Tumorigenesis and Neoplastic Progression

Trp53 Deletion Stimulates the Formation of Metastatic Pancreatic Tumors

Jennifer P. Morton,* David S. Klimstra,[†]
Michelle E. Mongeau,* and Brian C. Lewis*^{‡§}

From the Programs in Gene Function and Expression,* and
Molecular Medicine,[‡] and the Memorial Cancer Center,[§]
University of Massachusetts Medical School, Worcester,
Massachusetts; and the Department of Pathology,[†] Memorial
Sloan-Kettering Cancer Center, New York, New York

The presence of distant metastases is a common finding on diagnosis of pancreatic cancer; however, the mechanisms underlying the dissemination of this tumor type remain poorly understood. Loss of the p53 tumor suppressor protein has been associated with tumor progression and metastasis in several tumor types including pancreatic ductal adenocarcinoma. Here, we describe the generation of a progressive and metastatic pancreatic cancer mouse model after the somatic and sporadic delivery of avian retroviruses encoding the mouse polyoma virus middle T antigen to elastase-*tv-a* transgenic mice with a pancreas-specific deletion of the *Trp53* tumor suppressor locus. In this model, the tumors metastasize most frequently to the liver, consistent with human pancreatic carcinomas. Analysis of metastatic lesions demonstrated that concomitant loss of the *Ink4a/Arf* locus was not required for metastasis; however, pancreas-specific deletion of a single *Ink4a/Arf* allele cooperated with *Trp53* deletion in a haploinsufficient manner to accelerate tumor development. Thus, our findings illustrate the potential role of p53 loss of function in pancreatic tumor progression, demonstrate the feasibility of modeling pancreatic cancer metastasis after somatic and sporadic oncogene activation, and indicate that our model may provide a useful experimental system for investigation of the molecular mechanisms underlying pancreatic cancer progression and metastasis. (Am J Pathol 2008, 172:1081–1087; DOI: 10.2353/ajpath.2008.070778)

Pancreatic cancer is the fourth-leading cause of cancer-related mortalities in the United States, with ~32,000 deaths annually.¹ The median survival after diagnosis is 6

months, and the 5-year survival rate is only 5%. These statistics reflect the advanced stage at which most pancreatic tumors are identified, exemplified by extra-pancreatic invasion and metastasis to the liver and peritoneum, and the resistance of pancreatic cancers to conventional chemotherapeutic intervention.^{2,3} Thus, understanding the factors that contribute to pancreatic tumor progression and metastasis is important in combating this disease.

Mouse models are attractive experimental systems for exploring the genesis and behavior of human malignancies, and as a result, several mouse models for pancreatic cancer have been generated using transgenic approaches.^{2,4,5} These models, although valuable, were restricted in several respects including the expression of the oncogene throughout pancreatic development. Indeed, in some of the published models mice die shortly after birth, or perinatally, with architecturally abnormal pancreata.⁶ Further, because all cells of a particular lineage within the pancreas express the transgene, tumors arise in an environment of aberrant intercellular signaling, unlike the scenario of sporadic tumor development in humans, in which tumor cells are surrounded by genetically normal cells.

To address these issues, we recently generated a mouse model for pancreatic cancer using the RCAS-TVA gene delivery system.⁷ This system allows the sporadic postnatal delivery of oncogene-bearing avian retroviruses to targeted cells engineered to express the avian leukosis virus subgroup A (ALV-A) receptor TVA.⁸ Delivery of ALV-A-derived RCAS viruses encoding the mouse polyoma virus middle T antigen (PyMT) led to the formation of ductal precursor lesions in transgenic mice in which the elastase promoter directs *tv-a* expression.⁷ If the *Ink4a/Arf* locus was also inactivated in these mice,

Supported by the Burroughs Wellcome Fund career development award in the biomedical sciences (to B.C.L.) and the Worcester Foundation for Biomedical Research (to B.C.L.).

Accepted for publication January 2, 2008.

Present address of J.P.M.: Beatson Institute for Cancer Research, Glasgow, Scotland.

