Mouse models of pancreatic cancer: The fur is finally flying!

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Pancreatic cancer remains one of the most lethal of all human malignancies. Until recently, preclinical studies have been hampered by the absence of mouse models faithfully recapitulating critical elements of the human disease. However, recent months have witnessed a flurry of activity with respect to prospective mouse models. This progress now allows the evaluation of novel strategies for early detection, chemoprevention, and therapy and also provides new insights regarding the potential for differentiated and undifferentiated cell types to act as cells of origin for pancreatic ductal adenocarcinoma.

Among human cancers, adenocarcinomas of the lung, breast, prostate, colon, and pancreas represent the most common causes of cancer death in Western societies (Jemal et al., 2003). Over the past decade, mouse models of human malignancy have contributed significantly to our understanding of many of these human tumors. Mouse models have been employed to evaluate novel strategies for early detection, chemoprevention, and treatment, and classical mouse genetics have successfully identified modifier loci contributing to the risk of tumor initiation and/or progression. For example, multiple strategies for chemoprevention of intestinal polyposis have been evaluated in the *min*/*APC* model (Jacoby et al., 1996; Roberts et al., 2002; Torrance et al., 2000), and *aurora2*/*Stk6*/*STK 15* has been shown to be an important modifier of tumor multiplicity in a murine skin tumor model (Ewart-Toland et al., 2003). While fundamental differences in biology suggest the need for caution in equating mouse tumors with their human counterparts (Rangarajan and Weinberg, 2003; Van Dyke and Jacks, 2002), mouse models of malignancy nevertheless represent an important source of insight regarding human neoplasia.

For many human tumors, successful mouse modeling has been facilitated either by the availability of appropriate cell type-specific promoter elements for transgene targeting and conditional gene deletion (Hutchinson and Muller, 2000; Kasper et al., 1998), by an epithelium accessible and susceptible to chemical carcinogenesis (Bolt et al., 2000; Saran et al., 2000), or by the identification of heritable tumor predisposition following chemical mutagenesis (Su et al., 1992; Moser et al., 1992). For other tissues, significant rates of spontaneous or viral-mediated tumorigenesis have also provided effective mouse models (Hook et al., 2000; Malkinson, 2001). In stark contrast to the successful murine modeling of most common human tumors, the generation of appropriate mouse models of pancreatic cancer has remained an area of significant frustration. Combined with this frustration is a real sense of urgency. Pancreatic cancer is an almost uniformly fatal disease, accounting for 30,000 cancer deaths each year in the United States (Jemal et al., 2003). Among the five most common causes of cancer death listed above, pancreatic cancer is also the least accessible, with the retroperitoneal location of the pancreas rendering this tissue largely unavailable for routine tissue sampling or radiographic/endoscopic screening. As such, initiating events in human pancreatic cancer have been difficult to discern, and the potential benefits of a mouse model loom large.

Ironically, the mouse pancreas was one of the very first organs in which tissue-specific transgene expression was accomplished, and among the first tissues in which transgenic tumor induction was achieved (Ornitz et al., 1985, 1987; Quaife et al., 1987; Swift et al., 1984). These events were facilitated by the identification of tissue-specific promoter/enhancer elements in the rat elastase I locus. These elements predominantly target pancreatic acinar cells and produced acinar cell neoplasms when coupled to either activated H-ras or SV40 T-antigen. In contrast, expression of a *c-myc* transgene driven by the same promoter produced mixed acinar/ductal neoplasms (Sandgren et al., 1991), raising fascinating questions regarding the ability of specific oncogenes to either selectively initiate tumorigenesis in specific cell populations or to drive tumor differentiation in a directed manner. However, the generation of mouse models producing classic pancreatic ductal adenocarcinoma, the predominant form of human pancreatic cancer, was not achieved in these early studies.

Subsequent to these reports, the decade of the 1990s witnessed a dramatic expansion of knowledge in two important areas related to pancreatic tumorigenesis. First, the genetic basis of pancreatic ductal adenocarcinoma was revealed, with activation of KRAS and inactivation of the p16INK4a, p53, and SMAD4 tumor suppressor genes identified as characteristic features of invasive pancreatic cancer (Hruban et al., 2001b). Second, consensus was reached regarding the role of pancreatic intraepithelial neoplasia (PanIN) as a direct noninvasive neoplastic precursor to human pancreatic cancer (Figure 1; Hruban et al., 1999, 2001a). The identification of PanIN as a relevant precursor lesion further allowed the ordering of characteristic genetic events in a step-wise carcinogenesis paradigm, in which KRAS mutation and telomere shortening were characterized as early events, loss of p16INK4a as an intermediate event, and loss of p53 and SMAD4 as late events occurring in PanIN lesions of increasing severity (Edlund, 1999; Maitra et al., 2003; van Heek et al., 2002; Yeo et al., 2002).

