

# Pathology of Genetically Engineered Mouse Models of Pancreatic Exocrine Cancer: Consensus Report and Recommendations

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## Abstract

Several diverse genetically engineered mouse models of pancreatic exocrine neoplasia have been developed. These mouse models have a spectrum of pathologic changes; however, until now, there has been no uniform nomenclature to characterize these changes. An international workshop, sponsored by The National Cancer Institute and the University of Pennsylvania, was held from December 1 to 3, 2004 with the goal of establishing an internationally accepted uniform nomenclature for the pathology of genetically engineered mouse models of pancreatic exocrine neoplasia. The pancreatic pathology in 12 existing mouse models of pancreatic neoplasia was reviewed at this workshop, and a standardized nomenclature with definitions and associated images was developed. It is our intention that this nomenclature will standardize the reporting of genetically engineered mouse models of pancreatic exocrine neoplasia, that it will facilitate comparisons between genetically engineered mouse models and human pancreatic disease, and that it will be broad enough to accommodate newly emerging mouse models of pancreatic neoplasia. (Cancer Res 2006; 66(1): 95-106)

## Introduction and Objectives

The study of human pancreatic cancer is hampered by the advanced stage at which most patients present, the low neoplastic cellularity of most human pancreatic cancers, the inaccessibility of the organ to biopsy, and the extraordinarily high mortality rate of the disease (1). Genetically engineered mouse models, which recapitulate human pancreatic neoplasia, have the potential to advance our understanding of the pathobiology of noninvasive and invasive pancreatic neoplasia and to facilitate the development of novel tests for the early detection of pancreatic neoplasia and new therapies for invasive cancer (2).

A number of remarkable models of pancreatic disease have recently been developed in genetically engineered mice (2–9). These models use a variety of approaches to target the expression of mutant or endogenous genes in specific cellular compartments. It should therefore not be surprising that a broad spectrum of pathologic changes develop in these models. Some of these changes histologically mimic human disease, whereas others are not encompassed in the existing nomenclatures for human pancreatic neoplasia.

An international workshop, sponsored by The National Cancer Institute and the University of Pennsylvania, was held in Philadelphia, PA from December 1 to 3, 2004 with the goals of (a) describing the histopathology of pancreatic exocrine neoplasia in existing genetically engineered mouse models and (b) developing a standard nomenclature for these lesions. It is anticipated that this nomenclature will standardize the reporting of the pancreatic pathologic changes in genetically engineered mouse models and that it will facilitate comparisons among mouse models as well as between mouse models and human disease. Although it is obviously impossible to anticipate all of the morphologic patterns that may be seen in newly emerging mouse models, the proposed classification is intended to be flexible enough to accommodate variations in the existing morphologies, and by incorporating some of the more common patterns observed thus far only in humans, to enable continued use of the classification as new mouse models are developed. In addition, workshop participants thought it was important to specifically address the interpretation of mouse pancreatic intra-epithelial neoplasia (mPanIN), and that a collection of annotated images should be created to facilitate the promulgation of this nomenclature. The new nomenclature and selected images are presented here, and additional images are provided on the Web.<sup>21</sup>

Although the nomenclature was developed to parallel the existing nomenclature for human pancreatic exocrine neoplasia (1, 10), the group felt it was important to emphasize that genetically engineered mice differ significantly from humans, and that mouse

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<sup>21</sup> [http://www.abcc.ncifcrf.gov/LASP/lasp\\_index.php](http://www.abcc.ncifcrf.gov/LASP/lasp_index.php).

lesions histologically similar to human lesions may be genetically or biologically quite different. Thus, great care should be taken in applying findings in mice to the human condition without further investigation.

### Development of the Normal Pancreas

Before discussing the details of the pathologic changes in genetically engineered mouse models of pancreatic neoplasia, a brief review of the normal development of the mouse pancreas will provide a frame of reference for the genetic manipulations and subsequent changes in the models.

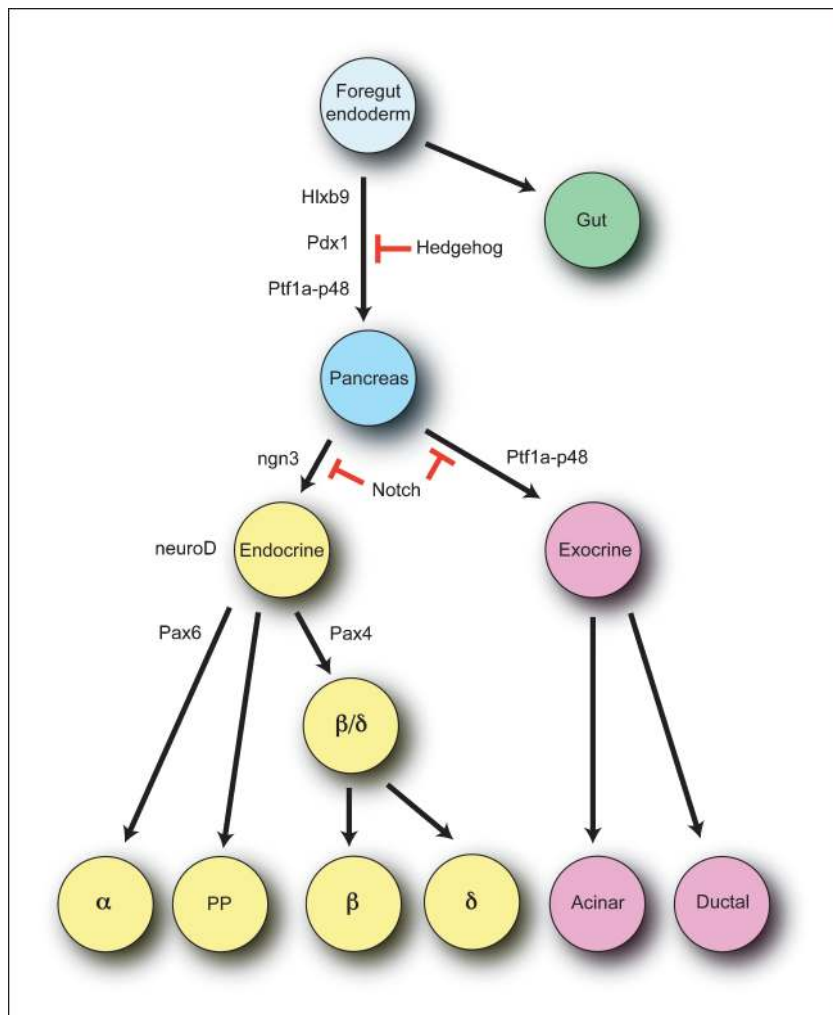
**Development of the exocrine pancreas.** Pancreatic development in the mouse is orchestrated by a cascade of transcription factors that are sequentially expressed in specific cell types as the organ develops (Fig. 1). Although molecular events involved later in pancreatic differentiation are similar in both the ventral and dorsal pancreatic anlagen, early specification of these two regions of endoderm towards a pancreatic fate differs (11). Development of the pancreas begins with inhibition of hedgehog signaling in a discrete region of dorsal endoderm by factors secreted by the notochord (12, 13). This absence of hedgehog signaling determines the pancreatic lineage of epithelium that becomes the dorsal pancreatic bud visible at E9.5 (13, 14). In contrast, the default developmental program for ventral endoderm in this region is

towards a pancreatic fate. The expression of fibroblast growth factor by cardiogenic mesoderm induces hedgehog expression and thereby specifies a hepatic fate in the foregut endoderm adjacent to the nascent ventral bud, thus limiting the anterior extent of the ventral pancreatic bud (15).