Address reprint requests to Brian C. Lewis, University of Massachusetts Medical School, 364 Plantation St., LRB 521, Worcester, MA 01605. E-mail: brian.lewis@umassmed.edu.

acinar carcinomas and ductal lesions, either precursor PanIN lesions or cystadenocarcinomas, developed. Interestingly, the tumors were positive for the progenitor cell marker Pdx1, and aberrantly expressed the neuroendocrine marker synaptophysin, whose expression within the pancreas is normally restricted to endocrine cells of the islets of Langerhans. The induction of multiple tumor types, and the expression of progenitor and endocrine markers, suggested that the tumors were derived from the transformation of pancreatic progenitor cells. Consistent with this hypothesis, introduction of RCAS viruses encoding c-Myc into elastase-*tv-a* transgenic mice deficient at the *Ink4a/Arf* locus led to the formation of pancreatic endocrine neoplasms exclusively.

These findings demonstrated the influence of the initiating oncogenic lesion on the tumor phenotype. Yet, the effects of tumor suppressor gene mutations on progression to invasive and metastatic disease in our model remain unclear, in part attributable to the shortened lifespan of animals with germline tumor suppressor gene deletion.^{9–11} Recently published studies by others suggested that tumor suppressor gene mutations could indeed influence the tumor phenotype. An endogenously expressed activated *Kras* allele induced moderately differentiated pancreatic ductal adenocarcinomas with metastatic potential in animals with pancreas-specific deletion of *Trp53*, but induced undifferentiated carcinomas that invade adjacent tissues, but do not readily form distant metastases, in animals with pancreas-specific *Ink4a/Arf* loss.^{12,13}

Here we report that pancreas-specific deletion of *Trp53* cooperates with somatic and sporadic PyMT expression to induce both acinar cell carcinomas and ductal adenocarcinomas of the pancreas, and additionally leads to the development of liver and lung metastases, similar to human pancreatic cancer. Thus, our findings demonstrate that pancreatic tumor progression and metastasis can be accurately modeled after the sporadic and somatic activation of oncogenes *in vivo*, and further show that our model is an attractive system in which to study factors regulating pancreatic tumor progression.

Materials and Methods

Genetically Modified Mice and Animal Care

The elastase-*tv-a*, *Trp53* flox, *Ink4a/Arf* flox, and *Ptf1a*-cre knockin mouse strains have been previously described.^{7,14–16} Animals were kept in specific pathogen-free housing with abundant food and water under guidelines approved by the University of Massachusetts Medical School Institutional Animal Care and Use Committee and Department of Animal Medicine.

Virus Delivery

The RCAS-GFP and RCAS-PyMT vectors have been previously described.^{7,17} DF1 chicken fibroblasts^{18,19} transfected with RCAS vectors were maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal

bovine serum in humidified 37°C incubators under 5% CO₂. Cells to be injected were harvested, washed once with phosphate-buffered saline (PBS), and resuspended in PBS at a final concentration of 10⁴ cells/μl. One hundred μl of the cell suspension was delivered intraperitoneally to 2- to 3-day-old animals using tuberculin syringes attached with 27-gauge needles.

Tumor Harvest and Histology

Animals were sacrificed with a lethal dose of CO₂ followed by cervical dislocation as per institutional guidelines. Pancreata were removed and either fixed in 10% buffered formalin overnight at room temperature or snap-frozen in liquid nitrogen. Fixed tissues were paraffin embedded and 5-μm sections placed on sialynated slides at Histoserv Inc. (Gaithersburg, MD).

Immunohistochemistry

Paraffin sections were deparaffinized and rehydrated by passage through Clear-Rite 3 and a graded alcohol series, and immunostaining performed as described.⁷ Primary antibodies used were mouse anti-human keratin 19 (Abcam, Cambridge, UK) 1:50; rabbit anti-mouse Pdx1 (gift of C.V. Wright, Vanderbilt University, Nashville, TN) 1:5000; mouse anti-human p16 (Santa Cruz Biotechnology, Santa Cruz, CA) 1:50; mouse anti-human E-cadherin (BD Pharmingen, Franklin Lakes, NJ) 1:100; mouse anti-human β-catenin (BD Pharmingen) 1:100; and rabbit anti-human synaptophysin 1:1000 (DAKO, Carpinteria, CA). For chymotrypsin staining, instead of citric buffer incubation, slides were pretreated with 0.05 μg/ml of protease 24 (Sigma, St. Louis, MO) for 10 minutes at 37°C. Sheep anti-human chymotrypsin antibody (Bioscience International, Saco, ME) was incubated at 4°C overnight at a dilution of 1:20,000.

