27. Pepe MS, Kerr KF, Longton G, et al. Testing for improvement in prediction model performance. Stat Med 2013;32:1467–1482.
28. Galbois A, et al. Mini review: prognostic scores for cirrhotic patients admitted to an intensive care unit: which consequences for liver transplant? Clin Res Hepatol Gastroenterol 2013;37:455–466.
29. Wlodzimirow KA, Eslami S, Chamuleau RA, et al. Prediction of poor outcome in patients with acute liver failure- systematic review of prediction models. PLOS One 2012;7:1–7.

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New Insights Into the Cell Lineage of Pancreatic Ductal Adenocarcinoma: Evidence for Tumor Stem Cells in Premalignant Lesions?

See "Identification and manipulation of biliary metaplasia in pancreatic tumors," by DelGiorno KE, Hall JC, Takeuchi KK, et al, on page 233 and "DCLK1 Marks a morphologically distinct subpopulation of cells with stem cell properties in preinvasive pancreatic cancer," by Bailey JM, Alsina J, Rasheed ZA, et al, on page 245.

Pancreatic ductal adenocarcinoma (PDA) is a deadly disease primarily because of its asymptomatic nature early in the disease process. As a result, the diagnosis is usually not established until the tumor has already become invasive. To improve detection and treatment options for PDA, efforts to better understand the early stages of PDA are clearly warranted. Invasive PDA is believed to arise from a spectrum of preneoplastic mucinous lesions, the most common of which are pancreatic intraepithelial neoplasias (PanINs). Although invasive tumors usually exhibit numerous oncogenic mutations, activating mutations of the *Kras* gene are already found in early premalignant PanIN lesions.[1] The significance of *Kras* mutations for PDA initiation has been demonstrated in mice, where induction of a constitutively active *Kras* allele in embryonic pancreatic progenitor cells induces PanINs and, after a significant latency period, also PDA.[2]

Because PanINs display a duct-like morphology and express ductal genes, it has long been thought that PanINs arise from pancreatic ducts. However, recent studies in mice have shown that adult pancreatic ductal cells are surprisingly resistant to *Kras*-induced neoplastic transformation, whereas acinar cells readily give rise to PanINs in response to Kras activation.[3–5] These findings have moved acinar cells into the spotlight as a possible cell of origin for PDA and suggest that *Kras*-induced transformation of acinar cells into duct-like cells is the primary event in PDA initiation. Previous findings showing that the inactivation of genes necessary for acinar-to-ductal metaplasia also inhibit PanIN formation[3,6,7] support the notion that acinar cells play a major role in tumor initiation. Although acinar cell-derived preneoplastic lesions clearly share morphologic and molecular features with pancreatic ducts, they also express markers characteristic of the gastric epithelium and embryonic pancreatic progenitor cells.[2,8,9] Thus, the cellular identity of preneoplastic lesions in the pancreas has remained enigmatic. In this issue of *Gastroenterology*, studies from DelGiorno et al[10] and Bailey et al[11] show that *Kras*-induced preneoplastic pancreatic lesions exhibit striking similarity to cells of the pancreatobiliary epithelium. Because pancreatobiliary cells share molecular characteristics with embryonic pancreatic progenitors, adult pancreatic ductal cells, and gastric epithelial cells, these novel findings explain previous observations and shed new light on the cellular identity of preneoplastic lesions in the pancreas.

With the goal of revealing possible heterogeneity in the cell composition of early PanIN lesions, DelGiorno et al[10] performed a meticulous analysis of epithelial growth factor signaling pathway activity in PanIN lesions of mice expressing active Kras in the pancreas. The authors identified a subset of PanIN cells with particularly high levels of epithelial growth factor receptor signaling. These cells could be further distinguished from adjacent cells by a unique arrangement of microfilaments and prominent microvilli on the apical surface. The morphologic features of these cells bear striking resemblance to the characteristics of a specialized cell type, called the tuft or brush cell, which is abundantly found in pancreatobiliary epithelium.[12] Further confirming the identity of these cells as tuft cells, the authors show that established markers of tuft cells, including Dclk1 and acetylated tubulin (AcTub),[13] are present in PanINs.[10] The presence of tuft cells in pancreatobiliary ducts, but not untransformed pancreatic ducts, led the authors to hypothesize that oncogenic Kras could transform acinar cells into a duct-like cell type with pancreatobiliary identity.