Two homeodomain transcription factors, HlxB9 and Pdx1 (pancreas duodenum homeobox 1) are also involved in early pancreatic cell fate determination. Pdx1 expression is critical to pancreatic development, and homozygous deletion of Pdx1 causes pancreatic agenesis (16). Pdx1 expression also extends into the surrounding foregut endoderm and is important to the development of the distal stomach, duodenum, and main bile duct. Loss of HlxB9 expression prevents the development of the dorsal but not the ventral pancreatic bud (17, 18). HlxB9 expression is extinguished by E12.5 but reappears later in developing islets of Langerhans (11).

The pancreatic epithelium proliferates and branches, and the dorsal and ventral buds ultimately fuse, resulting in an epithelial tubular complex containing all the precursor cells of the mature organ at E12.5 (11). These multipotential Pdx1-positive pancreatic precursor cells differentiate along islet, acinar, and ductal lineage pathways (Fig. 1) from E13.5 to E17.5 to give rise to the definitive cell types of the mature pancreas (11).

p48 is the pancreas-specific subunit of the heteromeric bHLH protein complex called PTF1 (19). Although, initially, p48 was



**Figure 1.** Gene expression during pancreatic development. Reprinted with permission of Jones and Bartlett Publishers from Pancreatic Cancer by Von Hoff et al., 2005.

**Table 1.** Genetically engineered mouse models included in the workshop

Submitter	Background	Model	Reference
Grippo	FVB	Ela-KRAS <sup>G12D</sup>	(6)
Sandgren and Schmid	B6	Ela-TGF $\alpha$	(5)
	B6/Balbc	Ela-TGF $\alpha$ ; p53 <sup>-/-</sup>	(5, 25)
Konieczny	B6/129SvJae	Mist1-Kras4B <sup>G12D</sup>	(26)
Guerra	B6/129SvJ/FVB	Ela-tTA; tet-o-cre; LSL-Kras <sup>G12V-ires-BGeo</sup>	<sup>22</sup>
Bardeesy	FVB/B6	Pdx1-cre; LSL-Kras <sup>G12D</sup> ; Ink4a/Arf <sup>fl/fl</sup>	(9)
Hingorani	B6/129SvJae	Pdx1-cre; LSL-Kras <sup>G12D</sup>	(7)
Hingorani	B6/129SvJae	Pdx1-cre; LSL-Kras <sup>G12D</sup> ; LSL-p53 <sup>R172H</sup>	(29)
Lewis	FVB/129/B6	TVA-RCAS-PyMT	(4)
Rustgi	B6/SJL	cK19- KRAS <sup>G12V</sup>	(3)
Means	B6/DBA2	Pdx1-HB-EGF	(30)
Thayer	B6/C3F1	Pdx1-SHH	(8)
Bar-Sagi	B6	Ela-PRSSI <sup>R122H</sup>	<sup>22</sup>

thought to be an exocrine-specific transcription factor, more recent evidence suggests it has a role earlier in pancreatic development and is required for the commitment of all three pancreatic cell lineages (20). Expression of p48 is restricted to the pancreatic anlagen of the Pdx1 expression domain, and the overlapping activities of Pdx1, p48, and pbx1, a member of the three amino acid loop extension class of homeodomain transcription factors, may delineate the endodermal domains committed to pancreatic development (11). The mechanism of final lineage commitment of Pdx1/p48-positive cells is not fully understood. Cells committed to the exocrine lineage lose Pdx1 expression but maintain p48 expression. Recent evidence suggests that terminally differentiated acinar cells are more closely related to islet cells than ductal cells, and that commitment to a ductal lineage occurs earlier than the divergence of acinar and islet cell lineages through a mechanism that is yet to be identified (11).

Although cells committed to the acinar lineage lose Pdx1 expression but maintain p48 expression, islet cell precursors maintain Pdx1 expression, lose p48 expression, and express the bHLH factor neurogenin 3 (21). Further lineage specification of islet cell precursors is regulated by neuroD (also known as  $\beta$  cell E-box transactivator-2), Pax6, Pax4 and Nkx2.2, Nkx6.1, and Glut2 (21). A small proportion of endocrine cells in the pancreas appears early in pancreatic development. This population of cells is thought to be analogous to the enteroendocrine cells located in the gastrointestinal tract. These cells are mostly glucagon immunoreactive, are not dependent on Pdx1 or p48 for development, and may contribute to the glucagon-positive mantle of mature islets (11). Finally, Notch signaling in developing pancreas inhibits p48 function and loss of Notch signaling permits acinar cell differentiation and subsequent production of pancreatic zymogens (22, 23). In addition, Notch acts to maintain a pool of undifferentiated cells in the mature organ, and Notch activity, as measured by Hes1 expression, is present in a small number of exocrine cells in the mature pancreas (23).

The histology of the normal mouse pancreas does not differ significantly from the histology of the normal human pancreas. An interesting feature of the normal mouse pancreas is the variability from mouse to mouse and region to region of the density of islets of Langerhans.

This framework for the normal development of the pancreas provides insight into approaches employed to generate the genetically engineered mouse models examined by this working

group. These approaches have also been guided by the growing body of information on the oncogenes and tumor suppressor genes targeted in human pancreatic adenocarcinomas (24) and by our current understanding of gene expression in normal mature acinar and ductal cells.

### Genetically Engineered Mouse Models

Twelve genetically engineered mouse models were submitted to the workshop participants (Table 1).

(a) An elastase (Ela)-Kras<sup>G12D</sup> transgenic mouse model was submitted by Grippo et al. (6). The model uses the rat elastase promoter to drive the expression of mutant *Kras* (a glycine to aspartate mutation). Elastase is a protein primarily expressed in cells committed to acinar differentiation.

(b) Sandgren and Schmid submitted a distinct transgenic model in which the expression of transforming growth factor- $\alpha$  (TGF- $\alpha$ ) is driven by the rat elastase promoter (5). Some of these mice were also crossed with p53<sup>+/-</sup> mice (25).

(c) In the model submitted by Konieczny, the expression of mutant *Kras* (Kras<sup>G12D</sup>) is driven by the endogenous Mist1 promoter (26). Mist1 is a transcription factor expressed in early development of the foregut endoderm (ed 10.5) and restricted to acinar cells in the adult pancreas (Fig. 1). In this model, the Kras<sup>G12D</sup> cDNA is “knocked-in” to the coding region of Mist1 by gene targeting in embryonic stem cells.

(d) A conditional Kras<sup>G12V</sup> model was submitted by Guerra et al. (27). This is a mutant Kras<sup>G12V-ires-BGeo</sup> “knock-in” model in which the endogenous *Kras* oncogene is transcriptionally silenced by a LoxP-flanked stop element, and expression is induced upon doxycycline administration (in a tet-o-cre; Elastase-rtTA background). The mice are constitutionally heterozygous for wild-type *Kras*.

(e) In the model submitted by Bardeesy, conditional Kras<sup>G12D</sup> mice (7) are crossed with conditional *Ink4a/Arf* null mice (9). In the conditional Kras<sup>G12D</sup> mice, endogenous expression of mutant *Kras* is activated by a Pdx1-Cre transgene (7). The *Ink4a/Arf* null mice have a conditional deletion of exons 2 and 3 of the *Ink4a/Arf* locus that concomitantly eliminates both p16<sup>INK4A</sup> and p19<sup>ARF</sup> in the same cells that activate Kras<sup>G12D</sup> expression.

<sup>22</sup> Unpublished.

(f) and (g) Hingorani submitted two models. The first model was the conditional  $Kras^{G12D}$  mice described above (7). In the second model, the conditional  $Kras^{G12D}$  mice were crossed with mice harboring a G-to-A substitution at nucleotide 515 of the endogenous p53 (p53+/515A) corresponding to the p53R175H hot-spot mutation in human cancers (28). Thus, in these models, both  $Kras^{G12D}$  and  $Trp53^{R172H}$  are expressed at physiologic levels in the context of their wild-type counterparts (29).

