It's a Free for All—Insulin-Positive Cells Join the Group of Potential Progenitors for Pancreatic Ductal Adenocarcinoma

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Despite significant efforts, the cellular compartment that gives rise to pancreatic ductal adenocarcinoma (PDAC), one of the most lethal human cancers, remains obscure. In this issue of *Cancer Cell*, Gidekel Friedlander and colleagues demonstrate that under conditions of tissue damage, mutant Kras can drive previously unsuspected insulin-positive endocrine cells into PDA.

Pancreatic ductal adenocarcinoma (PDAC) carries a dismal prognosis with a 5-year survival rate of less than 5% and, on average, mortality occurring within 6 months of diagnosis (Hezel et al., 2006). The disease is generally insensitive to chemotherapy and surgical resection is the most effective treatment. Generic symptoms and a lack of specific biomarkers frustrate early detection when surgery is possible, making the development of diagnostic tests paramount to increasing treatment options and efficacy. Determining whether the capacity to serve as a PDAC "cell of origin" is unique to a specific pancreatic compartment represents a key step in guiding this search for possible diagnostic and therapeutic measures. A study performed by Gidekel Friedlander et al. (2009) published in this issue of *Cancer Cell* contributes to this goal by implicating insulin-positive cells in the adult pancreas, a previously unsuspected cell type, as a possible source of PDAC.

PDAC and its suspected precursor lesions, such as pancreatic intraepithelial neoplasias (PanINs), possess ductal morphology and express markers of ductal differentiation. Therefore, PDAC was historically believed to develop from pancreatic duct cells. However, the recent development of PDAC mouse models has allowed nonductal cells to be evaluated for their ability to give rise to PanINs and PDAC. These models depend on a master role for deregulated Kras activity in PDAC development. In humans, activating mutations in Kras are found in nearly 100% of PDAC and can be frequently detected in early PanIN (Hezel et al., 2006). Seminal work by Hingorani et al. (2003) provided strong support to the notion that Kras mutation represents an initiating event in PDAC. These authors showed that expression of mutant Kras in the multipotent pancreatic progenitor pool can recapitulate the stepwise progression of PanINs and PDAC observed in the human disease. Since then, more sophisticated genetic tools have been used to test the ability of Kras to induce PanIN and PDAC when activated in subsets of developing and adult pancreatic cells. In particular, these data suggest that adult acinar cells, the cells that produce and secrete digestive enzymes into the duct network, possess the capacity to give rise to Kras-driven PanINs and PDAC (Habbe et al., 2008; De La et al., 2008; Ji et al., 2009). Furthermore, chronic pancreatitis, a significant PDAC risk factor in humans (Hezel et al., 2006), experimentally induced in mice through treatment with the cholecystikinin analog caerulein, can both promote and accelerate Kras-driven PanIN/PDAC originated from acinar and possibly centroacinar cells (a cell type located at the junction between acini and ducts) (Figure 1; Guerra et al., 2007).

Studying the response of the pancreas to inflammatory damage, in particular caerulein pancreatitis, has revealed crucial information regarding the inherent plasticity of cells in the adult pancreas and their possible capacity to contribute to PDAC. For example, during the early stages of regeneration after acute pancreatitis, acinar cells frequently assume a transient duct-like state (Strobel et al., 2007a; Fendrich et al., 2008). However, in the absence of oncogenic signals, persistent acinar to ductal reprogramming of adult acini appears to be tightly restricted. Even when subjected to prolonged caerulein pancreatitis (for example, 7–10 weeks), wild-type acini rarely assume a fixed ductal fate, although these ducts can resemble early PanIN lesions (Strobel et al., 2007a). Therefore, the ability of mutant Kras to spontaneously reprogram acini into ductal PanINs suggests that the oncogene permits the development of an otherwise generally inaccessible differentiation state. Gidekel Friedlander and colleagues contribute to this growing body of evidence that adult acini can give rise to PanINs and PDAC by targeting mutant Kras to a subset of acini utilizing a *ProCPA*^{ERT2} driver line. Similar to results from other models, adult acini targeted with this Cre line did give rise to PanINs, although infrequently. Consistent with previous findings, chronic chemical pancreatitis significantly accelerated the development of PanINs in these mice.