The pancreatobiliary duct is formed as the common bile duct from the liver merges with the main pancreatic duct. The pancreatobiliary duct then connects to the duodenum to deliver the contents of the pancreatic and biliary ductal system into the intestine. Developmentally, the pancreatobiliary duct and the pancreas have a common cellular origin and segregation of the 2 cell lineages is dependent on the transcription factor Sox17.[14] Unlike the pancreatic ductal

epithelium, the pancreatobiliary epithelium is thought to be slowly renewed from multiple outpockets of pancreatobiliary epithelium containing $Pdx1^{+}Sox17^{+}$ stem cells, much like stem cells in the intestinal crypts renew the villus epithelium.[15] Supporting the idea that preneoplastic lesions in the pancreas have pancreatobiliary identity, DelGiorno et al[10] found Sox17 and Pdx1 to be expressed in early metaplastic lesions and PanINs of all grades. Furthermore, forced misexpression of Sox17 in acinar cells was sufficient to induce widespread ductal metaplasia and enhanced *Kras*-induced PanIN formation. Importantly, the Sox17-induced metaplastic lesions also contain tuft cells, suggesting that Sox17 transforms acinar cells into a ductal epithelium that phenocopies most features of the pancreatobiliary epithelium. Together, these comprehensive studies establish that PanINs are formed by an acinar-to-pancreatobiliary cell type conversion, which is induced by Sox17.

In parallel studies, Bailey et al[11] also identified $Dclk1^{+}$ tuft cells in PanIN lesions. Similar to DelGiorno et al,[10] the authors found these cells to be extremely rare in normal pancreas.[11] Although the biological role of tuft cells in normal tissue homeostasis remains mysterious, recent studies have shown that $Dclk1^{+}$ cells in intestinal tumors have tumor stem cell properties and are important for tumor maintenance.[16] To determine whether $Dclk1^{+}$ cells could similarly function as tumor stem cells in pancreas, Bailey et al[11] activated *Kras* specifically in acinar cells and isolated acinar-derived $Dclk1^{HI}AcTub^{HI}$ cells from premalignant PanIN lesions. Using a sphere-forming assay, the authors showed that $Dclk1^{HI}AcTub^{HI}$ cells form spheres, whereas active Kras-expressing $Dclk1^{LO}AcTub^{LO}$ cells and wild-type acinar cells form few or no spheres, respectively. These findings provide compelling evidence that oncogenic Kras converts terminally differentiated acinar cells into $Dclk1^{+}$ cells with clonogenic potential. Although $Dclk1^{HI}AcTub^{HI}$ cells are the first population of sphere-forming cells identified in preneoplastic lesions, several cancer stem cell populations, characterized by expression of CD44/CD24/ESA, CD133 or high levels of aldehyde dehydrogenase, respectively, have previously been identified in PDA.[17–19] To determine whether $Dclk1^{HI}AcTub^{HI}$ cells are also present in PDA, Bailey et al[11] analyzed PDA cell lines for the presence of $Dclk1^{HI}AcTub^{HI}$ cells. The authors found that $Dclk1^{HI}AcTub^{HI}$ cells are specifically enriched in the $CD44^{+}/CD24^{+}/ESA^{+}$ and $CD133^{+}$ cell populations and can initiate tumors when transplanted into mice at limiting dilution. These findings identify Dclk1 and AcTub as new markers for a cancer stem cell population in pancreatic tumors. For therapeutic purposes, it will be important to understand which molecular mechanisms maintain the tumor-initiating potential of $Dclk1^{HI}AcTub^{HI}$ cells. Therefore, Bailey et al profiled the transcriptome of $Dclk1^{HI}AcTub^{HI}$ cells to catch a first glimpse into the molecular pathways that control the abundance and behavior of these cells. Their preliminary evidence suggests that Notch and Igf1 receptor signaling and the protooncogene *Abl1* could regulate the proliferation and survival of this novel cancer stem cell population.[11]