Results

Trp53 Loss Enhances Pancreatic Tumorigenesis

We previously showed that delivery of RCAS viruses encoding the mouse PyMT to elastase-*tv-a* transgenic mice initiates pancreatic tumorigenesis *in vivo*, and that germline deletion of the *Ink4a/Arf* locus cooperates with PyMT to induce both acinar and ductal tumors. However, we failed to observe grossly visible pancreatic tumors after RCAS-PyMT delivery to elastase-*tv-a*, *Trp53*-null mice because of the early demise of these animals attributable to lymphomas and sarcomas. To circumvent this problem, we crossed elastase-*tv-a* mice to mice bearing conditional mutant (floxed) alleles at the *Trp53* locus, and to mice expressing the cre recombinase from the *Ptf1a* locus.^{14,15} Analysis of genomic DNA isolated from the pancreata of *Trp53* flox, *Ptf1a*-cre mice demonstrated complete recombination of the *Trp53* locus (data not shown).

Table 1. Tumor Development in Eight-Month-Old Elastase-*tv-a* Mice Injected with RCAS-*PyMT*

Mouse ID	Trp53 status	Tumor histology	Metastasis
MM235.2	WT	Squamous metaplasia	None
MM235.3	WT	No pathology	None
MM236.1	WT	Squamous metaplasia	None
MM237.1	WT	Squamous metaplasia	None
MM237.2	WT	No pathology	None
MM237.3	WT	Squamous metaplasia, PanIN	None
MM238.1	WT	Squamous metaplasia, PanIN	None
MM239.1	WT	No pathology	None
MM239.3	WT	No pathology*	None
MM240.3	WT	Acinar carcinoma, PanIN	None
MM235.1	Null	Acinar carcinoma	None
MM236.2	Null	Acinar carcinoma	Diaphragm
MM236.3	Null	Acinar carcinoma w/ductal metaplasia	Liver
MM238.2	Null	Acinar carcinoma, ductal carcinoma	Diaphragm
MM238.3	Null	Acinar carcinoma, ductal carcinoma	None
MM238.4	Null	Ductal carcinoma	None
MM239.2	Null	Acinar carcinoma	None
MM240.2	Null	Acinar, ductal, sarcomatoid carcinomas	Liver, lung

All mice are elastase-*tv-a*, *Trp53* flox/flox. Pancreas *Trp53* status is determined by the presence (*Trp53*-null) or absence (*Trp53* WT) of the *Ptf1a-cre* allele.

*Animal presented with pancreatitis.

To determine whether *PyMT* and *p53* deficiency cooperate in pancreatic tumorigenesis in our mouse model, we delivered DF1 fibroblasts producing RCAS-*PyMT* to 2- or 3-day-old elastase-*tv-a*, *Trp53* flox litters containing cre-positive mice (resulting in pancreas-specific deletion of *Trp53*; hereafter referred to as *p53*-deficient) and cre-negative (*p53* wild type) littermates. Animals were sacrificed at 6 and 8 months of age, and their pancreata harvested and analyzed by histopathology. TVA-producing *p53* wild-type mice did not display any pancreatic pathology at 6 months of age. However, by 8 months of age 6 of 10 mice had pancreatic lesions, identified on sectioning of their resected pancreata (Table 1). All six of these animals had acinar lesions, some of which displayed histological features of immature squamous metaplasia (Figure 1A). The acinar character of the lesions was demonstrated by the expression of the acinar cell marker chymotrypsin. Interestingly, as was the case with *PyMT*-induced tumors in animals lacking *Ink4a/Arf*, the acinar lesions were also positive for the neuroendocrine marker synaptophysin, suggesting that the lesions might have originated from pancreas progenitor cells (Figure 1B). Consistent with this hypothesis, the acinar lesions were positive for Pdx1, a marker for pancreatic progenitor cells (Figure 1C).

