-derived suppressor cell signaling, thus improving the response to the immune checkpoint inhibitor nivolumab in PDAC GEMMs [100]. This combination is under investigation in a Phase II clinical trial for the treatment of metastatic PDAC (NCT03250273). Other epigenetic drugs such as the DNMT inhibitor azacitidine are in clinical trials in combination with immunotherapy (pembrolizumab, NCT03264404) or with first-line chemotherapy in patients with resected pancreatic cancer (NCT01845805).

RNA Regulation

One process by which RNA is regulated is by differential RNA splicing, which may result in aberrant protein isoforms and dysregulated gene expression. Because RNA splicing is a central biological process, targeting splice variants themselves represents a logical therapeutic approach. For example, the constitutive activation of the RON tyrosine kinase splice variant P5P6 is inhibited by BMS-777607 in PDAC [101]. Altered RNA splicing of Ras GTPase-activating protein (GAP) driven by *TP53* mutations has been shown to impact on the expression of RasGAP isoforms and activate oncogenic RAS signaling in PDAC [37]. Surprisingly, global inhibition of splicing shows that particular RasGAP isoforms are therapeutically vulnerable depending on *TP53* status [102]. This suggests that global targeting of RNA splicing could be utilized for targeted therapy in patients with specific *TP53* mutations.

efforts to mechanistically determine the transcription factors and epigenetic modifications that control these expression signatures. In addition, the role of ncRNAs, alternative splicing, and isoform stability as important modulators of oncogene expression has gained prominence. These efforts are important given that transcriptional addiction is a defining dependency of cancer [8]. Similar to the concept of oncogene addiction, dysregulated programs acquired during tumor development remain crucial for tumor maintenance. Therefore, identifying the aberrant ncRNA profiles, transcription factor nodes, and epigenetic modifications that orchestrate dysregulated signaling pathways may help to target PDAC vulnerabilities.

In the following we synthesize recent advances from genome-wide and reductionist studies in PDAC to identify drivers of gene expression dysregulation and highlight the clinical relevance of these findings. We focus on four driver mechanisms: mutations, transcription factors, epigenetic regulators, and RNA regulation. Given that these mechanisms frequently overlap, we highlight their contribution in the context of PDAC development, from initiation through to metastasis (Table 1).

Drivers of Aberrant Gene Expression That Facilitate PDAC Initiation

The normal pancreas is composed of several different cell types and can be divided into three major compartments based on histology: ductal, endocrine, and exocrine. Although

Table 1. Mechanisms Driving Dysregulated Gene Expression during PDAC Development[a]

Process	Genes altered	Type of alteration	PDAC stage/impact	Refs
Mutations	*KRAS*	GTPase-inactivating	Initiation, maintenance	[11,17,18,48]
	SMAD4, *CDKN2A*, *TP53*	Inactivation, deletion	Progression, metastasis	[11,19,66–68]
	KDM6A	Inactivation	TP63 activation	[87]
	GNAS	Activation	IPMN, epithelial differentiation	[22,23]
	RABL3	Nonsense (germline mutation)	Associated with PDAC incidence	[42]
	RNF43	Inactivation	Promotes PDAC growth	[43]
	PTPRN2, *LHX8*, *SLC12A8*, *TUSC7*	Non-coding (promoter mutations)	Promote PDAC growth	[44]
	TP63	Activation	Promotes a basal-like phenotype	[87]
	GLI2	Activation	Drives classical to basal subtype features	[90]
	MET	Inactivation	Promotes basal to classical transition	[86]
	GATA6, *SMAD4*	Amplification and deletion, respectively	Enriched in classical tumors	[84]
Transcription factors	*STAT3*	Phosphorylation	PanIN, ADM	[25,27]
	MYC	Amplification, activation	Progression, maintenance	[26]
	YAP1, *TAZ*	Activation	Initiation, ADM, drive basal subtype features	[27,28,89]
	SOX9	Upregulation	ADM, PanIN, progression	[29]
	HNF1A	Upregulation	Tumor growth, increases PCSC markers	[34]
	HNF4A	Gene Silencing	Classical to basal subtype reprogramming	[85]
	ZEB1	Loss of expression	Classical phenotype	[73]
	FOXA1	Upregulation	Metastasis, enhancer reprogramming	[75]
	YY1	Upregulation	Promotes PDAC invasiveness	[77]
	BLIMP1	Upregulation (hypoxia-induced)	Metastasis	[72]
	KLF5	Selective expression	Low-grade PDAC, epithelial identity control	[51]
RNA regulators	*LINC00673*	miRNA binding site mutation	Increases PDAC risk	[35]
	MST1R (RON tyrosine kinase receptor)	Splice variant (alternative splicing)	Transformation	[37]
	AGO2	Perturbation	PanIN to PDAC progression	[39]
	miR-489	Repression	Promotes PDAC invasiveness	[77]
Epigenetic regulators	*SIRT6*	Downregulation	Poor prognosis after resection	[52]
	Lin28b	Promoter hyperacetylation	PDAC development and metastasis	[52]
	ZEB1	Upregulation (APA)	PDAC cell survival, metastasis	[55]
	ALDOA, *FLNA*	Upregulation (APA)	PDAC growth-promoting genes	[56]
	KCNK15-AS1	Downregulation (demethylase loss)	Promotes PDAC cell migration and invasion	[59]

