PANCREATIC CANCER

Unlocking PDAC initiation with AP-1

Chronic pancreatitis is a risk factor for pancreatic cancer; however, the mechanisms underlying cellular susceptibility to oncogenic transformation are complex. A recent study reports a damage-associated progenitor cell state, controlled by the transcription factors KLF5 and members of the AP-1 family, that initiates tumorigenesis in mouse models of pancreatic cancer in which the proto-oncogene *KRAS* is altered.

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Cancer initiation is a lineage-dependent process that requires a permissive cell state amenable to both transformation and long-term propagation of oncogenic regulatory programs. Substantial efforts in cancer-biology research have focused on the identification of this initiating cell state (the 'cell of origin') to determine the unique susceptibilities of normal cells to oncogenic mutations across cancer subtypes[1,2]. As a result of the increasing resolution of single-cell transcriptomic and epigenomic technologies, the understanding of cellular identity is becoming increasingly complex, as normal cells may exist as heterogeneous cell states induced by various environmental factors, including tissue damage[3]. Furthermore, changes in these environmental factors may induce the disruption of lineage-specifying transcription factors (TFs) that are needed to maintain cell type–specific gene regulation, which results in a switch of cell identity. Recent research in the field has sought to identify how these environment-driven regulatory programs may predispose cells to cancer initiation[4]. In a study by Li et al. in this issue of *Nature Cancer*, the authors report their work seeking to understand the requirement for inflammation in transformation mediated by the small GTPase KRAS in pancreatic adenocarcinoma[5]. This work uncovers a progenitor cell state that arises in inflamed pancreatic cells, called 'pancreatic duct-like progenitor' (PDLP) cells, that are uniquely poised for oncogenic transformation into pancreatic adenocarcinoma.

Mouse models serve as powerful tools for studying the cell of origin of various cancers, such as through genetic labeling for in vivo lineage tracing of cell fates after oncogenic transformation. In mouse models, inflammation induced by cerulein promotes the development of pancreatic intraepithelial neoplasia in cells transformed by oncogenic *Kras*, which can ultimately lead to pancreatic adenocarcinoma[6]. Consistent with such studies in mice, pancreatitis is a common risk factor for pancreatic cancer in human patients[7]. Pancreatic adenocarcinomas morphologically resemble ductal cells; however, studies have shown that ductal cells are resistant to *Kras*-mediated transformation[8,9], whereas acinar cells are more readily transformed in mouse models[10]. Notably, pancreatic inflammation induces a transdifferentiation process called 'acinar-to-ductal metaplasia', whereby acinar cells transition to a duct-like cell state[6,10]. In the healthy pancreas, this process of transdifferentiation is resolved after several days by redifferentiation, and this transition is precluded in Kras-mutant cells[11].

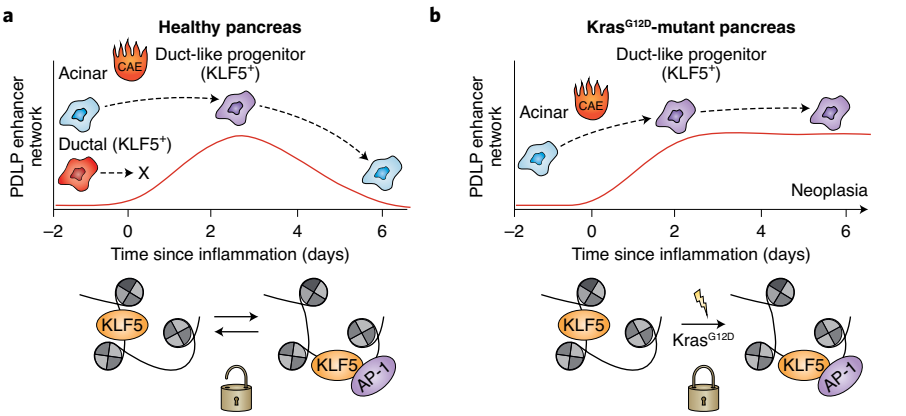

Fig. 1 | PLDPs serve as a cell of origin in Kras^{G12D}-mutant pancreatic adenocarcinomas. a, Trajectories of cellular identity after the induction of inflammation by cerulein (CAE). Acinar cells (blue) undergo acinar-to-ductal metaplasia after inflammation, unlike terminally differentiated ductal cells (red). Acinar-to-ductal metaplasia generates a duct-like progenitor cell called a 'PDLP' (purple). In the healthy pancreas, the PDLP transcriptional state is activated (vertical axis) but then recedes after the inflammation resolves (red line). KLF5 marks duct-like cells and PDLPs but not acinar cells. A transient (unlocked) PDLP-associated chromatin state arises in which KLF5 binds new enhancer sites with TFs of the AP-1 family. **b**, KLF5-expressing (KLF5$^+$) cells transformed with *Kras*^{G12D} form a PDLP state similar to that found in inflamed healthy pancreas; however, this PDLP state persists after oncogenic transformation (red line). The enhancer network induced by KLF5 and AP-1 factors becomes permanent (locked). Ultimately, the PDLP-like state gives rise to pancreatic intraepithelial neoplasia and pancreatic cancer.