(h) Lewis submitted a model that somatically delivers mouse polyoma virus middle T antigen (PyMT)-bearing avian retroviruses (RCAS) to mice that express TVA, the receptor for avian leucosis sarcoma virus subgroup A (ALSV-A) under control of the elastase promoter (4).

(i) The K19-KRAS model submitted by Rustgi was created using a cytokeratin 19 promoter to drive the expression of mutant  $Kras$  (a glycine-to-valine mutation; ref. 3).

(j) Means submitted a model in which the Pdx1 promoter is used to drive the expression of heparin-binding epidermal growth factor-like growth factor (HB-EGF; ref. 30).

(k) Thayer submitted a model in which the expression of full-length rat sonic hedgehog (Shh) is driven by the Pdx1 promoter (8).

(l) Bar-Sagi submitted a model of chronic pancreatitis in which the elastase promoter is used to drive the expression of a mutant trypsinogen gene ( $PRSSI^{R122H}$ ). This mutation removes an autocleavage site in the trypsin protein, leading to increased trypsin activity.

## General Approach to the Evaluation of Changes in the Pancreas

Because the spectrum of pathologies seen in genetically engineered mouse models is so broad, and to help accommodate emerging models, the working group first developed a general approach to the evaluation of genetically engineered mouse models of pancreatic neoplasia (Table 2). All changes in the genetically engineered mouse models should be evaluated relative to gender, age, and strain-matched controls, optimally using littermates.

**Gross evaluation.** The first step is a thorough gross examination of the pancreas. This examination should include careful documentation of the size, shape, consistency, and color of the gland, as well as the localization, size, and gross appearance of any lesions. Other organs, especially liver, should be inspected for evidence of metastases or other pathology. Because of the propensity of the pancreas to undergo autolysis rapidly following removal, it is important to complete the gross evaluation quickly if done before fixation of the organ.

After the gross examination is completed and tissues are harvested for any special studies, the remainder of the pancreas should be entirely submitted for microscopic examination. Because the relationship of the pancreas and the pancreatic ducts to the duodenum is often of interest, the duodenum can be included in the sections. Ten percent neutral buffered formalin is a versatile fixative for routine histology, but other fixatives may be used depending on the studies planned.

**Microscopic evaluation of the compartments affected.** The first step in the microscopic evaluation of the pancreas is to determine which cellular compartments (acinar, ductal, endocrine, and interstitial compartments) are affected. Each compartment should be evaluated systematically, and the involvement of more than one compartment should be documented. Although it is recognized that it is not always possible to determine with

**Table 2.** Overview of the general approach to the evaluation of genetically engineered mouse models of pancreatic neoplasia

1. Gross evaluation: size (weight), shape, consistency (firm, soft), and color of the gland, as well as the localization (head vs tail) and size of any focal lesions
2. Documentation of the compartments affected
  - a. Acinar
  - b. Ductal
  - c. Endocrine
  - d. Interstitial
3. Type of change within each compartment
  - a. Architectural
  - b. Cytologic
4. Description of the architectural changes
  - a. Structural alterations (solid, cysts, papillae, tubules)
  - b. Aberrantly located compartment
  - c. Focal, multifocal or diffuse
5. Description of the cytological changes
  - a. Hypertrophy/atrophy
  - b. Hyperplasia
  - c. Metaplasia
  - d. Proliferation-increased mitoses
  - e. Atypia
  - f. Cell death (apoptosis or necrosis)
  - g. Cell lineage definition
6. Description of the interstitial changes
  - a. Active fibrosis
  - b. Inactive fibrosis
  - c. Inflammation
  - d. Vascular alterations

certainty which compartments are affected by a process, the procedure of systematically assessing each compartment can provide a useful framework for beginning the challenging task of evaluating the complex changes possible in a genetically engineered mouse model.

The changes within each compartment can then be separated into architectural and cytologic changes. *Architectural changes* alter the relationships among cells or the organization of a compartment. Architectural changes therefore include (a) formation of new abnormal elements including masses (solid masses, papillae, cysts, etc.), (b) an aberrantly located compartment within the pancreas (e.g., ducts within an islet of Langerhans), (c) transformation within preexisting units (cystic change within ducts, etc.), and (d) infiltration by cells not normally found in the gland (inflammatory infiltrates, secondary tumors, etc.). Each of the architectural changes should be described and categorized as focal, multifocal, or diffuse.

Cytologic changes are alterations at the cellular and subcellular levels and include hypertrophy, atrophy, hyperplasia, metaplasia, proliferation, atypia, and cell death (apoptosis or necrosis). The cellular compartments in which each cytologic change occurs should be documented. Definitions of selected terms are provided in Table 3.

Specific interstitial changes should also be evaluated for each model. These include (a) desmoplasia, (b) active (cellular) fibrosis, (c) inactive (hypocellular) fibrosis, (d) inflammation, and (e) vascular alterations. Brief definitions of these interstitial changes are provided in Table 4. After a genetically engineered mouse model has been



**Table 3.** Selected definitions of cytologic changes

- A. Agenesis is the complete failure of a pancreas to develop.
- B. Hypoplasia is the development of a pancreas that is significantly smaller than that in paired control animals of similar gender, strain, and age.
- C. Hyperplasia is an increase in the number of epithelial cells compared with paired control animal of similar strain and age. Cells retain their normal architecture and lack cytologic atypia.
- D. Atrophy is a decrease in size of the adult pancreas relative to age-matched controls.
- E. Hypertrophy is an increase in cell size relative to age-matched controls.
- F. Metaplasia is the replacement of one adult cell type by another adult/mature cell type. Recognized by the replacement of normal epithelium by nonneoplastic squamous, mucinous, or acinar epithelium with cytologic features similar to other tissues in which these epithelia are found. Again, these changes are evaluated by comparing the findings with age-matched controls.

evaluated using the general approach outlined above, the model should be further evaluated using the specific classification/nomenclature for pancreatic tumors in mice provided below.

**Description and Nomenclature of Lesions of the Exocrine Pancreas**

The suggested classification broadly parallels the classification of pancreatic tumors in humans and is based on the direction of differentiation of the lesion (Table 5; refs. 1, 10).

**A. Ductal Neoplasm**

**Serous cystic neoplasms.** Serous cystic neoplasms are epithelial neoplasms composed of uniform cuboidal glycogen-rich cells that form numerous cysts containing serous fluid (1, 31).

**Cystic papillary neoplasms.** Cystic papillary neoplasms are large (>1 mm) cystic structures composed of usually papillary, noninvasive epithelial proliferations with varying degrees of cellular atypia (Fig. 2A). Lesions <1 mm may represent mPanIN (see below). The predominant direction of differentiation is along ductal cell lines. Those that are not predominantly ductal should not be classified as cystic papillary neoplasms but should instead be classified based upon the predominant line of differentiation (or under Mixed Neoplasms if there are significant components of more than one cell line; see below). An attempt should be made to determine whether the lesion is intraductal and, if so, if it is arising in the main pancreatic ducts or in a smaller peripheral duct. Cystic papillary neoplasms arising in the larger ducts may resemble human intraductal papillary mucinous neoplasms (32). These lesions may be associated with inflammatory/fibrotic changes in the surrounding pancreas.

**Mucinous cystic neoplasms (with ovarian stroma).** Cystic neoplasms with mucinous epithelium that contain “ovarian-type” stroma are distinctive human neoplasms (mucinous cystic neo-

plasms, MCN; refs. 1, 33), and if identified in the mouse, they should be distinguished from the cystic papillary neoplasms above that lack this stromal component. The dense ovarian-type stroma in MCNs is composed of closely packed spindle-shaped cells that often express progesterone and estrogen receptors. The presence or absence of an associated invasive carcinoma should be documented.