[a]Abbreviations: APA, alternative polyadenylation; IPMN, intraductal papillary mucinous neoplasms; MCN, mucinous cystic neoplasms; PanIN, pancreatic intraepithelial lesions.

the identity of PDAC precursor cells is not definitively established, PDAC is known to develop via transitional precursor lesions [3]. Three well-studied lesions are pancreatic intraepithelial lesions (PanIN), intraductal papillary mucinous neoplasms (IPMN), and mucinous cystic neoplasms (MCN). An activating *KRAS* mutation (most frequently G12D) that drives aberrant RAS signaling is the key event in PDAC initiation and development from PanINs and IPMNs [17,18]. PDAC development in the context of *KRAS* mutation can be associated with amplifications of oncogenes such as *MYC*, *YAP1*, and *NFKB*, or by chromothripsis and allelic imbalance accompanied by early *CDKN2A* deletion. The latter process is associated

with greater metastatic potential [19]. In either case, *KRAS* mutations are unequivocally associated with poor prognosis [20].

Mutations

Mutations in *GNAS*, encoding the G-protein Gαs subunit, are prevalent in IPMN [13,21,22]. Expression of a constitutively active $GNAS^{R201C}$ mutant in mice with *KRAS*-induced IPMN yielded a gene expression profile that overlapped with the ductal phenotype. Mechanistically, $GNAS^{R201C}$ activated the Hippo pathway and attenuated YAP1 signaling, leading to the formation of differentiated tumors. Therefore, mutant *GNAS* promotes an epithelial differentiation gene expression program on the background of mutant *KRAS* [23].

Transcription Factors and Epigenetic Regulators

Genetic alterations in precursor lesions disrupt crucial transcriptional programs, creating new signaling dependencies in PDAC. Downstream of *KRAS* mutations, aberrant transcription factor expression is a key driver of dysregulated gene expression programs that cooperate with pancreatitis to promote PDAC progression [24]. In a $Kras^{G12D}$, *Tp53* deletion mouse model, phosphorylated STAT3 (Tyr705) was upregulated in PanIN, whereas reduced STAT3 phosphorylation was observed in PDAC. This switch led to increased expression of mesenchymal markers and undifferentiated, malignant tumors [25]. Acute MYC activation can also trigger a $KRAS^{G12D}$-induced PanIN to PDAC transition *in vivo* [26]. MYC dysregulation (without overexpression or amplification) was sufficient to trigger hypoxia and desmoplasia within 24 h. This also reinforced the idea that persistent expression, and not elevated levels of MYC, promote its oncogenic activity. Untransformed acinar cells in the context of oncogenic *KRAS in vivo* exhibited activation of the YAP1 and TAZ transcription factors that redundantly mediate the JAK–STAT3 pathway to promote acinar to ductal metaplasia (ADM) [27,28]. The ataxia-telangiectasia (AT) group D-complementing *ATDC* gene was found to be required for *KRAS*-driven ADM progression to PanIN lesions via activation of β-catenin signaling and *SOX9* upregulation [29].

