A new mouse model of pancreatic cancer: PTEN gets its Akt together

***PTEN* (phosphatase and tensin homolog deleted on chromosome 10) is a negative regulator of the oncogenic PI3-K/Akt signaling pathway. Loss-of-function mutations of *PTEN* are seen in several human solid cancers. A murine model of conditional *Pten* inactivation in the pancreas is described that leads to acquisition of a profound metaplastic ductal phenotype accompanied by loss of differentiated acinar units. Evidence is presented for a centroacinar cell origin of the metaplastic "neoductules." These mice also develop invasive pancreatic adenocarcinomas at a low frequency, and provide a unique in vivo platform for exploring the role of PI3-K/Akt signaling in pancreatic neoplasia.**

Pancreatic cancer afflicts close to 32,000 individuals each year in the United States and 168,000 worldwide, and nearly all patients die from the ravages of their disease. Ductal adenocarcinoma, by far the most common histologic variant, is also the most challenging to diagnose and treat (Yeo et al., 2002). The last decade has provided several critical insights into the molecular pathogenesis of early pancreatic cancer. Notably, there is now strong evidence that invasive pancreatic adenocarcinoma proceeds through a morphologic spectrum of noninvasive ductal lesions known as pancreatic intraepithelial neoplasia (PanIN), and that histologic progression of these lesions toward invasive cancer is associated with the progressive accumulation of genetic abnormalities (Maitra et al., 2005). The identification of noninvasive precursor lesions of pancreatic cancer has enormous significance, since it provides an unprecedented opportunity for diagnostic and therapeutic interventions at a curable stage.

Despite the tremendous advances made in the field of pancreatic cancer genetics, there was, until recently, one remaining "bottleneck" on accelerating research into this disease, namely, the availability of a genetically engineered mouse model that would faithfully recapitulate the multistage progression of human pancreatic cancer. Although the pancreas was one of the first organs in which tissue-specific transgenesis was accomplished (Ornitz et al., 1987), many of the earlier studies utilized acinar-specific promoters (e.g., Elastase) for oncogene targeting. Not surprisingly, these models of murine exocrine pancreatic neoplasia reflected an acinar histogenesis (Sandgren et al., 1990), quite unlike the ductal adenocarcinomas that dominate human disease. Ironically, oncogene targeting utilizing a ductal promoter (cytokeratin 19) yielded only a minimal phenotype of perdicutal inflammation (Brembeck et al., 2003), implying that, at least in the mouse pancreas, this terminally differentiated compartment is unlikely to be the proximate cell-of-origin for ductal adenocarcinoma. A quantum leap toward establishing a phenocopy of the cognate human condition was made by virtue of targeting to the endogenous *KRAS* locus an oncogenic $KRAS^{G12D}$ allele, activated in the developing pancreas by *Cre*-mediated recombination (Hingorani et al., 2003); notably, *Cre* was expressed in this model as a *Pdx1* promoter-driven transgene (a near identical pancreatic phenotype was produced by $KRAS^{G12D}$ activation by Cre knocked in at the *Ptf1-p48* locus). These mice uniformly develop pancreatic intraductal lesions that mirror the histologic spectrum of human PanINs, and at a low frequency, develop invasive, widely metastatic pancreatic adenocarcinoma. *Pdx1* is a homeodomain protein that is a critical regulator of early pancreatic development, as targeted deletion of this gene during embryogenesis aborts pancreatic morphogenesis. The vast majority of differentiated cell types in the mature pancreas arise from a common *Pdx1*-expressing endocrine/exocrine precursor; postnatally, expression of *Pdx1* is essentially restricted to the islets of Langerhans (Song et al., 1999). The generation of a ductal neoplastic phenotype by oncogene targeting using *Pdx1* regulatory elements, but not mature acinar- or ductal-specific promoters, supports the notion of an undifferentiated precursor population as the initi cer. The identity of this precursor population has not been discerned, but it either preexists within the adult pancreas, or arises by "dedifferentiation" of differentiated elements, consistent with the reported plasticity of these mature components (Means et al., 2005).

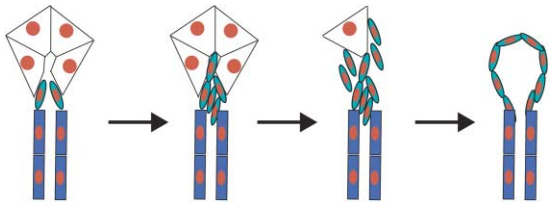

Figure 1. Model for *Pten* deletion and ductal metaplasia

Pten-deficient centroacinar cells (green ovals) begin to proliferate during development. Centroacinar cell expansion leads to apoptosis of acinar cells (white triangles) due to loss of acinar integrity or survival signals. Progeny of these centroacinar cells form tubular complexes with mucinous metaplasia. Reproduced with permission from Stanger et al. (2005).