**mPanIN.** Much of the discussion at the workshop centered on the definition of potential morphologic precursors to invasive ductal adenocarcinoma. In humans, clinical, morphologic, and molecular studies have all suggested that microscopic epithelial proliferations within the smaller (<5 mm in humans) pancreatic ducts progress to invasive ductal adenocarcinoma (34–36). In humans, these lesions are classified as PanIN, and in humans, PanINs can be graded based on the degree of cytologic and architectural atypia present as PanIN-1, PanIN-2, and PanIN-3 (Table 6; ref. 34).

Two critical issues were raised as the group attempted to develop a nomenclature for similar lesions in the genetically engineered mouse models. First, it was emphasized that the setting in which these lesions occur in genetically engineered mouse models is critical to their classification. Papillary proliferations of atypical epithelium in the background of essentially normal pancreatic parenchyma or focal lobulocentric atrophy more closely resemble human PanIN than do flat nonatypical epithelial lesions in the setting of diffuse acinar-ductal metaplasia (see Acinar-Ductal Metaplasia). Second, it was reemphasized that great care should be taken not to equate lesions in the mice with specific human disease processes. The group felt that although the generation of genetically engineered mouse models of lesions that mimic human PanIN have the potential to revolutionize our understanding of the precursors to invasive pancreatic ductal adenocarcinoma, direct extrapolation from lesions in the mice to humans would be

**Table 4.** Definitions of stromal/interstitial changes

- A. Desmoplasia is the cellular fibroinflammatory response, which may accompany an invasive ductal adenocarcinoma. Desmoplasia includes a hyperplasia of fibroblasts and the associated deposition of fibrous connective tissue. The term desmoplasia should not be used in the absence of an invasive carcinoma.
- B. Active fibrosis is the abnormal accumulation of collagen associated with an increased number of stromal cells (fibroblasts or myofibroblasts)
- C. Inactive fibrosis is the abnormal accumulation of collagen without an associated increase in the number of stromal cells (fibroblasts or myofibroblasts)
- D. Inflammation is the abnormal infiltration of inflammatory cells into the organ. At a minimum the type of inflammation should be designated as acute, chronic, or mixed
- E. Vascular alterations include increased or decrease vascularity of tissue compared to its normal counterpart. Vessel number or diameter may be altered.

**Table 5.** Classification of pancreatic exocrine tumors in genetically engineered mice

- A. Ductal neoplasms
1. Serous cystic neoplasms
  2. Cystic papillary neoplasm (without ovarian stroma)
    - a. Noninvasive
    - b. Cystic papillary neoplasm with an associated invasive carcinoma
  3. MCN (with ovarian stroma)\*
    - a. Mucinous cystadenoma (noninvasive)
    - b. Mucinous cystic neoplasm with an associated invasive carcinoma
  4. mPanIN
    - a. mPanIN-1A and mPanIN-1B
    - b. mPanIN-2
    - c. mPanIN-3
  5. Invasive ductal adenocarcinoma
    - a. Tubular adenocarcinoma
    - b. Adenosquamous carcinoma\*
    - c. Colloid (mucinous noncystic) carcinoma\*
    - d. Medullary carcinoma\*
    - e. Undifferentiated carcinoma
- B. Acinar cell neoplasms
- a. Acinar cell adenoma
  - b. Acinar cell carcinoma
  - c. Cystic acinar cell carcinoma
- C. Epithelial neoplasms with mixed or uncertain directions of differentiation
1. Mixed acinar-endocrine carcinoma
  2. Mixed acinar-ductal carcinoma\*
  3. Mixed ductal-endocrine carcinoma\*
  4. Mixed acinar-endocrine-ductal carcinoma\*
  5. Pancreatoblastoma\*
  6. Solid-pseudopapillary neoplasm\*

\*These entities, which are well characterized in humans, are included in this table for completeness, although a counterpart in the mouse has not yet been described.

dangerous. For example, if all lesions in genetically engineered mouse models that resemble human PanIN progress to invasive cancer, the conclusion should not be drawn that all human PanINs progress to invasive cancer. It was agreed, therefore, that the modifier “mouse” be added to PanIN terminology in the genetically engineered mouse models (mPanIN).

mPanIN was defined as a lesion that meets the following criteria: (a) It is a ductal epithelial proliferation confined to the native pancreatic ducts. (b) The involved ducts measure <1 mm. (c) It occurs in an appropriate setting. That is, mPanIN should be distinguished from acinar-ductal metaplasia. In restricting the classification of mPanINs to lesions that occur in the appropriate setting, we are not suggesting that small intraductal glandular proliferations that occur in the setting of acinar-ductal metaplasia are not biologically similar to mPanINs; rather, we are attempting to maintain close parallels with human disease. Developing insights may ultimately lead to change of definitions, but our current understanding of human intraductal epithelial proliferations is that the setting in which they occur determines their biology and setting is therefore reflected in the definition of mPanINs. (d) The lesion does not show significant acinar

differentiation. (e) Evidence suggests that the lesion is neoplastic (morphology, clonality, presence of additional genetic changes, progression, transplantation). Representative examples are illustrated in Fig. 2B-D. The degree of cytologic and architectural atypia in these lesions can be graded, and grading should parallel that in human PanIN (mPanIN-1, mPanIN-2, and mPanIN-3; see Table 6). mPanIN can occur in a pancreas with or without an associated invasive carcinoma, and evidence exists that mPanINs may progress to invasive carcinoma, because they appear before the invasive component in some models (7).

**Invasive ductal adenocarcinoma.** Invasive ductal adenocarcinomas constitute the vast majority of malignant neoplasms of the pancreas in humans. They are defined as malignant epithelial neoplasms with ductal differentiation that have penetrated through the ductal basement membrane (Fig. 3A). Ductal differentiation is primarily defined at the light microscopic level by the formation of glands and can be supported by special stains for mucin or immunolabeling for markers of ductal differentiation (e.g., cytokeratin 19). Invasive ductal adenocarcinomas are subdivided into five groups (see Table 5) as defined below. One of the characteristic features of invasive carcinomas of the pancreas in humans is that they are associated with an intense desmoplastic reaction. Desmoplasia is the cellular fibroinflammatory response, which may accompany an invasive ductal adenocarcinoma. The term desmoplasia should not be used in the absence of an invasive carcinoma.

Invasive ductal adenocarcinomas should be graded using the criteria that are applied to human cancers. Briefly, *well-differentiated* adenocarcinomas form well-defined glands. The cuboidal to columnar neoplastic cells have basally oriented uniform round to oval nuclei with evenly dispersed chromatin. Only minimal nuclear pleomorphism is seen. Mitotic figures are not numerous, nor are they atypical. *Moderately differentiated* adenocarcinoma has a disorganized growth pattern, and gland formation is less well defined (33, 37, 38). Nuclear pleomorphism is more prominent, and the nucleoli are larger and more irregular. Mitoses are more common, and some may be atypical. *Poorly differentiated* adenocarcinomas form small poorly defined glands, individual infiltrating cells, and solid areas (Fig. 3A; refs. 33, 37, 38). The neoplastic cells produce significantly less mucin than the better differentiated pancreatic cancers. Nuclear pleomorphism is prominent with occasional large, somewhat bizarre nuclei, and the nucleoli are large, multiple, and more irregular. Mitoses are common, as are atypical mitoses. Although no evidence exists for murine pancreatic neoplasms that this grading scheme correlates with biological behavior, it is recommended as a purely morphologic description of the extent to which the carcinoma recapitulates the normal ductal cytoarchitecture.