Minor populations of pancreatic cancer stem cells (PCSCs) have been identified in PDAC tumors that are distinguished by their cell-surface marker expression and can give rise to recurrent disease [30–32]. Although the basis of their aggressive behavior and progenitor role in PDAC initiation remains to be delineated, several regulatory molecules have been implicated in maintaining the PCSC state, including BMI-1, NOTCH, and SOX2. Notably, targeting the NOTCH-1 pathway with γ-secretase inhibitors depleted the PCSC population [33]. Recently, the endodermal lineage transcription factor HNF1A was found to promote tumor growth and maintain the unique transcriptomic signature of PCSCs via upregulation of the stem cell factor *OCT4* [34]. Knockdown of HNF1A depleted the PCSC activity of cells *in vivo*, and therefore targeting this axis may be important for treating recurrent disease.

RNA Regulation

The impact of RNA splicing and non-coding variants has gained attention as an important player in PDAC initiation. A recent study delineated the tumor-suppressive role of the ncRNA *LINC00673* in PDAC [35]. *LINC00673* was found to interact with and promote ubiquitination and degradation of the tyrosine phosphatase PTPN11. This led to downregulation of PTPN11 downstream signaling, including STAT1 response genes and SRC–ERK signaling, thereby inhibiting cell proliferation. A genome-wide association study (GWAS) found a G>A germline variant within *LINC00673* that is associated with pancreatic cancer risk [36]. This mutation creates a binding site for miR-1231 on *LINC00673*, causing its suppression, and this correlated with increased PDAC susceptibility. Recently, an alternatively spliced variant of the RON tyrosine kinase receptor was detected in a majority of pancreatic cancer cell lines and xenografts. This

isoform was found to transform human pancreatic ductal epithelial (HPDE) cells via activation of the AKT pathway [37].

Dysregulated miRNAs that alter PDAC gene expression programs play important roles in PDAC pathogenesis. In a study using a GEMM (genetically engineered mouse model) of Argonaute 2 (*AGO2*) loss, it was shown that depletion of this RNA-induced silencing complex component in the pancreas led to altered expression of multiple miRNAs. This depletion blocked PanIN to PDAC progression. In particular, the miR-29 and miR-30 families that have been strongly associated with oncogene-induced senescence were upregulated in PanIN lesions lacking *AGO2*, resulting in attenuated cell proliferation [38]. In addition, AGO2 is known to interact with KRAS to enhance cell proliferation. Therefore, the AGO2–KRAS interaction is a crucial and targetable dependency of PanIN to PDAC progression [39]. However, clinically actionable insights from mechanistic genomic and epigenomic studies have not yet gained traction. However, promising advances have been made in recent years. For example, using data from a limited cohort of 29 PDAC patients before and after resection, an exosomal miRNA signature that included miR10b and miR30c accurately established a PDAC diagnosis to differentiate between PDAC and chronic pancreatitis [40]. In addition, a recent study found that administering an amphiphilic nanocarrier in tumor-bearing mice that carried a combination of miR-34a (MYC targeting) and a PLK1-targeting siRNA showed an antitumor effect, suggesting the possibility of a nanotherapeutic [41]. Therefore, understanding the gene regulatory changes underlying PDAC onset may support not only the development of new therapies but also new diagnostics.

Molecular Drivers That Characterize the PDAC Phenotype and Its Maintenance

PDAC progression and maintenance involve multiple cooperating alterations and pathways. The molecular triggers of the transition of precursor lesions to PDAC may not be necessary or sufficient to maintain the PDAC phenotype. The following section aims to discuss the most recent findings associated with these processes. Although the studies described in the following text delineate dysregulated programs associated with PDAC progression or maintenance, these mechanisms may also play distinct roles in initiation or metastasis. A definitive understanding of these roles will require studies in the proper cellular context and model systems.