On the basis of those prior observations, and the finding that the TF KLF5 has high expression in pancreatic adenocarcinomas, Li et al. established a *Klf5*-knock-in mouse allele to track the fate of KLF5$^+$ cells in healthy and Kras^{G12D} (mutant) pancreas after the induction of acute inflammation by cerulein[5]. Lineage tracing of acinar-cell identity and KLF5 expression demonstrated the emergence of a KLF5$^+$ duct-like precursor, or PDLP, during inflammation. In the healthy pancreas, this KLF5$^+$ PDLP progenitor population reestablished acinar-cell identity after inflammation; however, in the Kras-mutant pancreas, cells remained in the PDLP state, which led to the rapid formation of pancreatic intraepithelial neoplasia (Fig. 1). Strikingly, inflammation

in both healthy pancreas and Kras-mutant pancreas resulted in the activation of a similar regulatory program, as analyzed by RNA sequencing; however, the Kras-mutant, KLF5+ PDLP cells seemed to over-activate this program.

TFs are context-dependent regulators of cellular identity and are clear candidates for remodeling chromatin after inflammation in the pancreas[12]. Li et al. hypothesized that the *Kras* mutation may act in cooperation with inflammation by irreversibly inducing a 'locked' chromatin state[5]. To clarify the role of gene regulation in the inflammation-induced PDLP state, the authors performed bulk and single-cell ATAC-seq (assay for transposase-accessible chromatin using sequencing)[3] in mouse and human pancreatic tumor samples to investigate differences in chromatin accessibility at DNA regulatory elements (enhancers). They identified a PDLP-specific enhancer program, distinct from that in normal ductal cells, that corresponded to increased chromatin accessibility at genes associated with pancreatic cell identity (i.e., those encoding KLF5 and the TF FOXA2), as well as AP-1 motifs. Activation of KRAS is required for the progression of pancreatic cancer[13]; notably, the authors also found that the permanence of the chromatin changes induced in PDLPs, mediated by TFs of the AP-1 family, was dependent on sustained activation of KRAS. These persistent chromatin alterations, activated by inflammation and maintained by oncogenic KRAS, are referred to as a 'locked' enhancer network by the authors.

The biology of AP-1 TFs in cancer initiation has remained underexplored, largely due to the broad family of regulators that bind a similar AP-1 motif. To investigate this biology, Li et al. used CRISPR screening, proteomic analyses and in vivo studies to systematically identify TFs relevant in maintenance of the 'locked' enhancer network[5]. A pooled CRISPR screen identified several AP-1 TFs, including FOSL1 and JUNB, that enhanced the proliferative capacity of pancreatic cancer cells. Inhibition of newly accessible AP-1-bound enhancers led to reduced expression of neighboring genes involved in proliferation. Furthermore, proteomic and in vivo studies determined that JUNB and FOSL1, but not lineage-defining TFs such as KLF5, were bound to chromatin only in the context of the 'locked' enhancer network, driven by aberrant activation of KRAS. The authors also found that AP-1 factors acted cooperatively with lineage-defining TFs, promoting the relocation of lineage-defining TFs across the genome. Depletion of JUNB, FOSL1 or KLF5 in vivo blocked progression in models of pancreatic cancer, which clearly links these TF regulators to cancer initiation and the persistence of inflammation-induced oncogenic programs. Together these data suggest a paradigm whereby TFs involved in the general inflammation response (such as AP-1 TFs) transiently activate oncogenic regulatory programs that are sustained when conditions are just right — here, with the presence of an activating mutation in *Kras* and induction of a PDLP cellular state — to drive oncogenesis.

The results from this study argue for an expanded definition of a cell of origin that includes environmentally induced cell states that may be more susceptible to oncogene-mediated transformation than unstimulated cells. Notably, environmental factors, such as inflammation in pancreatic cancer, may be needed to generate an emergent cell of origin absent from the healthy pancreas. However, further work is needed to achieve a better understanding of whether acutely induced inflammation in mouse models of pancreatic cancer initiates regulatory programs comparable to those of chronic pancreatitis in human patients. For example, are the effects of inflammation truly transient, or are some chromatin states irreversibly established following inflammatory stimulation? Given the work from Li et al.[5], the cell type–specific gene-regulatory consequences of environmental insults, coupled with their oncogene sensitivity, promises to be a ripe area for future exploration. Furthermore, this study[5] adds to the increasing evidence suggesting that normal cells must revert to a de-differentiated cell state to enable cancer initiation or progression[14].

TF complexes are cooperative and highly regulated; in turn, abnormal activation of TFs in the wrong cellular context can lead to an altered regulatory landscape that is fertile for cancer initiation and progression. The role of TFs that are more ubiquitously expressed, such as proteins of the AP-1 family, has been mystifying due to the sheer volume of different regulators that have the ability to bind similar motifs. Here, Li et al. demonstrated that the specificity of AP-1 TFs was altered by lineage-defining TFs and cell type–specific expression patterns[5], which are generally dictated by evolutionary history or oncogenic signaling. One might hypothesize that this concept is translatable to other cancer types susceptible to *KRAS* mutations, such as lung cancer, with its own repertoire of lineage-defining TFs and AP-1 TFs. Understanding the complex interplay of oncogenic signaling programs, lineage-defining TFs and more-general TFs will be critical for delineation of the complicated mechanisms of cancer progression. Furthermore, greater understanding of the chromatin-modifying enzymes that reorganize epigenomic states in cancer will refine the mechanistic understanding of tumor progression and help identify additional druggable targets[15]. Overall, the work by Li et al.[5] will motivate the study of environmentally induced cell states in cancer and provide a more precise understanding of context-specific sensitivities to oncogenic transformation. ❐

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Competing interests

The authors declare no competing interests.