Combined, these 2 studies provide new insight into the tumor cell lineage and development of PDA.[10,11] The work suggests a model whereby *Kras*-induced transformation of acinar cells into a pancreatobiliary epithelium is a critical tumor-initiating event (Figure 1). The similarity between normal pancreatobiliary ducts and early preneoplastic lesions in the pancreas extends beyond just the expression of a common set of markers. The pancreatobiliary epithelium harbors a stem-like cell population, which has been proposed to be central to normal tissue turnover and injury repair.[15] The study by Bailey et al shows that pancreatic preneoplastic lesions similarly contain a population of cells with clonogenic properties.[11] This suggests that the pancreatobiliary conversion of acinar cells could play a central role in producing cells susceptible to malignant transformation. Although a direct lineage relationship between clonogenic $Dclk1^{+}AcTub^{+}$ cells in preneoplastic PanIN lesions and cancer stem cells still needs to be demonstrated, the presence of $Dclk1^{+}AcTub^{+}$ tumor-initiating cells in human PDA hints at a possible lineage relationship.

Although the proposed lineage model for PDA is intriguing, several unanswered questions remain. The model suggests that $Dclk1^{+}AcTub^{+}$ tuft cells in PanIN lesions are tumor initiating and drive the progression of preneoplastic PanIN lesions into invasive PDA. A rigorous test of this hypothesis

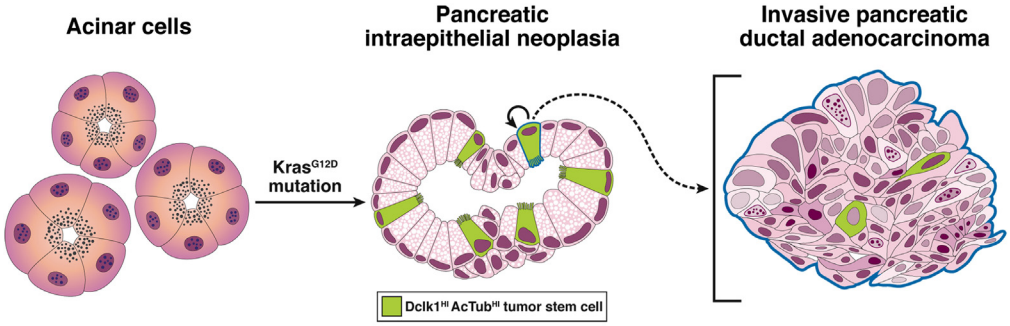

Figure 1. Proposed lineage model for pancreatic ductal adenocarcinoma. Expression of oncogenic Kras transforms acinar cells into pancreatic intraepithelial neoplasia (PanIN) with characteristics of pancreatobiliary epithelium, including the presence of mucin-producing cells and rare $Dclk1^{HI}AcTub^{HI}$ tuft cells (*green*). $Dclk1^{HI}AcTub^{HI}$ cells isolated from premalignant PanIN lesions are clonogenic. $Dclk1^{HI}AcTub^{HI}$ cells are maintained in invasive pancreatic ductal adenocarcinoma, where they function as cancer stem cells. The shared presence of clonogenic $Dclk1^{HI}AcTub^{HI}$ cells at early and late stages of the disease suggests a possible lineage relationship and points to $Dclk1^{HI}AcTub^{HI}$ cells in premalignant lesions as precursors for cancer stem cells.

will require lineage tracing of *Kras*-induced tuft cells in an in vivo context. The feasibility of such experiments, however, is currently limited by the lack of necessary genetic tools. In vivo lineage tracing of $Dclk1^+$ cells in the context of acinar-specific induction of oncogenic Kras will require the development of genetic models in which oncogenic Kras can be induced in a Cre recombinase-independent manner, using a tetracycline-inducible or Flp/Frt recombinase system. Such lineage analysis could also shed further light on the lineage relationship between $Dclk1^+AcTub^+$ tuft cells in PanIN lesions and previously characterized cancer stem cell populations in PDA. Another aspect that warrants further exploration, especially with regard to potential therapeutic implications, is the characterization of $Dclk1^+AcTub^+$ cells in premalignant lesions. For example, it should be determined whether $Dclk1^+AcTub^+$ cells in early PanINs also express other known markers of cancer stem cells. If they do not, when do $Dclk1^+AcTub^+$ cells acquire the expression of established pancreatic cancer stem cell markers and the ability to initiate tumors? Finally, selective ablation of $Dclk1^+AcTub^+$ cells by cell-specific expression of a suicide gene, like the diphtheria-toxin receptor, could help to determine whether $Dclk1^+AcTub^+$ cells are required for the progression of PanINs into PDA. Given that circulating pancreatic cells are already present in the blood stream at the PanIN stage,[20] identification of a tumor-initiating cell in PanINs would put these cells at center stage as a therapeutic target for PDA.