In addition to the acinar lesions, three of the mice had pancreatic lesions with the histological features of pancreatic intraepithelial neoplasia (PanINs), the presumptive precursor lesions of pancreatic ductal adenocarcinoma (Figure 1, D and E). Importantly, these lesions

Figure 1. *PyMT*-induced lesions in elastase-*tv-a* mice. **A:** H&E-stained section of an acinar lesion displaying features of squamous metaplasia, with nests of small cells having minimal cytoplasm and spindle-shaped nuclei. Immunostaining of metaplastic lesions (labeled as L) with antibodies against synaptophysin (**B**) and Pdx1 (**C**). N denotes normal pancreas acinar cells. **D:** H&E-stained section of a PanIN lesion induced by *PyMT*. **E:** Higher magnification view illustrating columnar epithelium, loss of polarity, and micro-papillary structures. **F:** Alcian blue staining of a PanIN lesion demonstrating the accumulation of apical mucins in this lesion. **G:** Immunostaining for Pdx1 in a PanIN lesion. **H:** Identification of synaptophysin-positive cells (arrows) in a PanIN lesion by immunostaining.

appeared without any evidence for acinar to ductal metaplasia. The lesions displayed invaginated epithelia with papillae, and columnar cells with abundant cytoplasm rich in apical mucins as highlighted by Alcian blue staining (Figure 1F). In addition, there was moderate nuclear atypia, mitotic figures, and loss of polarity. The presence of these features classifies these lesions as mouse PanIN-1 and -2, as determined by the Penn working group.²⁰ Consistent with our previously published findings, the PanIN lesions were positive for Pdx1 and contained scattered synaptophysin-positive cells (Figure 1, G and H). Thus, *PyMT* induces early acinar and ductal lesions in the mouse pancreas.

In contrast to the precursor lesions identified in wild-type animals injected with RCAS-*PyMT*, all *Trp53*-null littermates displayed multiple grossly visible tumors as early as 6 months of age. The three *Trp53*-null animals examined at this time had acinar cell carcinomas with similar histological features to those observed in RCAS-

Figure 2. PyMT-induced carcinomas in p53-deficient pancreata. **A:** H&E-stained section of an acinar cell carcinoma. The acinar cell carcinomas stained positive for chymotrypsin (**B**), nuclear Pdx1 (**C**), and synaptophysin (**D**). **E:** H&E-stained tissue section of a ductal adenocarcinoma displaying desmoplasia. The ductal tumors stained positive for cytokeratin 19 (**F**) but were negative for chymotrypsin (**G**). **H:** H&E-stained tissue section of an undifferentiated carcinoma displaying spindle-cell (sarcomatoid) morphology.

PyMT infected, *Ink4a/Arf*-null pancreata (data not shown).⁷ As was observed for the acinar cell carcinomas induced in *Ink4a/Arf*-null mice, all acinar cell carcinomas were positive for Pdx1 and synaptophysin (data not shown). Further, all eight *Trp53*-null animals analyzed at 8 months of age had multiple pancreatic tumors, some of which exceeded 1 cm in diameter (Table 1). Histological examination of these pancreatic tumors demonstrated that most were moderately to poorly differentiated acinar cell carcinomas that stained positive for chymotrypsin, Pdx1, and synaptophysin, consistent with the findings observed at 6 months of age, as well as our previous findings in *Ink4a/Arf*-null animals (Figure 2, A–D).⁷

Interestingly, half of the tumor-bearing mice sacrificed at 8 months of age also had ductal adenocarcinomas with individual tubular glands, mucin-producing cells, and stromal desmoplasia, findings not observed in mice with germline deletion of the *Ink4a/Arf* locus (Figure 2E). The ductal character of these lesions was confirmed by immunoreactivity with antibodies against keratin 19, which is specifically present within duct epithelial cells, and the absence of chymotrypsin labeling (Figure 2, F

and G). In addition, we observed an undifferentiated carcinoma with sarcomatoid features in one animal (Figure 2H). Interestingly, although early acinar lesions were identified in some p53-deficient pancreata, PanIN lesions were not seen in any of these mice, potentially indicating the rapid progression of preinvasive lesions in these mice. Consistent with our previously published findings, animals injected with DF1 cells producing RCAS-*GFP* did not harbor any pancreatic lesions, demonstrating that p53 deficiency is not sufficient to initiate pancreatic tumorigenesis within this time frame (Table 1). Thus, *Trp53* loss cooperates with PyMT to induce pancreatic tumors with acinar and ductal characteristics.