Mutations

Advances in gene editing and sequencing have facilitated studies that delineate the role of mutations in mediating gene expression changes in PDAC. A nonsense germline mutation was recently identified in the *RABL3* gene (RAS oncogene family-like 3) in a family with high PDAC incidence [42]. Dysregulation of KRAS activity and its downstream pathways was found to take place via increased prenylation of KRAS by mutant RABL3. Although *RABL3* mutation was proposed as a genetic testing target in familial PDAC, extensive genomic analyses and functional studies need to be carried out before clinical implementation. Another frequently mutated gene in PDAC is the ubiquitin ligase *RNF43*. Genome-wide CRISPR screens revealed that inactivating *RNF43* mutations in PDAC cells promoted cell growth via FZD5 receptor-dependent Wnt signaling, and demonstrated increased sensitivity to anti-FZD5 antibodies [43].

Non-coding mutations have gained recognition as an important contributor to the PDAC phenotype. Previously, challenges associated with the *in silico* identification of statistically significant mutations, coupled with their subtle and indirect influence on PDAC gene expression, had left this area relatively unexplored. However, recent studies have identified regulatory non-coding somatic mutations in the promoters of numerous genes (*PTPRN2*, *LHX8*, *SLC12A8*, *TUSC7*) that are distinct from PDAC coding mutations but converge on PDAC growth-promoting pathways, including the Wnt signaling pathway, cell adhesion, and axon guidance [44]. These *cis*-regulatory

promoter mutations significantly attenuated downstream gene expression. Importantly, low expression of two such genes, namely the protein phosphatase *PTPRN2* and the ion transporter *SLC12A8*, was associated with decreased patient survival, providing evidence for the clinical relevance of non-coding regulatory mutations in PDAC. Various GWAS and eQTL (expression quantitative trait locus) analyses in recent years have shed light on the genetic regulation of PDAC gene expression pathways and associated cancer risk [45,46]. A comprehensive eQTL study on 95 normal pancreas and 115 PDAC samples detected enrichment of eQTLs in regulatory regions of genes that are required for pancreas specification. The role of PDAC-specific altered eQTLs (in genes *ALOX5*, *DSCC1*, *CDCA7*) remains to be validated to determine their contribution to the PDAC phenotype [45]. A pathway-based analysis of GWAS data of 9040 PDAC cases and ~12 500 controls identified several SNPs and pathways associated with PDAC risk [46]. Although such GWAS and eQTL studies provide a strong rationale for follow-up mechanistic studies, their clinical impact remains unclear [36,45,47].

Transcription Factors and Epigenetic Regulators

Cancer cells often develop epigenetic dependencies that drive dysregulated expression programs integral to phenotype maintenance. Although mutant *KRAS* is a near-universal requirement in PDAC initiation, *KRAS*-independent maintenance of the PDAC phenotype has been frequently observed. A complete *KRAS* knockout in PDAC cell lines enhanced PI3K-dependent MAPK signaling to maintain the PDAC phenotype [48], thereby increasing sensitivity to PI3K inhibitors. The role of a YAP1-mediated transcriptional program in KRAS-independent PDAC maintenance has been shown in GEM models [49]. YAP1 is required for PDAC maintenance via MYC transcription and prevention of ductal cell redifferentiation [49,50]. MYC inhibition triggers a transcriptional program that rapidly reverses the PDAC phenotype to PanIN, leading to tumor regression in mice [26]. The transcription factor *KLF5* is selectively expressed in low-grade PDAC and is required for differentiated epithelial identity. KLF5 maintains the acetylation of a group of enhancers regulating the epithelial gene expression program, and loss of their acetylation state was associated with a partial loss of epithelial identity in high-grade PDAC [51]. The histone deacetylase sirtuin 6 (SIRT6) was identified as a PDAC tumor suppressor [52]. Patients with low *SIRT6* who underwent resection exhibited poorer prognosis in comparison to those with high *SIRT6* levels. SIRT6 inactivation led to promoter hyperacetylation of the *let-7* miRNA negative regulator, *Lin28b*. This resulted in increased expression of key let-7 target genes such as *HMGA2* and *IGF2BP1*, hastening PDAC development and metastasis in GEMMs. Recently, the redox regulator NRF2 was found to stimulate mRNA translation in PDAC by maintaining the reduced state of cysteine residues in proteins regulating translation. Loss of NRF2 led to impaired autocrine EGFR signaling, regulatory protein oxidation, and consequently inefficient mRNA translation. This resulted in PDAC cell proliferation defects in both *in vitro* and *in vivo* mouse models [53]. Inhibition of the heat-shock protein HSP90 in PDAC cell lines downregulated a subset of DNA methyltransferases (DNMTs), driving altered methylation patterns. This resulted in re-expression of tumor-suppressive genes such as *CDKN2A/P16*^{INK4A}, *MLH-1*, and *SPARC*, leading to inhibition of cell proliferation, suggesting that HSP90 might be a targetable link in PDAC [54].