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References

1. Kanda M, Matthaei H, Wu J, et al. Presence of somatic mutations in most early-stage pancreatic intraepithelial neoplasia. Gastroenterology 2012;142:730–733.
2. Hingorani SR, Petricoin EF, Maitra A, et al. Preinvasive and invasive ductal pancreatic cancer and its early detection in the mouse. Cancer Cell 2003;4:437–450.
3. Kopp JL, von Figura G, Mayes E, et al. Identification of Sox9-dependent acinar-to-ductal reprogramming as the principal mechanism for initiation of pancreatic ductal adenocarcinoma. Cancer Cell 2012;22:737–750.
4. Carriere C, Seeley ES, Goetze T, et al. The Nestin progenitor lineage is the compartment of origin for pancreatic intraepithelial neoplasia. Proc Natl Acad Sci U S A 2007;104:4437–4442.
5. Guerra C, Schuhmacher AJ, Cañamero M, et al. Chronic pancreatitis is essential for induction of pancreatic ductal adenocarcinoma by K-Ras oncogenes in adult mice. Cancer Cell 2007;11:291–302.
6. Ardito CM, Grüner BM, Takeuchi KK, et al. EGF receptor is required for KRAS-induced pancreatic tumorigenesis. Cancer Cell 2012;22:304–317.
7. Navas C, Hernández-Porras I, Schuhmacher AJ, et al. EGF receptor signaling is essential for K-ras oncogene-driven pancreatic ductal adenocarcinoma. Cancer Cell 2012; 22:318–330.
8. Prasad NB, Biankin AV, Fukushima N, et al. Gene expression profiles in pancreatic intraepithelial neoplasia reflect the effects of Hedgehog signaling on pancreatic ductal epithelial cells. Cancer Res 2005;65:1619–1626.
9. Miyamoto Y, Maitra A, Ghosh B, et al. Notch mediates TGF alpha-induced changes in epithelial differentiation during pancreatic tumorigenesis. Cancer Cell 2003;3:565–576.
10. DelGiorno KE, Hall JC, Takeuchi KK, et al. Identification and manipulation of biliary metaplasia in pancreatic tumors. Gastroenterology 2014;146:233–244.
11. Bailey JM, Alsina J, Rasheed ZA, et al. DCLK1 Marks a morphologically distinct subpopulation of cells with stem cell properties in preinvasive pancreatic cancer. Gastroenterology 2014;146:245–256.
12. Sato A. Tuft cells. Anat Sci Int 2007;82:187–199.
13. Gerbe F, Legraverend C, Jay P. The intestinal epithelium tuft cells: specification and function. Cell Mol Life Sci 2012;69:2907–2917.
14. Spence JR, Lange AW, Lin S-CJ, et al. Sox17 regulates organ lineage segregation of ventral foregut progenitor cells. Dev Cell 2009;17:62–74.
15. Carpino G, Cardinale V, Onori P, et al. Biliary tree stem/progenitor cells in glands of extrahepatic and intrahepatic bile ducts: an anatomical in situ study yielding evidence of maturational lineages. J Anat 2012;220:186–199.
16. Nakanishi Y, Seno H, Fukuoka A, et al. Dclk1 distinguishes between tumor and normal stem cells in the intestine. Nat Genet 2013;45:98–103.
17. Li C, Heidt DG, Dalerba P, et al. Identification of pancreatic cancer stem cells. Cancer Res 2007;67:1030–1037.
18. Rasheed ZA, Yang J, Wang Q, et al. Prognostic significance of tumorigenic cells with mesenchymal features in pancreatic adenocarcinoma. J Natl Cancer Inst 2010;102:340–351.
19. Hermann PC, Huber SL, Herrler T, et al. Distinct populations of cancer stem cells determine tumor growth and metastatic activity in human pancreatic cancer. Cell Stem Cell 2007;1:313–323.
20. Rhim AD, Mirek ET, Aiello NM, et al. EMT and dissemination precede pancreatic tumor formation. Cell 2012;148:349–361.

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