Trp53 Deficiency Leads to Metastatic Disease

Our earlier studies with *Ink4a/Arf*-null mice failed to identify metastatic lesions in the lymph nodes, liver, or lungs, common sites of spread in pancreatic cancer patients, potentially because of the sacrifice of these animals between 6 and 7 months of age. However, in four of eight tumor-bearing p53-deficient mice sacrificed at 8 months of age, we observed metastasis to the liver, lungs, and/or diaphragm (Figure 3, A–C). The metastatic lesions had undifferentiated histology, and formed discrete nests in the distant tissue without any signs of invading further within these organs. Interestingly, although the metastatic lesions frequently maintained synaptophysin expression (Figure 3D), Pdx1 was localized in the cytoplasm (Figure 3, E and F), in contrast to the majority of primary tumors in which Pdx1 was localized to the nucleus (Figure 2C).

Pancreatic acinar and ductal epithelial cells display membrane localization of the adhesion molecule E-cadherin and its binding partner β -catenin (Figure 3, G and H). Loss of E-cadherin expression often correlates with tumor cell invasion and metastasis, and is frequently associated with the epithelial-to-mesenchymal transition, which is postulated to be involved in tumor metastasis.²¹ We therefore determined whether loss of E-cadherin expression and mislocalization of β -catenin occur in primary and metastatic lesions in our model. By immunostaining, we observed that both primary and metastatic lesions display reduced levels of membrane-associated E-cadherin and β -catenin, consistent with the loss of tight junctions that characterize epithelial cells (Figure 3, I–L). Importantly, there were no discernible differences between primary and metastatic lesions, or lesions present in mice without distant metastases.

Deletion of the Ink4a/Arf Locus Accelerates Tumorigenesis

The inactivation of the *Ink4a/Arf* locus is a common early event in pancreatic tumorigenesis.² We therefore determined whether the tumors induced in p53-deficient pancreata retained expression of the *Ink4a/Arf* gene products p16 and p19. We performed PCR on DNA extracted from p53-deficient tumors and confirmed that the locus was not deleted (data not shown). We additionally performed

Figure 3. Characterization of metastatic lesions. p53-deficient, PyMT-induced pancreatic tumors metastasize to the liver (metastasis denoted as T, normal liver as L) (A), the lungs (B), and diaphragm (C). Liver metastases (denoted as T) display cytoplasmic staining for synaptophysin (D) and Pdx1 (E). Normal liver tissue is denoted as L. Cytoplasmic Pdx1 is also observed in metastatic lesions to the diaphragm (F) and lungs. Normal pancreas parenchyma displays membrane localized staining for E-cadherin (G) and β -catenin (H). Primary tumors (I, J) and metastases (K, L) display loss of membrane-associated E-cadherin and β -catenin staining.

reverse transcriptase-polymerase chain reaction analysis for p16 and p19 on RNA extracted from several primary tumors. All analyzed tumor samples retained expression of these gene products, indicating that the locus was not silenced by promoter methylation as commonly occurs in human pancreatic ductal adenocarcinomas.² We further confirmed the expression of p16 by immunostaining of primary and metastatic tumors. p16 was detected in all lesions analyzed, although one primary tumor, and its associated lung metastasis, contained some p16-negative cells (Figure 4, A–C; and data not shown). Thus, inactivation of the *Ink4a/Arf* locus is not required for tumor metastasis.

We next asked whether the loss of a single *Ink4a/Arf* allele could cooperate with p53 deficiency in pancreatic tumor development in our mouse model. We crossed elastase-*tv-a*, *Trp53* flox, *Ptf1a-cre* mice to mice bearing floxed alleles at the *Ink4a/Arf* locus to generate *tv-a* transgenic animals with pancreas-specific deletion of *Trp53* and a single copy of the *Ink4a/Arf* locus. We found that delivery of RCAS-PyMT to these animals led to the rapid formation of pancreatic tumors in five of seven injected animals. Mice displayed distended abdomens and tumors exceeding 2 cm in diameter as early as 14 weeks of age, and all tumor-bearing mice had to be sacrificed by 18 weeks of age. All tumors were undifferentiated, and the tumor cells displayed sarcomatoid morphology with regions of ductal differentiation (Figure 4, D and E). Immunostaining confirmed the presence of p16 in the tumors, demonstrating retention of the wild-type *Ink4a/Arf* allele (Figure 4F). Injection of *Trp53*, *Ink4a/Arf* double-null mice with RCAS-GFP did not result in tumor formation

Figure 4. *Ink4a/Arf* deficiency accelerates tumor development. Expression of p16 is maintained in most primary tumors (A) and metastases (B) as determined by immunostaining. C: A sarcomatoid carcinoma with p16-negative cells (arrowheads). Concomitant deletion of *Trp53* and a single *Ink4a/Arf* allele induces undifferentiated carcinomas (D) with regions of ductal differentiation (E). F: Expression of p16 is maintained in these tumors as determined by immunostaining.

through 12 months. Thus, the *Ink4a/Arf* locus demonstrates a haploinsufficient phenotype in collaboration with p53 loss.