A regulatory mechanism recently gaining recognition as an important modulator of gene dysregulation in PDAC is alternative polyadenylation (APA) (Box 2). APA controls the length of the mRNA 3'-untranslated region (UTR), and thus affects mRNA stability and localization. Gemcitabine treatment of PDAC cells led to an APA-mediated increase of the epithelial-to-mesenchymal transition (EMT)-related transcription factor ZEB1 [55]. An in-depth tumor type-specific analysis of APA revealed overexpression of the APA machinery and widespread 3'-UTR shortening events in PDAC [56]. These shortening events were associated with overexpression of PDAC growth-

Box 2. Alternative Polyadenylation (APA) as a Driver of Oncogene Expression in Cancer

APA is a post-transcriptional mRNA process that generates distinct mRNA isoforms. APA can occur within a gene, thereby producing different protein products. This type of APA has been implicated in leukemia but not in solid tumors [102]. The most common type of APA occurs within the 3' untranslated region (3'-UTR) and generates the same protein product but with distinct 3'-UTR lengths [103]. This is facilitated by the presence of multiple polyadenylation sites (PASs) mostly located within the 3'-UTR [104] and by a set of core APA factors that bind to the 3'-UTR in a sequence-dependent manner [105]. Both PASs and APA factors are crucial elements for the selection and cleavage of the 3'-UTR before the addition of the poly(A) tail. The 3'-UTR also contains gene regulatory elements such as miRNA and RNA-binding protein (RBP) binding sites that are crucial for mRNA stability, translocation, and translation. The choice of PAS dictates whether the resulting transcript is short or long. Short transcripts result in loss of multiple miRNA- and RBP-binding sequences, directly impacting on gene expression. In the past decade, APA has emerged as a key gene regulatory mechanism in cancer. Pan-cancer analyses of 3'-UTR usage revealed that global shortening of 3'-UTRs and upregulation of APA factors is associated with overexpression of many oncogenes across multiple tumor types [106]. For example, the cyclin D2 (*CCND2*) and *IMP1* oncogenes with short 3'-UTRs escape miRNA repression and are stable and overexpressed in cancer [107]. Regarding the APA factors, overexpression of *CSTF2*, for instance, increases the usage of the short *RAC1* 3'-UTR, thereby stimulating cancer cell proliferation, migration, and invasion [108]. In PDAC, gemcitabine treatment leads to APA-mediated increase in the expression of ZEB1 protein, an EMT-related transcription factor [109]. This suggests that APA plays a role in the chemoresistance of pancreatic cancer. An in-depth PDAC-specific analysis of APA revealed widespread 3'-UTR shortening events and an overexpression of APA factors in pancreatic cancer [55]. Although APA shortening events are widespread among different cancer types, whether such alterations are cancer-specific or there is commonality between cancer types is not known. Also unknown is how specific driver mutations in cancer contribute to these APA changes and how APA mediates drug resistance.

promoting genes (e.g., the aldolase dehydrogenase *ALDOA* and the filamin *FLNA*) and loss of highly conserved miRNA binding sites. Finally, patterns of APA were associated with poor prognosis in PDAC patients, suggesting that APA may be a key player in PDAC oncogenesis.