The most surprising finding by Gidekel Friedlander and colleagues is that insulin-positive endocrine cells can serve as PDAC progenitors in the context of Kras mutation and inflammatory damage. The first indication for endocrine involvement comes from experiments in which a *Pdx1-Cre*^{ER} driver line was utilized to activate Kras in cells expressing the key pancreatic transcription factor Pdx1, which is found in a subset of adult acini and ducts, but mainly restricted to specific classes of adult endocrine cells. Whereas *Pdx1-Cre*^{ER}-driven recombination was detected in both the exocrine and endocrine compartment, PanINs were frequently and unexpectedly found embedded in

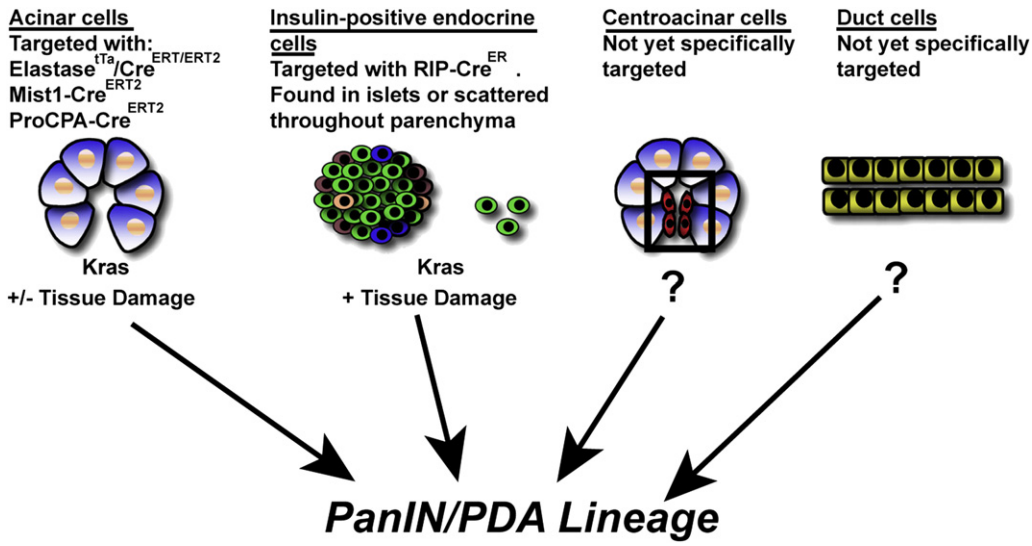

Figure 1. The Epithelium of the Adult Pancreas Consists of Diverse Cell Types
The exocrine compartment contains enzyme secreting acinar cells, duct cells, and centroacinar cells that connect acini and ducts. The cells of the endocrine compartment are mainly located in islets of Langerhans and secrete hormones directly into the bloodstream. The insulin producing β cells (green) are the predominant endocrine cell type. Whereas mutant Kras has been targeted to all pancreatic cell types nonspecifically during pancreatic development, Figure 1 illustrates which adult cell types have been specifically targeted with mutant Kras and under what conditions their contribution to PanIN/PDAC formation has been verified.

islets, potentially indicating that mutant Kras can reprogram endocrine cells into PanINs. It is key to note that PanIN lesions in these mice occurred in the absence of caerulein administration, suggesting that adult Pdx1-positive cells are sensitive to reprogramming by Kras even in the absence of tissue damage and inflammation. This is unexpected because direct genetic evidence of in vivo endocrine to ductal metaplasia is lacking. In fact, lineage tracing of insulin-positive cells in mice subjected to chronic caerulein pancreatitis showed no evidence of these cells contributing to persistent mucinous metaplastic ductal lesions (Strobel et al., 2007b).

By utilizing a *RIP-Cre^{ER}* (rat insulin promoter) line to target Kras activity to adult insulin-postive cells, Gidekel Friedlander and colleagues further show that the ability of mutant Kras to exploit tissue damage and inflammation to initiate the PanIN to PDAC sequence can be extended to the endocrine compartment. Although the mechanism of this shift in cell plasticity is unknown, this study shows that given a permissive state of tissue damage, mutant Kras can drive insulin-positive cells into an otherwise restricted duct-like state. One theory posited by the authors is that chronic damage amplifies an insulin-positive cell type that is sensitive to Kras activity. Elements of embryonic pancreatic development (e.g., Notch signaling, Pdx1 and Nestin expression) are reactivated in the exocrine compartment in response to caerulein pancreatitis (Jensen et al., 2005; Fendrich et al., 2008). Theoretically, a similar "dedifferentiation" could occur in insulin-positive cells, rendering a cell state that can be reprogrammed into PanINs and PDAC.

This study has many implications on our understanding of PDAC biology. It demonstrates that PanINs and PDAC can arise from nonexocrine cell types, so it begs the question as to whether all pancreatic cell types inherently possess the ability to give rise to PDAC. Taken together with recent studies targeting mutant Kras to adult acini, the capacity of the remaining non-β-cell endocrine cells (such as glucagon-positive α-cells), adult centroacinar, and duct cells to be reprogrammed into PanIN/PDAC remains to be specifically interrogated (Figure 1). Furthermore, it highlights the ability of tissue damage, specifically that characteristic of chronic pancreatitis, to provide a permissive environment for Kras-induced reprogramming of a "PanIN/PDAC lineage" from both exocrine and endocrine cells. Determining what cell-autonomous and extrinsic changes occur during chronic pancreatitis that permit this normally restricted shift in differentiation may provide key diagnostic or therapeutic targets for the earliest stages of PDAC.