Concomitant with these advances in the understanding of human pancreatic cancer, significant progress was made in pancreatic developmental biology, with the identification of multiple transcription factors and signaling pathways required for normal foregut patterning, branching morphogenesis, and establishment of endocrine and exocrine cell lineages (Ahlgren et al., 1996; Edlund, 1999; Kim and MacDonald, 2002). Among these factors, the homeodomain protein Pdx1 and the basic helix-loop-helix protein Ptf1-p48 were implicated as critical regulators of early pancreatic development, with targeted deletion

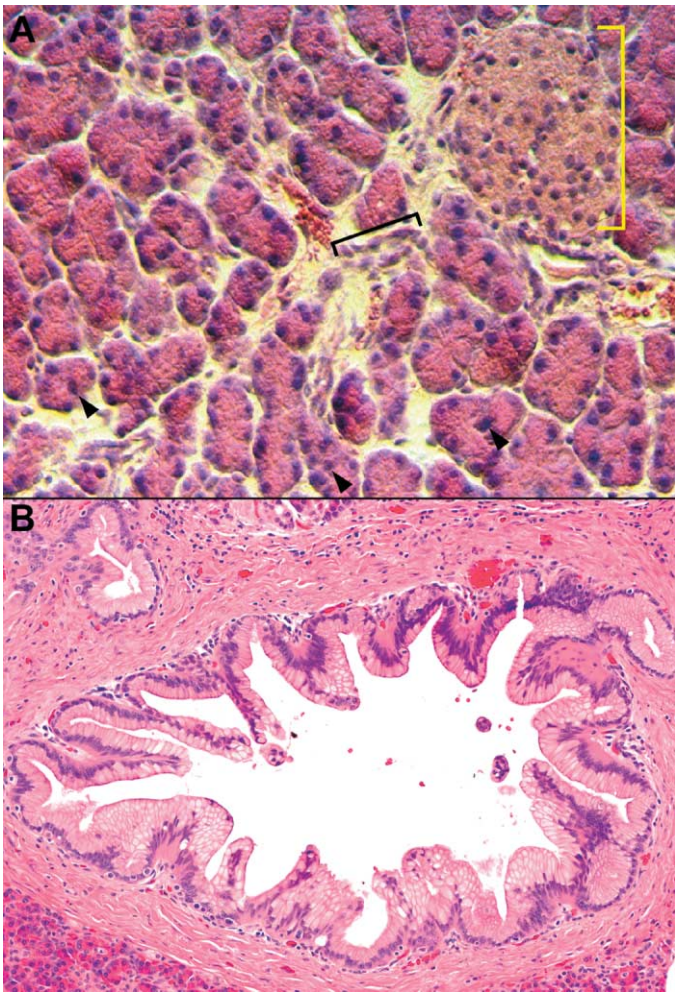

Figure 1. Histology of normal and neoplastic pancreas

A: Normal mouse pancreas demonstrating preponderant acinar cell mass, terminal intralobular ductal epithelium (black bracket), centroacinar cells (arrowheads), and endocrine islet (yellow bracket). Within this complex epithelial tissue, the location of dedicated epithelial precursor cells is unknown, and the cell of origin for pancreatic intraepithelial neoplasia is not yet defined.

B: Human pancreatic intraepithelial neoplasia (PanIN), demonstrating columnar epithelium with papillary architecture and surrounding desmoplastic stroma. PanIN image courtesy of Dr. Ralph Hruban, Johns Hopkins University.

of either of these genes resulting in severely aborted pancreatic morphogenesis and a paucity of differentiated cell types (Ahlgren et al., 1996; Kawaguchi et al., 2002; Krapp et al., 1998; Offield et al., 1996). More recent lineage tracing studies and single cell expression profiling have demonstrated that the vast majority of differentiated cell types are generated from a common endocrine/exocrine precursor pool expressing both Pdx1 and Ptf1-p48 (Chiang and Melton, 2003; Gu et al., 2003; Kawaguchi et al., 2002). Additional studies have defined specific roles for the Hedgehog, EGF, and Notch signaling pathways in regulating normal pancreatic development in the mouse. Among these, soluble Hedgehog signals appear to play a critical role in foregut patterning, acting to restrict pancreatic development to nascent dorsal and ventral buds, and promoting gut and liver differentiation programs in adjacent nonpancreatic endoderm (Apelqvist et al., 1997; Deutsch et al., 2001; Hebrok et al., 2000). Within the pancreatic buds, EGF signaling appears to drive proliferation of undifferentiated precursor cells (Cras-Meneur et al., 2001), while Notch acts to prevent both endocrine and exocrine differentiation (Apelqvist et al., 1999; Hald et al., 2003; Jensen et al., 2000), effectively preserving an undifferentiated precursor pool.

