Deploying Mouse Models of Pancreatic Cancer for Chemoprevention Studies

Paul J. Grippo and David A. Tuveson

Abstract

With the advent of mouse models that recapitulate the cellular and molecular pathology of pancreatic neoplasia and cancer, it is now feasible to recruit and deploy these models for the evaluation of various chemopreventive and/or anticancer regimens. The highly lethal nature of pancreatic ductal adenocarcinoma (PDAC) makes multiple areas of research a priority, including assessment of compounds that prevent or suppress the development of early lesions that can transform into PDAC. Currently, there are over a dozen models available, which range from homogeneous preneoplastic lesions with remarkable similarity to human pancreatic intraepithelial neoplasms to models with a more heterogeneous population of lesions including cystic papillary and mucinous lesions. The molecular features of these models may also vary in a manner comparable with the differences observed in lesion morphology, and so, navigating the route of model selection is not trivial. Yet, arming the community of cancer investigators with a repertoire of models and the guidance to select relevant models that fit their research themes promises to produce findings that will have clinical relevance. Cancer Prev Res; 3(11): 1382–7. ©2010 AACR.

Introduction

Pancreatic cancer develops insidiously, recurs quickly following surgical resection, and metastasizes widely, resulting in nearly uniform lethality. Although grim, these clinical characteristics nonetheless represent several opportunities to interrupt disease progression and improve the outcome for pancreatic cancer patients. Indeed, increased surveillance of individuals with a strong family history of pancreatic cancer has prompted the development of endoscopic, pathologic, and radiological methods that allow for highly beneficial prophylactic surgery (1). Furthermore, the limited but measurable benefit of adjuvant chemotherapy supports the premise that systemic treatments can also affect pancreatic cancer progression (2, 3); the challenge, though, is to identify the most effective systemic approaches for different stages of disease. The recent advent of genetically engineered mice that accurately develop and advanced forms of the most common type of pancreatic cancer, pancreatic ductal adenocarcinoma (PDAC), may provide preclinical model systems to address these issues.