RNA Regulation

Long ncRNAs (lncRNAs) and miRNAs have also emerged as key regulators of gene expression in tumor maintenance [47,57,58]. Next-generation sequencing studies comparing the non-coding transcriptome of six PDAC patients and five control samples confirmed significantly different expression signatures of miRNAs and lncRNAs that regulate the expression of genes such as *TCF4* [58]. The lncRNA *KCNK15-AS1* was found to be a target of ALKBH5, a demethylase that is downregulated in PDAC. Downregulation of *KCNK15-AS1* in PDAC cells promoted cell migration and invasion [59]. Using The Cancer Genome Atlas (TCGA) pancreatic adenocarcinoma (PAAD) dataset and a microdissected dataset of PDAC tumors, a recent study generated a collection of PDAC-associated lncRNAs, identified lncRNAs that regulate the transcriptional profile of PDAC tumors, and determined associated SNPs in genomic regions of lncRNAs that correlated with PDAC risk [60,61]. This endeavor identified relevant lncRNAs in PDAC, providing a resource for functional validation studies.

Several splice variants and epigenetic markers have been proposed as novel targets and/or diagnostic markers for PDAC [41]. For example, promoter methylation of *ADAMTS1* and *BNC1* was identified as a potential diagnostic biomarker in cell-free tumor DNA. However, the clinical relevance remains to be established.

Gene Expression Dysregulation That Drives Metastatic PDAC

Metastasis, where migratory tumor cells expand in a new tissue environment, represents an advanced stage in PDAC progression. Most sequencing studies have focused on primary PDAC tumors because of difficulty in obtaining metastatic clinical samples. However, there has been a recent surge in genomic and transcriptomic analyses of distant and local metastatic tissue that have provided deeper mechanistic insights into metastatic programs. Furthermore, the ability to culture metastasis-derived organoids has provided a unique model system for functional studies [7,62–64]. This section seeks to discuss key findings that establish the drivers of

dysregulated gene expression that orchestrate the newly acquired migratory properties in metastatic cells.

Investigating the mutational landscape of metastases can help to explain the acquisition of migratory and invasive properties. *SMAD4* loss is known to drive metastasis in GEM models [65]. Concordant with this finding, a retrospective study showed that *SMAD4* loss was associated with higher rates of distant recurrence in surgically resected patients [66]. A recent targeted exome sequencing study of ten resected primary tumors and matched recurrences or distant metastases showed that recurrent disease was associated with increased mutational burden [66–68]. These recurrences were enriched for alterations that activated MAPK and the PI3K–AKT signaling pathways, revealing key clinical dependencies of recurrent disease.

The exact role of mutant *KRAS* and the EMT transcriptional program in driving PDAC metastasis remains to be resolved. Although high mutant *KRAS* expression was shown to drive metastasis in mice and the induction of EMT genes in human PDAC cell lines [69], it has also been shown that *KRAS* knockout in human PDAC cell lines led to the induction of metastatic genes and the EMT phenotype [19]. Recent studies on treatment-naïve patient autopsy samples revealed limited heterogeneity in driver mutations between primary and metastatic lesions [48]. Interestingly, this pattern has been observed in several other untreated metastatic cancers [70]. Although each metastatic lesion (liver, lymph, lung, and peritoneum) was found to arise from an independent primary tumor subclone, there was no specific driver mutation that accounted for differences between the primary and distinct metastatic PDAC lesions. Instead, the acquisition of passenger mutations of unclear functional relevance contributed to the observed intratumoral heterogeneity between lesions. This leads us to two conclusions. First, driver mutation-specific treatments could target primary and metastatic lesions uniformly, and therefore represent a useful treatment strategy in metastatic pancreatic cancer. Second, prioritizing the study of non-genetic drivers of metastasis will be crucial for unraveling the mechanistic players of this clinically advanced event.