Against this backdrop of advances in pancreatic cancer molecular genetics, consensus regarding PanIN precursors, and identification of transcription factors and signaling pathways regulating normal pancreatic development, it might be expected that progress on mouse models of pancreatic cancer would finally be made. A series of recent reports validates this expectation. After years of lagging behind, mouse models of pancreatic cancer can now be said to have equaled, and in certain respects surpassed, their counterparts in other tissues.

Specifically, a recent report by David Tuveson and colleagues at the University of Pennsylvania (Hingorani et al., 2003) reports the generation of progressive PanIN lesions and low-frequency progression to invasive and metastatic adenocarcinoma following activation of oncogenic KRAS in mouse pancreas. In this report, mice expressing a Cre-activated $KRAS^{G12D}$ allele inserted into the endogenous KRAS locus were crossed with mice expressing Cre recombinase in pancreatic tissue, either by virtue of a Pdx1 promoter-driven transgene or by Cre knockin at the Ptf1-p48 locus. Prior lineage studies suggest that both of these lines express Cre in a common endocrine/exocrine precursor cell during development, while expression in adults is retained in mature islet cells in the case of Pdx1-Cre transgenics and in mature acinar cells in the case of the Ptf1-$p48^{+/Cre}$ knockin (Gu et al., 2003; Kawaguchi et al., 2002).

Notably, both the islet and acinar compartments in mice expressing Pdx1-Cre- or Ptf1-$p48^{+/Cre}$-activated $KRAS^{G12D}$ appeared histologically normal, at least prior to the onset of progressive fibrosis. Instead, activation of oncogenic KRAS in either Cre line resulted in progressive intraductal lesions recapitulating multiple aspects of human PanIN. Specifically, the normal cuboidal epithelium of small interlobular ducts was converted to a columnar morphology, with minimal involvement of larger ducts. These changes were apparent as early as 2 weeks postpartum. Over the course of several months, these early PanIN lesions were replaced by lesions of progressive architectural and cytologic abnormality, characterized by the development of papillary or micropapillary lesions, loss of cell polarity, and increasing nuclear atypia. By demonstrating this progression in the mouse, these studies have provided confirmation of the PanIN progression model initially proposed in human pancreas, which has by necessity been generated largely from examination of static specimens without the benefit of timed initiation or serial sampling (Brat et al., 1998; Hruban et al., 1999; Maitra et al., 2003). PanIN lesions from these mice also demonstrated evidence of Notch pathway activation, as well as expression of both cyclooxygenase 2 (COX2) and matrix metalloproteinase 7 (MMP7). These pathways are typically quiescent in normal ductal epithelium, but are commonly active in human PanIN (Crawford et al., 2002; Maitra et al., 2002; Miyamoto et al., 2003). In addition, serum from these mice demonstrated a characteristic proteomic signature, even in the absence of invasive disease. While it remains uncertain whether this serum response represents a specific marker of PanIN as opposed to other consequences of KRAS activation in pancreatic tissue, the results certainly provide an important

precedent supporting similar efforts to detect pre-invasive lesions in human pancreas.