Discussion

Pancreatic cancer is a deadly disease with a median survival after diagnosis of 6 months. This poor prognosis is attributable in large part to extra-pancreatic metastases at the time of diagnosis, which renders patients ineligible for surgical resection, the best potential curative option.³ Thus, understanding the mechanisms by which pancreatic tumors metastasize to distant sites is of critical importance in combating this disease.

Mouse models are attractive systems for the analysis of tumor development and progression. We have previously described a mouse model in which the somatic and sporadic delivery of oncogenes induces the formation of pancreatic tumors. In our current study, we demonstrate that metastatic pancreatic cancers can be induced after the postnatal and sporadic induction of oncogene expression using this model system. Consistent with features of the human disease, the tumors spread most frequently to the liver, abdominal surface, and lungs.²² Notably, our tumors and metastatic lesions were induced after somatic and sporadic introduction of the PyMT oncogene. An important consequence of this strategy, absent in previous models, is that tumor progression and metastasis are not influenced by the expression of the oncogene in neighboring cells. This attribute makes it easier to identify cell autonomous factors that contribute to these processes, which is an important avenue of investigation in ongoing efforts to understand the mechanisms of tumor spread in pancreatic cancer. Thus, the findings described here demonstrate that metastatic pancreatic cancer can be induced after postnatal oncogene activation, and suggest that our model system may be useful for the elucidation of the molecular mechanisms that underlie the development and progression of pancreatic carcinomas.

The loss of the *Ink4a/Arf* locus is an early and common event in the genesis of human pancreatic ductal tumors. We were therefore interested in identifying whether loss of this locus is required for tumor development in our model. Using amplification of genomic DNA and mRNA, we found that the locus remains intact and that the p16 and p19 tumor suppressor gene products are expressed. Thus, loss of this locus is not required for pancreatic tumor formation. Further, we demonstrated by immunostaining that p16 expression is retained in metastatic cells, and therefore its loss is not required for metastatic disease. These findings are consistent with those of the Tuveson¹³ and Depinho²³ laboratories in other mouse models.

However, loss of the *Ink4a/Arf* locus is important in pancreatic tumorigenesis. Delivery of RCAS-PyMT to *tv-a* transgenic mice with pancreas-specific deletion of *Trp53* and a single *Ink4a/Arf* allele led to acceleration of pancreatic tumor formation, and conferred an undifferentiated histology on the resulting tumors. Importantly, the locus displays a haploinsufficient phenotype as the wild-

type locus is retained and expressed in the developed tumors. These findings are consistent with the early loss of the locus during pancreatic adenocarcinoma formation,² and our observation that the loss of this locus, but not the loss of *Trp53*, enhances the proliferation of primary pancreatic duct epithelial cells in culture (J.P.M and B.C.L., unpublished observations).

During immunohistochemical analysis of tumor specimens, we observed that the metastatic lesions were negative for nuclear localized Pdx1, whereas nuclear localized Pdx1 is a commonly observed feature in primary pancreatic lesions in mouse models.¹³ Instead, the metastatic lesions displayed cytoplasmic localization of this transcription factor. We also observed cytoplasmic localization of Pdx1 in some of the larger primary tumors, whereas precursor PanIN lesions and smaller primary tumors contain nuclear Pdx1. Together, these findings suggest that loss of Pdx1 nuclear function, or the gain of cytoplasmic Pdx1 function, may be important for pancreatic tumor progression. Alternatively, the cytoplasmic localization of Pdx1 may reflect the activation of signaling pathways, such as those regulated by c-jun N-terminal kinase (JNK), that regulate Pdx1 activity during pancreatic development and β -cell function.^{24,25} Future exploration of these issues may provide important information regarding pancreatic cancer metastasis.