Using GEMMs, it was shown that the transcription factor *BLIMP1* is a key player that orchestrates the metastatic properties of cells in response to hypoxia [71]. Although the exact role of EMT in metastasis is debated, the EMT transcription factor ZEB1 was found to drive metastatic pancreatic cancer in a mutant *Kras* and *Tp53* (KPC) mouse model [72]. Epigenetic profiling of cell lines generated from primary and metastatic tumors showed global loss of histone H3 lysine 9 (H3K9) and H4K20 methylation that correlated with metastasis [73]. Surprisingly, this global reprogramming and dysregulated gene expression program that promotes invasion was exhibited by distant, but not local, metastases, and could be reversed by inhibiting a key enzyme dependency. This enzyme, 6-phosphogluconate dehydrogenase, was found to modulate the increased activity of the oxidative pentose phosphate pathway in distant metastases. In PDAC organoids, the transcription factor FOXA1 drives enhancer reprogramming, in particular an increase in histone H3 acetylation (H3K27ac) and methylation (H3K4me1) marks near foregut endoderm development genes to promote PDAC metastasis [74]. Genes in this pathway encode for proteins such as the Ral guanine exchange factor, RGL1, that promote invasive properties in PDAC, although this pathway has not been implicated in metastasis previously [75]. KRAS signaling represses miR-489 expression via upregulation of the transcription factor YY1 through the NF-κB pathway. This was found to promote PDAC invasiveness in cell lines owing to increased expression of the metalloprotease genes *ADAM9* and *MMP7* [76]. Overall, a recent influx of metastatic PDAC studies utilizing human samples has provided insights that may uncover new targetable dependencies.

Inter- and Intratumoral Transcriptional Heterogeneity That Drives PDAC Subtypes

Recent genomic studies have identified prominent intertumoral transcriptional heterogeneity and used this information to delineate PDAC subtypes [77]. The goal of subtyping in pancreatic cancer is ultimately to stratify patients so as to inform prognosis and personalized treatment. Although PDAC subtyping has greatly enhanced our fundamental understanding of the complex transcriptional heterogeneity in pancreatic cancer, it has not yet led to clinical breakthroughs. Prioritizing research into the underlying transcriptional factors and epigenetic modifications that drive the formation of each subtype can contribute to the identification of targets regulating phenotypic plasticity. Although there are excellent reviews on classification of PDAC subtypes, this section aims to highlight recent findings regarding the mechanisms that drive transcriptional heterogeneity [16,60,78–80].

The current consensus based on gene expression programs, epigenetic modifications, histology, and genomic aberrations in numerous model systems classifies PDAC tumors as basal/quasimesenchymal or classical, where the basal subtype exhibits a worse prognosis. ncRNA signatures that associate with the classical and basal subtypes have also been identified [5,81,82]. Evaluation of intrapatient sample heterogeneity identified the basal subtype to be a subclonal population within a classical tumor. These basal regions are significantly enriched in chromatin-modifier gene mutations and *MYC* amplifications [13,61]. *SMAD4* loss and *GATA6* amplifications were primarily enriched in classical tumors, whereas genome duplication-driven imbalances in *KRAS* were associated with the basal subtype [83]. Cells exhibiting basal and classical signatures form a gene expression continuum and exist intratumorally [84].