While spontaneous invasion and metastasis were observed at low frequency following activation of oncogenic KRAS in mouse pancreas, a subsequent report from Ronald DePinho, Nabeel Bardeesy, and colleagues at the Dana Farber Cancer Institute of Harvard Medical School demonstrated accelerated PanIN formation, rapid tumor progression, and fatal metastatic disease when Pdx1-Cre-activated $KRAS^{G12D}$ activation is combined with tissue-specific Ink4a/Arf deficiency (Aguirre et al., 2003). In this study, mice expressing Pdx1-Cre-activated $KRAS^{G12D}$ developed PanIN lesions and progressive pancreatic fibrosis, but no evidence of invasive cancer, as assessed up to 30 weeks of age. However, when expressed in the context of combined Cre-mediated excision of loxP-flanked Ink4a/Arf alleles, all mice succumbed to invasive and metastatic pancreatic cancer within 11 weeks. Pdx1-Cre;$Ink4a/Arf^{lox/lox}$ mice lacking the Cre-activated $KRAS^{G12D}$ transgene failed to develop pancreatic tumors, specifically implicating a role for oncogenic KRAS in tumor initiation and a role for Ink4a/Arf in tumor progression. Additional molecular changes observed in these aggressive tumors included amplification of the mutant KRAS allele and evolving expression of EGFR and HER2/NEU. In contrast, no evidence of acquired mutation in SMAD4 or p53 was observed, suggesting that (in the mouse) these tumor suppressors are not rate limiting for generation of invasive and metastatic pancreatic cancer, at least in the presence of combined Ink4a/Arf deficiency.

Combined with other recent papers evaluating oncogenic KRAS targeting to either mature acinar cells using the elastase 1 promoter (Grippo et al., 2003) or mature ductal epithelium using the cytokeratin 19 promoter (Brembeck et al., 2003), these studies raise additional fascinating questions regarding the true cell of origin for pancreatic ductal adenocarcinoma. While these studies are unable to provide definitive resolution of this question, the fact remains that KRAS targeting to acinar cells appears to result in acinar or mixed acinar/ductal tumors, while targeting mature ductal epithelium results in periductal inflammation, without characteristic PanIN formation. Together with the observation of normal islet and acinar cell populations in mice with oncogenic KRAS activated by either the Pdx1-Cre transgene or the Ptf1-$p48^{+/Cre}$ knockin (Hingorani et al.), these observations suggest that PanIN lesions may not arise from differentiated exocrine or endocrine cell types, but rather from undifferentiated precursor cells targeted by Pdx1 and/or Ptf1-p48 regulatory elements.

Further insights regarding this issue are provided by a recent study reported by Harold Varmus and collegues from the Memorial Sloan-Kettering Cancer Center (Lewis et al., 2003). Using transgenic mice expressing TVA, the receptor for avian leukosis sarcoma virus subgroup A (ALSV-A), under control of elastase 1 promoter elements, these investigators used ALSV-A-based RCAS vectors to selectively deliver either polyoma virus middle T-antigen (*PyMT*) or *c-myc* to elastase-expressing cells in mouse pancreas. Although the genetic alterations introduced in these mice are not typically observed in human pancreatic cancer, this novel method for tissue-specific gene delivery appears to carry multiple advantages, including the ability to introduce a variety of different oncogenes to specific target cells using a single transgenic line, stochastic oncogene activation in a limited number of cells, and the benefit of temporal control based on timed intraperitoneal delivery of the viral vector.

In this study, expression of the TVA transgene appeared to be limited to elastase-expressing acinar cells, although expression in a low-frequency nonacinar precursor cell cannot be excluded. Delivery of *PyMT* to this population resulted in a variety of tumor types showing varying degrees of either acinar or ductal differentiation, as well as occasional cystadenocarcinomas. A subset of ductal lesions displayed features of PanIN. Both acinar and ductal tumors expressed Pdx1 and demonstrated either uniform or focal expression of synaptophysin, suggesting possible involvement of a multipotent precursor cell. As in the case of $KRAS^{G12D}$-induced tumors, tumor progression appeared to be accelerated in the absence of Ink4a/Arf. In contrast, delivery of *c-myc* to elastase-expressing cells resulted in tumor formation only in the context of Ink4a/Arf deficiency, and all tumors demonstrated an endocrine pattern of differentiation. These tumors closely resembled well-differentiated human pancreatic endocrine tumors, in which enhanced c-*myc* expression is often observed.

In addition to providing exciting new models with which to conduct preclinical studies, these reports highlight a number of considerations likely to inform future work in the field. First, the cumulative results of these and other studies suggest that differentiated pancreatic cell types may be relatively resistant to KRAS-driven generation of PanIN lesions, and may not represent the proximate cell of origin for classical pancreatic ductal adenocarcinoma. Instead, these tumors may be derived from either undifferentiated precursor cells or dedifferentiated derivatives of mature cell types, reflecting a significant capacity for plasticity among differentiated exocrine and endocrine cells (Meszoely et al., 2001). Second, advances in the field of pancreatic developmental biology will likely continue to be informative with respect to ongoing refinement and characterization of these and other mouse models. In addition to adding to the reagent toolbox, improved understanding of pancreatic developmental biology has stimulated identification of the Hedgehog and Notch signaling pathways as characteristic features of both PanIN and invasive human pancreatic cancer (Berman et al., 2003; Miyamoto et al., 2003; Thayer et al., 2003). Together with the established ability of EGF receptor signaling to regulate pancreatic epithelial differentiation and initiate pancreatic tumorigenesis (Wagner et al., 2001, 2002), these pathways not only represent intriguing targets for chemoprevention but may also provide a molecular handle for identification of putative precursor cells responsible for PanIN initiation. While it is clear that the study of pancreatic development has had significant impact on our view of pancreatic neoplasia, we should also expect that these new tumor models may in turn provide much needed enlightenment regarding the presence, location, and identity of dedicated precursor cells in adult and embryonic pancreas.