In this issue of *Cancer Cell*, Stanger et al. (2005) utilize a mouse model of targeted *PTEN* gene deletion to provide some elegant insights into mechanisms of homeostasis in the pancreas, and in the process, generate a new model of murine preinvasive and invasive adenocarcinoma. The *PTEN* (phosphatase and tensin homolog deleted on chromosome 10) tumor suppressor gene is frequently deleted or mutated in human cancers. Germline mutations of *PTEN* result in a group of related disorders known as "*PTEN* hamartoma tumor syndrome," characterized by multiple hamartomas and an increased predisposition to solid cancers of the breast and thyroid. The tumor suppressor function of the Pten phosphatase is related to its antagonism of the PI3-K/Akt signaling pathway. Aberrant activation of the PI3-K/Akt pathway is extremely common in many solid cancers, including pancreatic cancers, and as such can arise via several mechanisms, including oncogenic mutations in the activating components (e.g., *PIK3CA* or *AKT2* genes) or loss of function mutations in the inhibitory components (e.g., *PTEN* or the *TSC1/2* genes) (Vivanco and Sawyers, 2002). The $Pten^{lox/lox}$ mouse model described in this article has previously been utilized for tissue-specific gene deletion in the prostatic and ovarian

epithelia, resulting in the full histologic spectrum of lesions ranging from preneoplasia to metastatic carcinomas (Dinulescu et al., 2005; Wang et al., 2003). Thus, given its established pedigree as a tumor suppressor at multiple anatomic sites, a putative role for *Pten* in pancreatic exocrine neoplasia would seem almost intuitive. Nevertheless, this study yields novel, and somewhat unexpected, information regarding the influence of the PI3-K/Akt signaling pathway on the balance of differentiated cell types in the mature pancreas.

By selectively deleting *Pten* in Pdx1-expressing progenitor cells within the developing pancreas of bitransgenic *Pdx1-Cre*; *Pten*$^{lox/lox}$ mice, the overwhelming phenotype to emerge is one of ductal metaplasia. Beginning at approximately 3 weeks of age, there is progressive effacement of acinar structures by "neoductules," with the metaplastic epithelium eventually replacing entire lobules. Symptoms of systemic distress develop in these mice by approximately 2–3 months, and at necropsy, cystic lesions that histologically correspond to cystically dilated ductular complexes are often observed. Metaplastic ductal epithelium is no stranger to the world of genetically engineered mouse models of pancreas cancer, and in fact, the recent consensus conference on histologic classification of murine pancreatic lesions defines "acinar-ductal-metaplasia" (ADM) as an entity distinct from murine pancreatic intraepithelial neoplasia (mPanIN) lesions (Hruban et al., unpublished data). While ADM is most prominent in mouse models that rely on acinar promoters for gene targeting (Sandgren et al., 1990), such lesions are at least focally observed in other systems (e.g., *Pdx1-Cre*; *LSL-KRAS*^{G12D} mice), and in certain human disease states (e.g., chronic pancreatitis). Implicit in the moniker of ADM is the understanding that these lesions arise via transdifferentiation of acinar cells into ductular epithelium (Means et al., 2005). However, Stanger et al. (2005) provide a compelling argument that in the *Pdx1-Cre*; *Pten*$^{lox/lox}$ mice, the "neoductules" do not arise via acinar-ductal transdifferentiation, but rather from expansion of an existing centroacinar cell population with concomitant replacement of acini. The evidence provided *against* acinar-ductal transdifferentiation is more conclusive than the evidence *for* centroacinar cell expansion; for example, it is noted that the neoductular population expresses the Notch pathway target gene *Hes1*, which is also a marker of normal centroacinar cells (Miyamoto et al., 2003). In the ductal metaplasia model proposed by Stanger et al. (Figure 1), *Pten*-deficient centroacinar cells begin to proliferate during development. By a few weeks of age, centroacinar cell expansion results in apoptosis of acinar cells, accompanied by formation of tubular complexes with mucinous metaplasia. The definitive proof of this model will require lineage-tracing studies with *Hes1* regulatory elements. However, this study provides the first indication that *Pten*, and by extension the PI3-K/Akt pathway, maintains the balance between various cell types in the adult pancreas.

In addition to the prominent metaplastic phenotype, *Pdx1-Cre*; *Pten*$^{lox/lox}$ mice develop invasive pancreatic adenocarcinomas at a low frequency. The precise ontogenic relationship between the metaplastic epithelium and ductal neoplasia is uncertain. The rapid onset of metaplasia with accompanying debilitation likely offsets the possibility of developing PanIN lesions with high penetrance, as observed in the *Pdx1-Cre*; *KRAS*^{G12D} mice. Nonetheless, the occurrence of invasive adenocarcinomas in a subset of mice confirms the importance of the PI3-K/Akt pathway in the pancreas. In the central nervous system, *Pten* negatively regulates stem/progenitor cell numbers (Groszer et al., 2001), and it is tempting to speculate that loss of *Pten* function within *Pdx1*-expressing compartment of the developing pancreas results in the persistence of multipotent progenitor cells susceptible to neoplastic transformation under appropriate conditions. For example, one can envision the simultaneous targeting of an oncogenic *Kras* allele to the putative *Pten*-deficient progenitor cell population as an instance of a highly conducive genetic environment for inducing neoplasia; in fact, similar oncogenic cooperativity between *Pten* and *Kras* results in metastatic ovarian adenocarcinomas in mice (Dinulescu et al., 2005). From a translational perspective, these results further establish the emerging role of PI3-K/Akt pathway as a therapeutic target in established human pancreatic cancers (Asano et al., 2005). In addition, given the putative role of *Pten* dysregulation in the "primordial" lesions of pancreatic neoplasia, the prospect of treating pancreatic cancer at its earliest stages, or aborting the disease altogether, might become a real possibility in predisposed individuals.

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