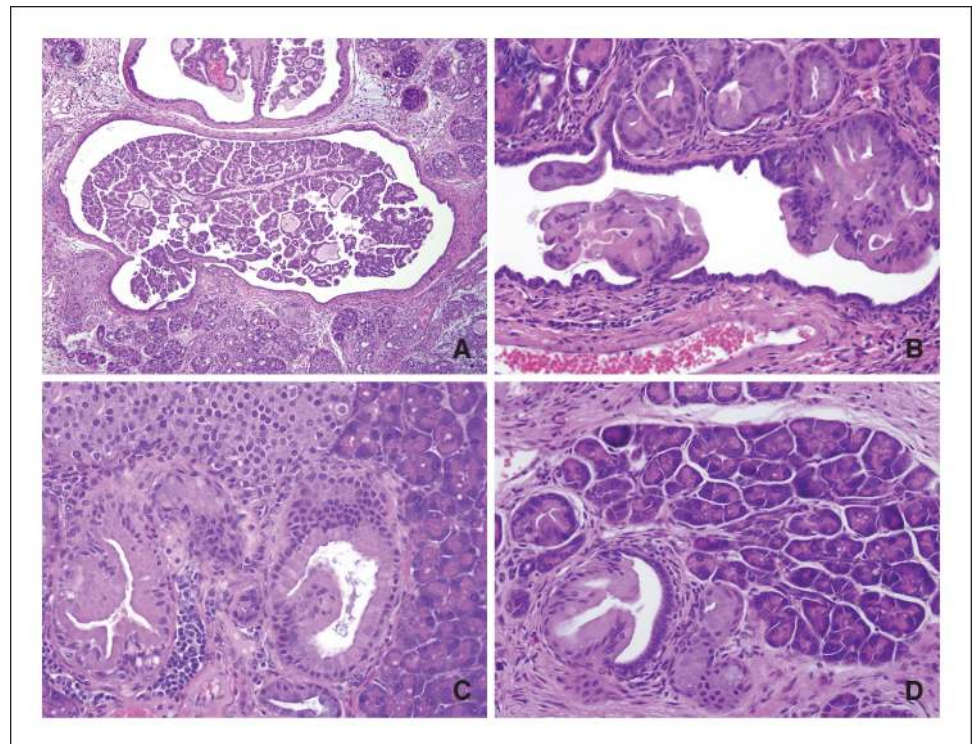
The subtypes of invasive adenocarcinoma include:

a. Tubular adenocarcinoma is an invasive malignant epithelial neoplasm with ductal differentiation and without a predominant component of any of the other carcinoma types (Fig. 3A; refs. 1, 10). Evidence of ductal differentiation can include morphology supported by special stains for mucin or immunolabeling (e.g., for cytokeratin 19).

b. Adenosquamous carcinoma is a malignant epithelial neoplasm of the pancreas with significant components of both ductal and squamous differentiation (1, 10).

c. Colloid (mucinous noncystic) carcinoma is an infiltrating adenocarcinoma in which  $\geq 80\%$  of the neoplasm is characterized

**Figure 2.** A, cystic papillary neoplasm. The cystic structures are composed of papillary, noninvasive epithelial proliferations with varying degrees of cellular atypia. B-D, mPanIN. These glandular epithelial proliferations are confined to the pancreatic ducts, the ducts measure <1 mm, and they occur in an appropriate setting. A, H&E-stained section from the Ela-KRAS<sup>G12D</sup> model; B and D, from the Pdx1-cre; LSL-Kras<sup>G12D</sup> model; C, from the Ela-tTA; tet-o-cre; LSL-Kras<sup>G12V-ires-BGeo</sup> model.



by mucin-producing neoplastic epithelial cells suspended in large pools of extracellular mucin (39). Thus far only described in humans, colloid carcinomas are usually associated with intraductal papillary mucinous neoplasms (40).

d. Medullary carcinoma is a malignant epithelial neoplasm characterized by poor differentiation, a syncytial growth pattern, pushing borders, and necrosis. To date, it has only been reported in humans (41).

e. The undifferentiated carcinoma is an invasive, malignant epithelial neoplasm that shows no glandular, acinar, endocrine,

or squamous differentiation (1, 10). Undifferentiated carcinoma can have a variety of microscopic appearances. Anaplastic giant cell carcinomas are composed of relatively noncohesive pleomorphic mononuclear cells admixed with bizarre, frequently multinucleated giant cells containing abundant eosinophilic cytoplasm (1). When the spindle cell morphology is predominant, the neoplasm may resemble a sarcoma, and this form of undifferentiated carcinoma can be designated sarcomatoid carcinoma (Fig. 3B; ref. 1). Sometimes, there is sufficient mesenchymal differentiation to result in heterologous stromal

**Table 6.** Grading of mPanIN (33)

Normal: The normal ductal and ductular epithelium is a cuboidal to low-columnar epithelium with amphophilic cytoplasm. Mucinous cytoplasm, nuclear crowding, and atypia are not seen.

Squamous (transitional) metaplasia: A process in which the normal cuboidal ductal epithelium is replaced by mature stratified squamous or pseudostratified transitional epithelium without atypia.

mPanIN-1A: These are flat epithelial lesions composed of tall columnar cells with basally located nuclei and abundant supranuclear mucin.

The nuclei are small and round to oval in shape. When oval, the nuclei are oriented perpendicular to the basement membrane. It is recognized that there may be considerable histologic overlap between nonneoplastic flat hyperplastic lesions and flat neoplastic lesions without atypia.

mPanIN-1B: These epithelial lesions have a papillary, micropapillary, or basally pseudostratified architecture but are otherwise identical to mPanIN-1A.

mPanIN-2: Architecturally, these mucinous epithelial lesions may be flat but are mostly papillary. Cytologically, by definition, these lesions must have some nuclear abnormalities. These abnormalities may include some loss of polarity, nuclear crowding, enlarged nuclei, pseudostratification, and hyperchromatism. These nuclear abnormalities fall short of those seen in mPanIN-3. Mitoses are rare, but when present, are nonluminal (not apical) and are not atypical. True cribriform structures with luminal necrosis and marked cytologic abnormalities are generally not seen and, when present, should suggest the diagnosis of mPanIN-3.

mPanIN-3: Architecturally, these lesions are usually papillary or micropapillary; however, they may rarely be flat. True cribriforming, the appearance of "budding off" of small clusters of epithelial cells into the lumen, and luminal necrosis should all suggest the diagnosis of mPanIN-3. Cytologically, these lesions are characterized by a loss of nuclear polarity, dystrophic goblet cells (goblet cells with nuclei oriented toward the lumen and mucinous cytoplasm oriented toward the basement membrane), mitoses that may occasionally be abnormal, nuclear irregularities, and prominent (macro) nucleoli. The lesions resemble carcinoma at the cytonuclear level, but invasion through the basement membrane is absent.