Mouse Models of Pancreatic Cancer for Potential Chemoprevention Studies

Persistent research has culminated in the generation of genetically engineered mouse models that represent different stages of human PDAC. These models are now available to investigate the basic and translational aspects of this malignancy (4, 5). Models of neoplasms such as murine pancreatic intraepithelial neoplasia (mPanIN), intraductal papillary mucinous neoplasia (mIPMN), and mucinous cystic neoplasia (mMCN) have all been described (although mIPMN and mMCN should be further characterized for a more complete validation). These genetically engineered mouse models may be appropriate for assessing the role of genes, environmental conditions such as tobacco exposure and diet, comorbidities including pancreatitis, and the influence of immunologic and pharmacologic interventions on the development of invasive PDAC, as highlighted in Table 1. Models of localized PDAC have also been reported and could be used for neoadjuvant, adjuvant, and anti-metastatic approaches to prevent relapse and dissemination. Most of the genetically engineered models include Pdx1-Cre/Lox-Stop-Lox (LSL)-Kras or p48
textsuperscriptCr/LSL-Kras mice, which were further modified by conditional deletion or mutation of the p16/ 19 (these mice also develop sarcomatoid lesions; ref. 6), p53 (7), smad4 (8, 9), or transforming growth factor β receptor II (TGFbRII) loci (10). The results of these combined genotypes were a multiplicity of preinvasive lesions of all grades, invasive adenocarcinoma, and metastasis to other organs, ultimately leading to significantly reduced median survival. The most robust murine models of neoplasms (earlier-stage lesions, as opposed to later-stage...
<table>
<thead>
<tr>
<th>GEM</th>
<th>Time of express</th>
<th>Preneoplastic/neoplastic lesions</th>
<th>Mesenchymal</th>
<th>Parenchymal</th>
<th>Cancer phenotype</th>
<th>Survival (mo)</th>
<th>Caveats to consider</th>
<th>Potential uses</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>EL-Kras</td>
<td>E.14</td>
<td>+/−</td>
<td>+</td>
<td>+</td>
<td>CPN</td>
<td>+</td>
<td>No cancer</td>
<td>No cancer</td>
<td>18+</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>No cancer driven off EL promoter</td>
<td>(70)</td>
</tr>
<tr>
<td>Pdx1-Cre LSL-Kras</td>
<td>E8.5</td>
<td>++</td>
<td>++</td>
<td>+++</td>
<td>PDAC</td>
<td>6+</td>
<td>Yes</td>
<td>Express in GI tract; oral and genital papillomas</td>
<td>(22)</td>
</tr>
<tr>
<td>p48Cre LSL-Kras</td>
<td>E9.5</td>
<td>++</td>
<td>++</td>
<td>+++</td>
<td>PDAC</td>
<td>8+</td>
<td>Yes</td>
<td>Evaluate effect on PanIN lesions</td>
<td>(22)</td>
</tr>
<tr>
<td>+Mist1Kras</td>
<td>E10.5+ in adults</td>
<td>+/−</td>
<td>+/−</td>
<td>CPN</td>
<td>Mixed histo</td>
<td>3</td>
<td>Yes</td>
<td>Evaluate effect on PanIN lesions</td>
<td>(71)</td>
</tr>
<tr>
<td>Nest-Cre LSL-Kras</td>
<td>E10.5</td>
<td>++ 1 and 2 (-100%)</td>
<td>+/−</td>
<td>+++</td>
<td>No cancer</td>
<td>No</td>
<td>No</td>
<td>Expression in brain</td>
<td>(72)</td>
</tr>
<tr>
<td>EL-rTA TRE-Cre LSL-Kras</td>
<td>E.16.5</td>
<td>3 mo</td>
<td>++</td>
<td>+++</td>
<td>PDAC</td>
<td>12</td>
<td>Yes</td>
<td>Kras mt G12V</td>
<td>(13)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Chemoprevention before express</td>
<td></td>
</tr>
<tr>
<td>P60</td>
<td></td>
<td>None</td>
<td></td>
<td></td>
<td>PDAC</td>
<td>No cancer</td>
<td>No</td>
<td>Term at 6 months</td>
<td></td>
</tr>
<tr>
<td>KS-COX-2</td>
<td>E13.5</td>
<td>+/−</td>
<td>CN</td>
<td>+++</td>
<td>Near PDAC</td>
<td>No</td>
<td>No</td>
<td>10-week = CP; bladder abnormal</td>
<td>(24)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Ability to inhibit COX-2</td>
<td></td>
</tr>
<tr>
<td>Pdx1-CreERT LSL-Kras</td>
<td>E.10.5</td>
<td>2-4 mo</td>
<td>++</td>
<td>+++</td>
<td>Study terminated at 4 mo</td>
<td></td>
<td></td>
<td>Addition of R26; increase number and severity of mPanINs</td>
<td>(11)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Chemoprevention before express</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Targeting of liver cells</td>
<td>(14)</td>
</tr>
<tr>
<td>EL-CreERT LSL-Kras</td>
<td>P42</td>
<td>1-30%</td>
<td>++</td>
<td>++</td>
<td>No cancer</td>
<td>No</td>
<td>No</td>
<td>Chemoprevention before express</td>
<td>(11,14)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Targeting of liver cells</td>
<td></td>
</tr>
<tr>
<td>Mist1CreERT LSL-Kras</td>
<td>P42</td>
<td>2 mo</td>
<td>++</td>
<td></td>
<td>No cancer</td>
<td>No</td>
<td>No</td>
<td>Chemoprevention before express</td>
<td>(14)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(like EL target)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Chemoprevention before express</td>
<td></td>
</tr>
<tr>
<td>EL-CreERT cLGL-Kras</td>
<td>E16+</td>
<td>0-2 = 1.2</td>
<td>++</td>
<td>+++</td>
<td>CPC PDAC</td>
<td>Yes</td>
<td>Yes</td>
<td>Cre expression without tamoxifen</td>
<td>(15)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Chemoprevention before express</td>
<td></td>
</tr>
<tr>
<td>pCPA1CreERT iLSL-Kras</td>
<td>P14,21,24,07,56</td>
<td>1-10%</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>No cancer</td>
<td>No</td>
<td>Addition of cerulein increase number and severity of mPanINs</td>
<td>(12)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Chemoprevention before express</td>
<td></td>
</tr>
<tr>
<td>RipCreERT iLSL-Kras</td>
<td>P14,21,24,07,56</td>
<td>None</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>No cancer</td>
<td>No</td>
<td>Up to 8 months</td>
<td>(12)</td>
</tr>
</tbody>
</table>

Table 1. Phenotypic comparisons

NOTE: Similarities and differences among genetically engineered mouse models of pancreatic neoplasia for chemoprevention studies. Abbreviations: ADM, acinar-ductal metaplasia; AH, acinar hyperplasia; CN, cystic neoplasms; CP, chronic pancreatitis; CPC, cystic papillary carcinoma; CPN, cystic papillary neoplasms; EL-rTA, EL tetracycline transactivator; Fibros, fibrosis; GEM, genetically engineered mouse; histo, histology; Inflam, inflammation; Inv, invasive; Met, metastatic; Mist1Cre, knock-in of Cre upstream of the Mist1 coding region; Mist1Kras, knock-in of mutant Kras upstream of the Mist 1 coding region; NR, not reported; p48Cre, knock-in of Cre upstream of the p48/Ptfa coding region; pCPA1CreERT, procarboxypeptidase A1–responsive CreERT; RipCreERT, rat insulin promoter–responsive CreERT; TRE-Cre, tetracycline-responsive Cre.
lesions such as PanIN3) display complete penetrance (all mice with a gene mutation have phenotypic manifestation of that disease) and express an endogenous or transgenic oncogenic Kras allele in pancreatic exocrine and/or progenitor cells. When combined with various tumor suppressor mutations, these models oftentimes yield invasive and metastatic PDAC and related epithelial histologies (see Table 2 in ref. 5). Models using inducible alleles of Cre recombinase, such as estrogen receptor–Cre fusion genes (CreER or CreERT) and tetracycline-responsive Cre expression alleles (TRE-Cre), are capable of being temporally controlled and thus initiated selectively in adult pancreata, better reflecting the somatic acquisition of genetic mutations thought to occur in humans (11–15).