Finally, this work further entrenches mutant Kras as a master regulator of the differentiation process that gives rise to PDAC. In demonstrating that PDAC can arise from cellular compartments as distinct as the exocrine and endocrine, this study displays a clear need to closely dissect which molecular events are critical for Kras to first initiate the precursor state and then drive this sensitized cell toward PDAC. Such analysis may identify a tractable node for hobbling this devastating disease, regardless of its cell of origin.

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Edging toward New Therapeutics with Cyclin D1 Egl'ng on Cancer

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In this issue of *Cancer Cell*, Zhang et al. reports that the iron-dependent 2-oxoglutarate dioxygenase or prolyl hydroxylase EglN2 induces Cyclin D1 levels, egging on breast tumorigenesis. Their observations through loss of function studies suggest the potential for drug-like molecules inhibiting EglN to serve as new cancer therapeutics.

EglN2 (also known as PHD1) is among three known EglNs, with EglN1 (PHD2) being key to the hydroxylation and degradation of the hypoxia inducible factor 1α (HIF-1α) (Berra et al., 2006; Epstein et al., 2001; Kaelin and Ratcliffe, 2008). Although the direct substrates for hydroxylation by EglN2 and EglN3 are not well understood, Zhang et al. document that EglN2 activity positively influences the level of Cyclin D1, independent of HIF, in a series of in vitro experiments and in genetically engineered mice (Zhang et al., 2009). The authors linked EglN2 to Cyclin D1 in breast tumorigenesis, partly on the basis of their genetic connection in *Drosophila* and the fact the EglN2 is estrogen inducible, such that loss of EglN2 activity diminishes breast tumorigenesis that could be rescued by ectopic Cyclin D1 expression.

Prolyl hydroxylases belong to the superfamily of iron-dependent, 2-oxoglutarate dioxygenases that use molecular oxygen to hydroxylate specific prolyl residues of substrates whose functions may be altered, or the hydroxylated substrates are destined for degradation through the proteasome (Kaelin and Ratcliffe, 2008). EglNs have Km values for oxygen that are higher than oxygen concentrations in mammalian tissues, causing these enzymes to be highly sensitive of oxygen levels, which are naturally diminished in areas of tissues distal from a blood vessel or abruptly decreased by blockage of the blood supply. With decreased oxygen concentration, the EglNs are thought to not only be inactivated by the lack of its substrate oxygen but may also be disabled by increased reactive oxygen species (ROS) resulting from inefficient mitochondrial respiration. ROS oxidizes and inactivates the catalytic EglN ferrous ion, resulting in loss of prolyl hydroxylation and stabilization of the EglNs' substrates. Chief among the EglNs' substrates is HIF-1α, which is a ubiquitous transcription factor mediating cellular genomic responses to hypoxia and a substrate of EglN1 (Figure 1) (Semenza, 2007).

Zhang et al. noted on the basis of earlier work by Frei and Edgar that the sole EglN, Egl9, in *Drosophila* interacts genetically with Cyclin D1 (Figure 1) (Frei and Edgar, 2004). Specifically, Frei and Edgar observed that Cyclin D1 overexpression in flies resulted in eye overgrowth, a phenotype that could be extinguished by the concurrent loss of Egl9 function. By examining *EglN2*$^{-/-}$ mice, which are grossly normal, Zhang et al. found that Cyclin D1 mRNA and protein levels are diminished in the mutant murine embryonic fibroblasts. They also document hypoproliferation of mammary glands in older EglN2 null mice reminiscent of the more severe phenotype reported for the *Cyclin D1*$^{-/-}$ mice. These observations in genetically engineered mice suggest that loss of EglN2 function phenocopies decreased Cyclin D1 activity in mammary tissue. Intriguingly, although it is documented that hypoxia inhibits cell proliferation partly through the induction of p27 putatively downstream of HIF-1 in mammalian cells (Gardner et al., 2001), the connection between EglN and Cyclin D1 in *Drosophila* and mice suggests a possible alternative pathway to inhibit cell proliferation when oxygen is lacking. In this regard, EglN could play a direct role in regulating the cell cycle by suppressing a putative suppressor of Cyclin D1 (Figure 1).

Cyclin D1 promotes the phosphorylation of the retinoblastoma protein (pRB),