So where do we stand? For the first time, we have a model of pre-invasive pancreatic epithelial neoplasia that faithfully recapitulates multiple aspects of the human disease. The model provides a unique opportunity to evaluate strategies for early detection of noninvasive disease, perhaps taking advantage of global or specific changes in serum protein profiles, as suggested by Hingorani et al. (2003). In addition, the mouse PanIN model now allows formal evaluation of novel chemoprevention strategies, with pharmacologic inhibiton of COX2, MMP7, EGF, Notch, and Hedgehog all providing exciting possibilities. Moreover, the combination of targeted $KRAS^{G12D}$ activation and Ink4a/Arf deletion reported by Aguirre et al. (2003) provides the ability to study the impact of various therapeutic interventions on invasive and metastatic disease developing along a canoni-

cal PanIN-to-carcinoma sequence. Combined with the recent insights regarding Notch and Hedgehog signaling alluded to above, the past few months have witnessed a period of remarkable progress in modeling and understanding this disease.

Caution certainly remains necessary in assuming that further information generated by these models will be directly translatable to human pancreatic cancer. However, at the very least, these new models should facilitate ongoing efforts to clarify basic biologic questions regarding pancreatic ductal adenocarcinoma and its noninvasive precursors: what is the cell of origin? How do stromal factors influence pathogenesis? What are the effects of epithelial injury on relevant precursor cells, as well as on tumor initiation and/or progression? Are nonmutational changes in EGF, Notch, and Hedgehog signaling required to initiate, modify, or enforce the phenotypic response to oncogenic KRAS, or do these represent downstream sequelae? How do commonly observed metaplastic changes in epithelial differentiation contribute to PanIN initiation?

In order to productively address these questions, it will be critical to recognize that no single mouse model is likely to mirror the full spectrum of human pancreatic ductal adenocarcinoma and its precursors. While recent efforts have emphasized the role of PanIN as a direct precursor for invasive pancreatic cancer, our view of the human disease remains largely static. It is likely that not all pancreatic ductal adenocarcinoma is generated by way of a canonical PanIN sequence, and that multiple pathways and multiple trajectories exist by which transformed pancreatic epithelial cells may arrive at an apparently common phenotype. It is therefore important to recognize that each of the models in our collective mouse colony, even those not directly mimicking human PanIN, may provide important insights regarding underlying biology.

This may also be true for studies of experimental therapeutics. For evaluation of novel therapies targeting specific molecular defects, molecular mimicry of human tumors may prove to be more important than histologic mimicry (Van Dyke and Jacks, 2002). Mouse models displaying high degrees of molecular homology with their human counterparts (Wagner et al., 2001) may therefore remain highly informative with respect to preclinical studies, even if differences exist with respect to histologic progression.

In association with the recent proliferation of available mouse models, it will also be critical to develop consensus regarding the spectrum of neoplastic phenotypes observed in mouse pancreas and to apply stringent criteria and a well-defined nomenclature when relating mouse phenotypes to human disease. The successful development of such criteria for human PanIN has required extensive efforts to achieve consensus (Hruban et al., 2001a). Only a short time ago, there might have been little enthusiasm for a proposed consensus conference on mouse pancreatic cancer pathology, based on a paucity of material to review. Fortunately, such a conference now appears both timely and necessary.

Acknowledgments

This work was supported by NIH grants DK-61215 and DK-56211 and by the Paul K. Neumann Professorship in Pancreatic Cancer at Johns Hopkins University. The author wishes to thank Nabeel Bardeesy, Robert Coffey, Paul Grippo, Sunil Hingorani, Ralph Hruban, Brian Lewis, Dan Longnecker, Anirban Maitra, Anil Rustgi, Eric Sandgren, Roland Schmid, and David Tuveson for many stimulating discussions regarding this topic.

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