The generation of multiple tumor types (acinar, ductal) in our model, and the expression of progenitor markers (Pdx1) and markers for multiple lineages (chymotrypsin, synaptophysin) raise the issue of the cell of origin. In our model, expression of *tv-a* is controlled by the elastase promoter, which is believed to be acinar-specific.²⁶ However, the presence of ductal tumors, in the absence of any evidence for metaplasia, suggests that the tumors may be derived from multipotential progenitors, consistent with our previously published data.⁷ The identity of this progenitor remains unknown, although the hypothesis that the *elastase-tv-a* transgene may be expressed in progenitors is consistent with published findings by Chiang and Melton²⁷ that indicated that mRNAs for lineage-specific genes are detected in individual cells simultaneously with mRNAs associated with early pancreas development. Recent studies have suggested that centroacinar cells or nestin-positive cells may act as progenitor cells in the pancreas and may be target cells for transformation in pancreatic tumorigenesis.^{28,29} Whether either, or both, of these cell types are the cell of origin in our model is a provocative idea that remains to be elucidated.

Acknowledgments

This work was initiated in the laboratory of Harold Varbus, and we thank him for his support and guidance, and we thank Michael Twarog for technical assistance, Paul Krimpenfort and Anton Berns for the *Trp53* and *Ink4a/Arf* conditional mutant mouse strains, Chris Wright for the *Ptf1a-cre* mouse line, Kirsten A. Hubbard for critical re-

view of the manuscript, and members of the Lewis Lab for helpful discussions.