Recently it was found that the siRNA-mediated depletion of the endodermal specification gene *HNF4A* in PDAC patient-derived cell lines was sufficient to switch their metabolic profiles from the classical to the basal subtype [84]. This switch was associated with upregulation of glycolysis gene expression programs regulated by downstream molecules including ALDOA, HK, and GSK-3β. Targeting glycolysis using GSK-3β inhibitors revealed selective sensitivity of the basal subtype. This selective sensitivity was attributed to distinct patterns of chromatin accessibility, emphasizing the relevance of chromatin profiling for patient stratification. Chromatin immunoprecipitation-sequencing experiments on patient-derived xenografts uncovered two distinct epigenomic landscapes that characterized the classical and basal subtypes. Classical tumors were associated with transcription factors involved in pancreas development and RAS signaling, whereas the basal phenotype expressed proliferative and EMT-associated transcriptional markers [85]. In particular, *MET* was identified as an essential molecular player for the basal phenotype, and *MET* depletion reverted the gene expression signature to a more classical subtype. Although loss of *ZEB1* expression was associated with a transcriptional program that characterizes a classical phenotype, single-cell sequencing data confirmed the association between EMT and the basal expression program [86].

Recently, a network of transcription factors that drive the basal subtype via a group of specific superenhancers were identified. It was found that ΔNp63 (TP63) was a required dependency for these superenhancers to drive gene activation in basal subtypes. Depletion of the demethylase *KDM6A* (that is known to be mutated in PDAC) led to activation of TP63 to drive a basal-like phenotype in pancreatic cancer cells and greatly increased their sensitivity to BET inhibitors [73,84]. Interestingly, TP63 activates the Hippo pathway coactivator YAP1 in other cancers [87]. Although this link remains to be explored in pancreatic cancer, YAP1 is necessary to maintain, and sufficient to drive, basal subtype features in PDAC cells, and its expression is associated with poor survival [88]. Activation of the transcription factor GLI2 is sufficient to drive classical PDAC cells to acquire basal subtype features [89]. Although there is evidence

for YAP1-mediated activation of GLI2 in other cancers [90], this link in basal PDAC remains to be determined.

Overall, although PDAC subtyping studies in the past decade have been crucial in delineating tumoral heterogeneity and the transcriptional programs that are associated with PDAC maintenance and metastases, there has been modest progress with respect to clinical relevance of this classification system. For example, a recent study delineated a transcriptomic signature of high replication stress that was enriched in the basal subtype and predicted response to DNA damage inhibitors. However, this study was limited to patient-derived cell lines and organoids, and used preclinical stage inhibitors [91]. Accounting for molecular pathology and master transcriptional regulators driving dysregulated programs might be key to improving tumor classification systems that would facilitate patient selection for personalized therapy.

Concluding Remarks and Future Perspectives

Dissection of the dysregulated gene expression programs that drive PDAC initiation, maintenance, and metastasis is necessary to identify patient-specific dependencies and improve patient survival. In the past decade, the rapid development of sequencing technologies, PDAC GEMMs and organoid models that reliably recapitulate the patient PDAC phenotype, and studies on PDAC metastatic patient samples have provided genome-wide and mechanistic insights into disease progression. Moreover, an increased understanding of the complex PDAC stroma, including multiple subtypes of cancer-associated fibroblasts and immune cell infiltrates, has raised the possibility of modulating the tumor microenvironment for improved drug delivery and immunotherapy response [92]. However, clinical progress has been incremental, and we have a long journey to traverse in the PDAC landscape (see Outstanding Questions). Greater commitment to delineating the mechanistic triggers of dysregulated gene expression programs, exploiting these insights to develop new therapies, and the development of a clinically pertinent patient classification system will hasten personalized treatment strategies.

Outstanding Questions

Can we identify a set of master transcriptional and epigenetic molecules that are necessary and sufficient to drive and/or maintain the PDAC phenotype? How many of these are targetable?

How can we reconcile transcriptomic findings from different PDAC model systems to develop a comprehensive model of PDAC progression based on dysregulated gene expression programs?

Can the current subtype classification system be further refined based on drivers of gene expression to aid clinical stratification and treatment benefits?

How can we improve the scope of current studies that propose oligonucleotide-based targeting approaches and RNA-based biomarkers to provide clinically actionable insights?

What is the basis of the aggressive behavior of PCSCs? Can we characterize their gene signature and use this information to target transcriptional dependencies in PCSCs for the treatment of recurrent disease?

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Declaration of Interests

The authors declare no conflicts of interest.

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