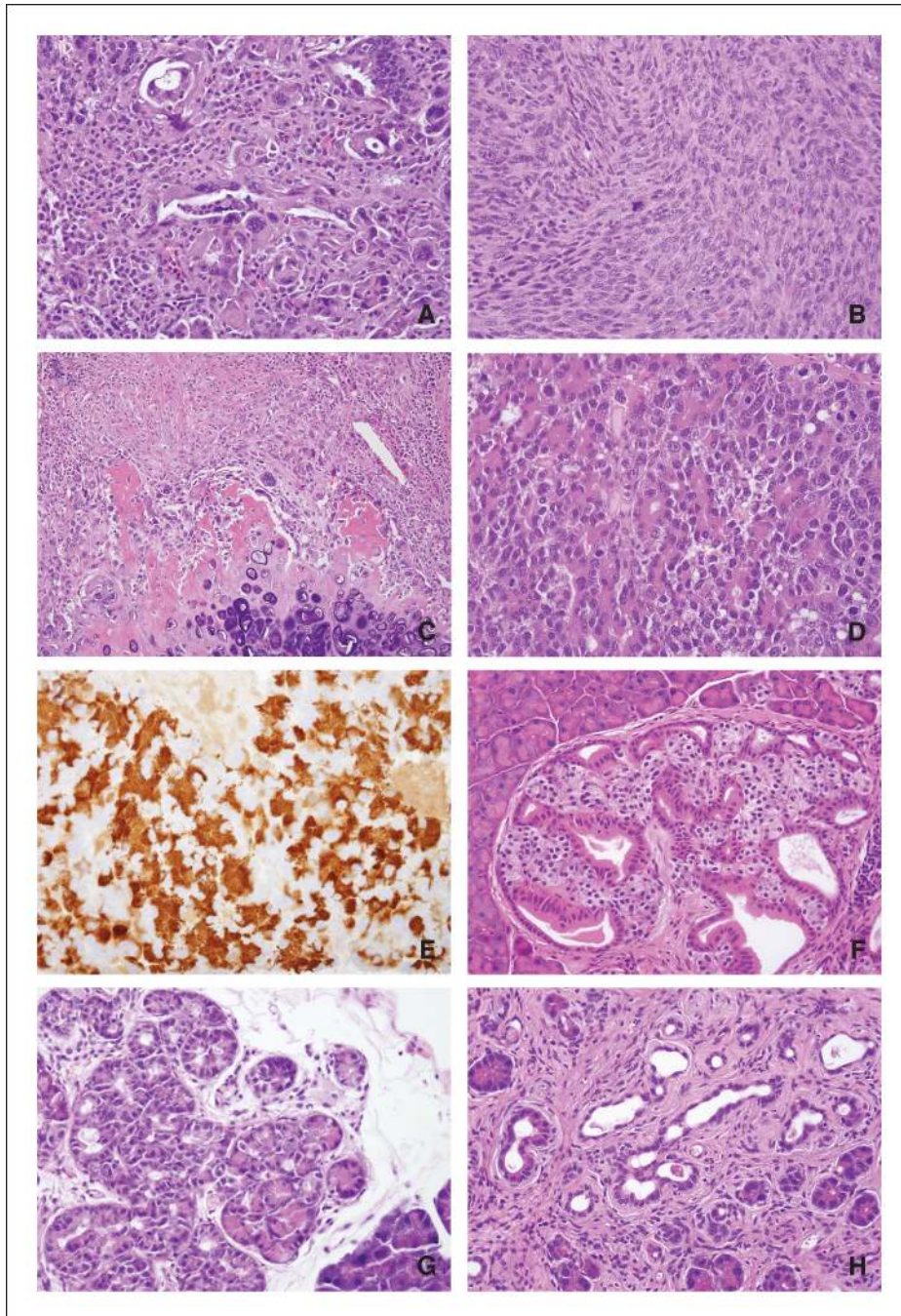


elements, such as bone, cartilage, or striated muscle (Fig. 3C; refs. 1, 10). Such carcinomas should be designated as adenocarcinomas with a metaplastic component, and the metaplastic component should be named.

### B. Acinar Lesions

Acinar cell lesions include both neoplasms and hyperplastic lesions. Acinar cell lesions are microscopically distinct epithelial lesions with evidence of pancreatic acinar differentiation. This differentiation can be identified at the light microscopic level but is best shown by immunohistochemical labeling for pancreatic exocrine enzymes (lipase, amylase, trypsin, or

chymotrypsin), or by the ultrastructural demonstration of zymogen granules within the neoplastic cells. Historically, well-differentiated noninvasive lesions have been classified according to size. Focal hyperplasia is used to designate lesions <1 mm; acinar cell nodule designates lesions 1 to 5 mm; and acinar cell adenoma lesions >5 mm. If dysplasia (atypia) is present in these lesions, it should be documented. The predominant acinar neoplasm seen in the mouse models is the acinar cell carcinoma (Fig. 3D and E). Acinar cell carcinoma is a solid or cystic invasive epithelial neoplasm with acinar differentiation (morphology supported by immunolabeling for exocrine enzymes [e.g., chymotrypsin and amylase] or electron microscopy; ref. 42).



**Figure 3.** A, invasive ductal adenocarcinoma. This malignant epithelial neoplasm shows prominent ductal differentiation and has penetrated through the basement membrane. B, sarcomatoid carcinoma. A spindle cell morphology predominates in this undifferentiated carcinoma. C, undifferentiated carcinomas with a metaplastic component. Note the cartilage formation. D and E, acinar cell carcinoma. Note the formation of acini (D) and the expression of chymotrypsin (E). F, ductular-insular complex. G and H, acinar-ductal metaplasia. Note the formation of tubular structures with both ductal and acinar differentiation that replace acinar parenchyma. The ductular and acinar units in (H) are separated by mature fibrous tissue, which is characteristic in this model and exemplifies involvement of the interstitial compartment. H&E-stained sections from the Pdx1-cre; LSL-Kras<sup>G12D</sup>; LSL-p53<sup>R172H</sup> model (A), from the Pdx1-cre; LSL-Kras<sup>G12D</sup>; Ink4a/Arf<sup>fl/fl</sup> model (B), from the TVA-RCAS-PyMT model (C), from the Ela-TGF $\alpha$ ; p53<sup>-/-</sup> model (D), the Pdx1-HB-EGF model (F), the Ela-KRAS<sup>G12D</sup> (G) model, and the Ela-TGF $\alpha$  model (H). Immunolabeling for chymotrypsin in the Ela-TGF $\alpha$ ; p53<sup>-/-</sup> model (E).



### C. Epithelial Neoplasms with Mixed or Uncertain Directions of Differentiation

**Neoplasms with multiple lines of differentiation** (“mixed neoplasms”). It is recognized that pancreatic neoplasms, particularly those neoplasms that arise in genetically engineered mouse models, can show more than one direction of differentiation. Each component comprising >20% of the neoplasm should be designated. Examples include mixed acinar-endocrine, ductal-acinar, ductal-endocrine, and ductal-endocrine-acinar carcinomas. If a minor (<20%) component of a secondary line of differentiation is detected, the neoplasm should be classified based upon the predominant line of differentiation. Pancreatoblastoma, a human neoplasm containing squamoid nests and commonly exhibiting acinar, endocrine, and ductal differentiation, is also regarded as a type of neoplasm with multiple lines of differentiation.

Cystic papillary neoplasms should not be confused with solid-pseudopapillary neoplasms (1, 43). Solid-pseudopapillary neoplasms have not been reported in genetically engineered mouse models. In humans, solid-pseudopapillary neoplasms are epithelial neoplasms composed of noncohesive cells that surround delicate blood vessels and form solid masses with frequent cystic degeneration (1, 43).

### D. Other Lesions

**Ductulo-insular lesion.** Ductulo-insular lesion is the aberrant presence of proliferating ductules within an islet of Langerhans (Fig. 3F). These should not be considered mPanINs as they are not arising in the appropriate setting.

**Acinar-ductal metaplasia.** Acinar-ductal metaplasia is a common finding in genetically engineered mouse models, particularly those models that use acinar promoters, such as elastase. Acinar-ductal metaplasia is characterized by the abnormal formation of tubular structures (i.e., tubular complexes) with both ductal and acinar differentiation that replace acinar parenchyma (Fig. 3G and H). The sharp transition between normal caliber ducts and acini is lost. The process is usually diffuse, it can be proliferative, it can be mucinous, and it can have scattered endocrine cells. Those with mucinous lining, if examined out of context, are very similar to human PanIN-1A. Importantly, the lesions are located within the acinar compartment of the pancreas rather than in the native ducts. A similar acinar-ductal metaplasia can be seen in human pancreata, particularly in the setting of chronic pancreatitis. Although some lesions of acinar-ductal metaplasia may progress to neoplastic lesions and models, which generate these lesions, may provide useful insight into human disease, the group felt that it is important to clearly distinguish acinar-ductal metaplasia from intraductal lesions that meet the criteria for mouse PanIN (see above). This distinction will help establish the importance (or lack thereof) of the setting in which intraductal lesions develop.