References

- Jemal A, Siegel R, Ward E, Murray T, Xu J, Smigal C, Thun MJ: Cancer statistics, 2006. *CA Cancer J Clin* 2006, 56:106–130
- Hezel AF, Kimmelman AC, Stanger BZ, Bardeesy N, Depinho RA: Genetics and biology of pancreatic ductal adenocarcinoma. *Genes Dev* 2006, 20:1218–1249
- Warshaw AL, Fernandez-del Castillo C: Pancreatic carcinoma. *N Engl J Med* 1992, 326:455–465
- Hruban RH, Rustgi AK, Brentnall TA, Tempero MA, Wright CV, Tuveson DA: Pancreatic cancer in mice and man: the Penn Workshop 2004. *Cancer Res* 2006, 66:14–17
- Leach SD: Mouse models of pancreatic cancer: the fur is finally flying! *Cancer Cell* 2004, 5:7–11
- Quaife CJ, Pinkert CA, Ornitz DM, Palmiter RD, Brinster RL: Pancreatic neoplasia induced by ras expression in acinar cells of transgenic mice. *Cell* 1987, 48:1023–1034
- Lewis BC, Klimstra DS, Varmus HE: The c-myc and PyMT oncogenes induce different tumor types in a somatic mouse model for pancreatic cancer. *Genes Dev* 2003, 17:3127–3138
- Orsulic S: An RCAS-TVA-based approach to designer mouse models. *Mamm Genome* 2002, 13:543–547
- Donehower LA, Harvey M, Slagle BL, McArthur MJ, Montgomery CA Jr, Butel JS, Bradley A: Mice deficient for p53 are developmentally normal but susceptible to spontaneous tumours. *Nature* 1992, 356:215–221
- Jacks T, Remington L, Williams BO, Schmitt EM, Halachmi S, Bronson RT, Weinberg RA: Tumor spectrum analysis in p53-mutant mice. *Curr Biol* 1994, 4:1–7
- Serrano M, Lee H, Chin L, Cordon-Cardo C, Beach D, DePinho RA: Role of the INK4a locus in tumor suppression and cell mortality. *Cell* 1996, 85:27–37
- Aguirre AJ, Bardeesy N, Sinha M, Lopez L, Tuveson DA, Horner J, Redston MS, DePinho RA: Activated Kras and Ink4a/Arf deficiency cooperate to produce metastatic pancreatic ductal adenocarcinoma. *Genes Dev* 2003, 17:3112–3126
- Hingorani SR, Wang L, Multani AS, Combs C, Deramaudt TB, Hruban RH, Rustgi AK, Chang S, Tuveson DA: Trp53R172H and KrasG12D cooperate to promote chromosomal instability and widely metastatic pancreatic ductal adenocarcinoma in mice. *Cancer Cell* 2005, 7:469–483
- Jonkers J, Meuwissen R, van der Gulden H, Peterse H, van der Valk M, Berns A: Synergistic tumor suppressor activity of BRCA2 and p53 in a conditional mouse model for breast cancer. *Nat Genet* 2001, 29:418–425
- Kawaguchi Y, Cooper B, Gannon M, Ray M, MacDonald RJ, Wright CV: The role of the transcriptional regulator Ptf1a in converting intestinal to pancreatic progenitors. *Nat Genet* 2002, 32:128–134
- Krimpenfort P, Quon KC, Mooi WJ, Loonstra A, Berns A: Loss of p16Ink4a confers susceptibility to metastatic melanoma in mice. *Nature* 2001, 413:83–86
- Holland EC, Li Y, Celestino J, Dai C, Schaefer L, Sawaya RA, Fuller GN: Astrocytes give rise to oligodendrogliomas and astrocytomas after gene transfer of polyoma virus middle T antigen in vivo. *Am J Pathol* 2000, 157:1031–1037
- Himly M, Foster DN, Bottoli I, Iacovoni JS, Vogt PK: The DF-1 chicken fibroblast cell line: transformation induced by diverse oncogenes and cell death resulting from infection by avian leukosis viruses. *Virology* 1998, 248:295–304
- Schaefer-Klein J, Givol I, Barsov EV, Whitcomb JM, VanBrocklin M, Foster DN, Federspiel MJ, Hughes SH: The EV-O-derived cell line DF-1 supports the efficient replication of avian leukosis-sarcoma viruses and vectors. *Virology* 1998, 248:305–311
- Hruban RH, Adsay NV, Albores-Saavedra J, Anver MR, Biankin AV, Boivin GP, Furth EE, Furukawa T, Klein A, Klimstra DS, Kloppel G, Lauwers GY, Longnecker DS, Luttges J, Maitra A, Offerhaus GJ, Perez-Gallego L, Redston M, Tuveson DA: Pathology of genetically engineered mouse models of pancreatic exocrine cancer: consensus report and recommendations. *Cancer Res* 2006, 66:95–106
- Thiery JP: Epithelial-mesenchymal transitions in tumour progression. *Nat Rev Cancer* 2002, 2:442–454
- Li D, Xie K, Wolff R, Abbruzzese JL: Pancreatic cancer. *Lancet* 2004, 363:1049–1057
- Bardeesy N, Aguirre AJ, Chu GC, Cheng KH, Lopez LV, Hezel AF, Feng B, Brennan C, Weissleder R, Mahmood U, Hanahan D, Redston MS, Chin L, Depinho RA: Both p16(Ink4a) and the p19(Arf)-p53 pathway constrain progression of pancreatic adenocarcinoma in the mouse. *Proc Natl Acad Sci USA* 2006, 103:5947–5952
- Kaneto H, Xu G, Fujii N, Kim S, Bonner-Weir S, Weir GC: Involvement of c-Jun N-terminal kinase in oxidative stress-mediated suppression of insulin gene expression. *J Biol Chem* 2002, 277:30010–30018
- Kawamori D, Kajimoto Y, Kaneto H, Umayahara Y, Fujitani Y, Miyatsuka T, Watada H, Leibiger IB, Yamasaki Y, Hori M: Oxidative stress induces nucleo-cytoplasmic translocation of pancreatic transcription factor PDX-1 through activation of c-Jun NH(2)-terminal kinase. *Diabetes* 2003, 52:2896–2904
- Ornitz DM, Palmiter RD, Hammer RE, Brinster RL, Swift GH, MacDonald RJ: Specific expression of an elastase-human growth hormone fusion gene in pancreatic acinar cells of transgenic mice. *Nature* 1985, 313:600–602
- Chiang MK, Melton DA: Single-cell transcript analysis of pancreas development. *Dev Cell* 2003, 4:383–393
- Carriere C, Seeley ES, Goetze T, Longnecker DS, Korc M: The Nestin progenitor lineage is the compartment of origin for pancreatic intraepithelial neoplasia. *Proc Natl Acad Sci USA* 2007, 104:4437–4442
- Stanger BZ, Stiles B, Lauwers GY, Bardeesy N, Mendoza M, Wang Y, Greenwood A, Cheng KH, McLaughlin M, Brown D, Depinho RA, Wu H, Melton DA, Dor Y: Pten constrains centroacinar cell expansion and malignant transformation in the pancreas. *Cancer Cell* 2005, 8:185–195