**Ductal-intestinal metaplasia.** Ductal-intestinal metaplasia is the replacement of the normal exocrine pancreas with cells showing mature intestinal differentiation (Fig. 4A). Intestinal differentiation is defined by light microscopic examination and can be supported by stains for mucin and immunohistochemical labeling for markers of intestinal differentiation, such as *cdx2*.

**Acute pancreatitis.** Acute pancreatitis is characterized by an inflammatory cell infiltrate that includes neutrophils and is usually associated with necrosis of the pancreatic parenchyma or adjacent fat. An absence of significant replacement fibrosis supports the reversibility of the process when it is of mild severity.

**Chronic pancreatitis.** Chronic pancreatitis is the irreversible loss of acinar tissue associated with replacement fibrosis (Fig. 4B). An inflammatory cell infiltrate is usually present and is often mixed, composed of lymphocytes, plasma cells, and macrophages. In some instances, an acute inflammatory cell infiltrate may be present, reflecting ongoing acute pancreatitis.

For purposes of comparison, representative examples of human infiltrating ductal adenocarcinoma, pancreatic intraepithelial neoplasia, and acinar carcinoma are illustrated in Fig. 4C-F.

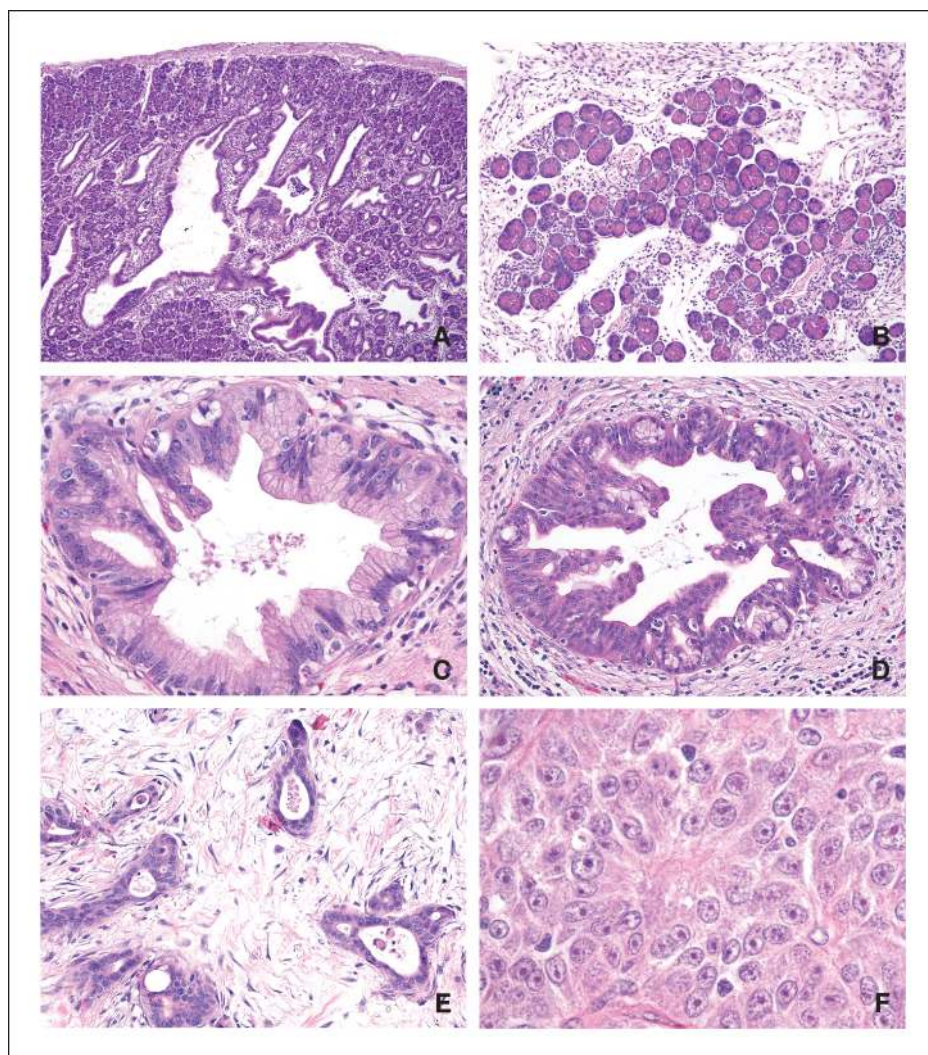
### General Comments about Existing Models

In the last phase of the workshop, the group reviewed each of the 12 submitted existing genetically engineered mouse models of pancreatic exocrine neoplasia (Tables 1 and 7; refs. 3–9, 26, 29, 30). This provided an opportunity to validate the new nomenclature, as well as a framework in which to categorize the existing genetically engineered mouse models. Investigators from each of these genetically engineered mouse models were invited to submit representative formalin-fixed, paraffin-embedded tissue blocks to the workshop. H&E-stained sections were prepared from each block, as well as a mucicarmine stain and a panel of immunohistochemically labeled slides. These included immunohistochemical labeling for synaptophysin (DakoCytomation, Copenhagen, Denmark; 1:100), cytokeratin 19 (DakoCytomation, 1:50), and chymotrypsin (Bioscience International, Saco, ME; 1:100). The mucin-stained slides and the panel of immunohistochemically labeled slides were sent to workshop participants before the workshop, and a complete set of slides and multi-headed microscopes were available for additional slide reviews at the workshop. The group felt that although there was significant variation among the models, the three acinar models could be broadly grouped together, as could the three endogenous *Kras* models.

**Acinar promoter models.** The three models that predominantly used acinar promoters included the rat *Ela-Kras*<sup>G12D</sup> model submitted by Grippo et al. (6), the rat *Ela-TGF- $\alpha$*  model submitted by Sandgren and Schmid (5), and the endogenous *Mist1-Kras*<sup>G12D</sup> model submitted by Konieczny (26). All three of these genetically engineered mouse models of pancreatic neoplasia were characterized by prominent acinar-ductal metaplasia (Fig. 3G and H), the formation of cystic acinar neoplasms, the majority of which were carcinoma *in situ* (Fig. 2A). A variety of invasive carcinomas developed in these models. Most were acinar cell carcinomas (Fig. 3D and E), but undifferentiated carcinomas and carcinomas with mixed differentiation were also seen. Extensive metastases developed in the *Mist1-Kras*<sup>G12D</sup> model.

Because the intraductal lesions in the small ducts in these models developed in the setting of extensive acinar-ductal metaplasia, the group felt that these models did not develop mPanIN as strictly defined above despite the presence of mucin staining that could be shown in metaplastic ductal structures that could resemble mPanIN in isolation.

**Endogenous *Kras* models.** Four models were broadly grouped together as endogenous *Kras* models. These included the *Kras*<sup>G12V-ires-BGeo</sup> model submitted by Guerra et al. (27) and the *Kras*<sup>G12D</sup> model developed by Hingorani (7). The *Kras*<sup>G12D</sup> model was subsequently used to develop the *Kras*<sup>G12D</sup> + *Ink4a/Arf* model submitted by Bardeesy (9) and the *Kras*<sup>G12D</sup> + *p53*<sup>R172H</sup> model submitted by Hingorani et al. (29). Acinar-ductal metaplasia was much less extensive in these models than it was in the three



**Figure 4.** A, ductal-intestinal metaplasia. The normal exocrine pancreas is replaced with cells showing mature intestinal differentiation. B, chronic pancreatitis with acinar dropout, fibrosis, and chronic inflammation. C and D, human PanIN grades PanIN-2 (C) and PanIN-3 (D). E, human infiltrating tubular (ductal) adenocarcinoma. Note the haphazard arrangement of glands and the desmoplasia. F, human acinar cell carcinoma. Note the granular cytoplasm and single prominent nucleoli. H&E-stained sections of the Pdx1-Shh model (A), the Ela-PRSS1 model (B), and surgically resected human pancreata (C-F).

acinar promoter models. The four endogenous *Kras* models developed mPanIN lesions, including mPanIN-1, mPanIN-2, and mPanIN-3 (Fig. 2B-D; ref. 34). All developed invasive ductal adenocarcinomas, some with metastases. The carcinomas that developed in these models were well differentiated, poorly differentiated, and undifferentiated (Fig. 3A and B).

**Lewis TVA-RCAS model.** The TVA-RCAS model submitted by Lewis was relatively unique (4). The PyMT model developed cystic papillary neoplasms with ductal differentiation and carcinomas with mixed acinar-endocrine differentiation when deficient for *Ink4a/Arf* and undifferentiated carcinomas in mice null for *p53* and heterozygous for *Ink4a/Arf* in the pancreas (Fig. 3C).

**Rustgi K19-KRAS.** The K19-KRAS model submitted by Rustgi showed minimal morphologic changes, although cells isolated from the pancreata of these animals showed an interesting *in vitro* phenotype (3).

**Pdx1-HB-EGF.** The *pdx1*-HB-EGF model submitted by Means developed ductulo-insular lesions (Fig. 3F; ref. 30). No mPanIN lesions were identified in the slides submitted for review, and the animals did not develop invasive carcinoma.

**Pdx1-Shh.** The *Pdx1*-Shh model submitted by Thayer showed extensive ductal-intestinal metaplasia with atypia (Fig. 4A; ref. 8).

No mPanIN lesions were identified, and the animals did not develop invasive carcinoma.

**ELA-PRSS1.** The ELA-PRSS1<sup>R122H</sup> model submitted by Bar-Sagi showed chronic pancreatitis with a lymphocytic infiltrate and acinar-ductal metaplasia (Fig. 4B). No mPanIN lesions were identified, and the animals did not develop invasive carcinoma.

Thus, the pathologic changes identified in all of the models submitted to the meeting could be classified using the new nomenclature and classification system. The group of pathologists reviewing the pathology felt that the two-step process in which the general features of the model were evaluated and then the specific changes classified was very useful.

## Conclusions

Early transgenic models of exocrine pancreatic cancer relied on the elastase promoter and primarily yielded acinar cell carcinomas (44). In the last decade, there has been a wonderful growth in the number of genetically engineered mouse models of pancreatic neoplasia (2–9, 27, 29). A variety of promoters can now be exploited to target specific cell types in the pancreas at different stages of development. The changes produced in these genetically engineered mouse models are complex, making it difficult to compare

**Table 7.** Pathology of the mouse models reviewed

Model	Acinar ductal metaplasia	mPanIN	Cystic papillary neoplasia	Invasive ductal carcinoma	Acinar cell carcinoma	Other	Penetrance; latency	Metastasis	Mortality; median survival
Ela-Kras <sup>G12D</sup>	+ (P, D)	—	+ (P)	—	adenoma	Strain dependent*	50, 1 y	No	0; >1 y
Ela-TGF- $\alpha$	+ (P, D)	—	+ (P)	—	+		30, 1 y	No	30; 1 y
Ela-TGF- $\alpha$ ; p53 <sup>+/-</sup>	+ (P, D)	—	+ (P)	—	+		100; 3 mo	Yes	100; 5 mo
MIST1-Kras4B <sup>G12D</sup>	+ (P, D)	—	+ (P)	—	+		100; birth	Yes	100; 11 mo
Ela-tTA;tet-o-cre; Kras <sup>G12V-ires-BGeo</sup>	F	+	—	+	—		100; 3 mo	Rare	10; >1 y
Kras <sup>G12D</sup> ; Pdx1-cre INK4a/ARF <sup>fl/fl</sup>	F	+	—	+	—	Sarcomatoid	100; 1 mo	Yes	100; 10 wk
Kras <sup>G12D</sup> ; Pdx1-cre	F	+	—	+	—		100; 1 mo	Yes	100; 16 mo
Kras <sup>G12D</sup> ; Trp53 <sup>R172H</sup>	F	+	—	+	—	Glandular	100; 1 mo	Yes	100; 5 mo
TVA-RCAS-PyMT; (Ink4a/Arf, p53)	—	—	+ (P)	+ mixed <sup>†</sup>	+ mixed	Mixed histology	75; 3-6 mo	Rare	0; ?
K19-KRAS <sup>G12V</sup>	—	—	—	—	—	<i>In vitro</i> phenotype	NA	NA	0; ?
Pdx1-HB-EGF	—	—	—	—	—	Ductular-insular complexes	40	NA	0; ?
Pdx1-Shh	—	—	—	—	—	Ductal intestinal metaplasia	100, birth	NA	100; <1 mo
Ela-PRSS1 <sup>R122H</sup>	—	—	—	—	—	Chronic pancreatitis	40, 7 wks	No	0; ?

NOTE: + = positive.  
 Abbreviations: D, diffuse; P, prominent; F, focal; NA, not available.  
 \*B6/FVB F1 have 100% penetrance at 6 months.  
<sup>†</sup>Setting of (p53<sup>-/-</sup>, p16/19<sup>+/-</sup>).

the findings in the models. Here, we present a proposed nomenclature for the pathology of genetically engineered mouse models of pancreatic neoplasia that we hope will gain international acceptance.

A two-phase approach to the evaluation of genetically engineered mouse models of pancreatic neoplasia is presented. Models should first be evaluated using a general approach to describe the pathologic changes in a broad sense: the compartments that are affected and the general nature of the process within each compartment. After this general description, a more specific approach can be taken to establish a specific pathologic diagnosis.

The review of a large number of mouse models also provided an opportunity to identify several general differences between the pathology identified in the genetically engineered mouse models and the pathology seen in humans. First, human pancreatic ductal adenocarcinoma tends to be moderate or poorly differentiated, whereas many of the models produced anaplastic carcinomas. Second, most neoplasms in humans show a single direction of differentiation, whereas multilineage differentiation, including acinar differentiation, was often seen in the genetically engineered mouse models. Third, pancreatic intraepithelial neoplasia in humans often, although not always, occurs in the relatively intact pancreatic parenchyma. By contrast, many of the duct lesions in genetically engineered mouse models arose in the background of diffuse acinar-ductal metaplasia. Fourth, most human pancreatic carcinomas are solitary, whereas multifocality seems to be common in the genetically engineered mouse models. Finally, intense desmoplasia is a characteristic feature of invasive

ductal adenocarcinoma in humans. By contrast, little to no desmoplasia was seen in most carcinomas in the genetically engineered mouse models.

The group felt it was important to emphasize two points. First, the classification of genetically engineered mouse models of pancreatic neoplasia is not a static process. It is fully anticipated that new models will be created and that some of these new models will develop lesions that are not easily classifiable using the proposed framework. Furthermore, the classification system needs to be further tested and validated. As such, the classification system presented should be viewed as a “working formulation” and not as a final unchangeable product. Second, the group felt that each of the models submitted for review has its own unique merits in advancing our understanding of pancreatic neoplasia. The classification system proposed is not meant to be used to value one model over another. Rather, the purpose of the classification is to provide an internationally acceptable framework to facilitate comparisons between genetically engineered mouse models and human pathology.

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