Experimental Design/Strategies and Criteria for Evaluating Interventional Effects

The following pertinent parameters are included among those to consider for pancreatic cancer chemoprevention studies: (a) the optimal in vivo model system for the prevention of invasive cancer or metastasis; (b) criteria for assessing a significant response; and (c) targets of chemoprevention including the relevant molecular pathways, cell types, and environmental conditions promoting the progression of PanIN to PDAC.

Considerations for model selection

Murine models of preneoplasms that progress to invasive PDAC and murine models of focal PDAC that progresses to metastatic disease allow distinct questions to be addressed for cancer prevention and therapy studies (see Table 4.1 in ref. 16). From the perspective of chemoprevention research, perhaps the most critical feature is activation of the target pathway, where the molecular profile of the model is identified before the preclinical trial. Timing of delivery may also be a key factor because late administration in models that progress to PDAC may yield only a modest, if any, response. The onset, penetrance, frequency, and latency of progression of these various models should be considered during the design of prevention studies. Some models have a very homogeneous population of neoplastic lesions that may create an ideal environment for studying a single species of lesions. Other models have a variety of lesions that may offer a broader platform for evaluation. Furthermore, the type of preneoplasms (e.g., mPanIN, mIPMN, and mMCN) that develop should be considered. These parameters will define the number of mice necessary to achieve adequate statistical power for the analysis, the length of the study, the cellular response, and an indication of the potential mechanisms involved. At times, it may be important to consider the background strain of the mice, the presence of cell surface antigens, and the source and type of oncogene mutation, particularly as it relates to immunologic studies. Other types of lesions and/or abnormalities in the parenchymal and mesenchymal compartment may also play a role, albeit smaller than that of the previously mentioned parameters, in choosing ideal models for preclinical chemoprevention trials and evaluations of the interplay between epithelial and stromal components.

Parameters for assessing response to treatment

A variety of approaches is often used to measure the efficacy of interventions. Direct evaluation of the neoplastic tissue, where onset, incidence, frequency, size, and proliferative/apoptotic indices are assessed, is normally the first point of analysis. This evaluation is often accompanied by molecular evaluations of certain cellular markers and/or factors involved in signaling pathways, especially targets of the chemopreventive agent under assessment. More complex evaluations can include surrogate biomarker investigations in the blood and radiological assessments of cellular/tissue response via small-animal imaging including high-resolution ultrasound and magnetic resonance (17). Such approaches will optimize meaningful analyses of response when done in tandem.

Targets for chemoprevention

Inflammation. A pivotal question about cancer prevention study—for which mouse models are ideally suited—is whether the target is contained in the preneoplastic cells, the microenvironment, or both. This is particularly germane in pancreatic cancer because pancreatitis, which causes both the death of acinar cells and a reactive stromal fibrosis, promotes the development of PDAC in patients carrying the PRSS1 allele (18) and in mice treated with the secretagogue cerulein (12, 13, 19–21). Thus far, chemoprevention that suppresses inflammation has been somewhat limited, with a primary focus on elevated levels of cyclooxygenase-2 (COX-2) in mPanIN cells in Pdx1-Cre/LSL-KrasG12D mice (22). Treatment with the nonsteroidal anti-inflammatory drug (NSAID) nimesulide inhibited COX-2 and led to reduced mPanINs, particularly later-stage lesions (23). Similar results were observed in K5-COX-2 transgenic mice (24). Furthermore, a successful preclinical trial of the selective COX-2–inhibiting NSAID celecoxib, a MUC1 peptide, and gemcitabine led to a complete lack of development of invasive disease and significant suppression of mPanIN2 and mPanIN3 in p48Tg/LSL-Kras/ MUC1 mice, supporting COX-2 and MUC1 as cancer-chemopreventive targets in the mouse pancreas (25). Complementary genetic approaches to determine whether the elastase-driven (EL)–PRSS1 allele (19, 21) cooperates with oncogenic Kras in PanIN/PDAC progression, and conversely whether the conditional deletion of COX-2 (26) in pancreatic cells or surrounding stroma inhibits PanIN/PDAC formation, are technically feasible but hitherto unreported.

The NF-κB pathway, a central mediator of inflammatory signaling in neoplastic and microenvironment cells, has been implicated in pancreatic cancer biology (27). Treatment with aspirin as a surrogate pharmacologic inhibitor of the NF-κB pathway inhibited orthotopic tumor formation in mice (28, 29). Therefore, more precise inhibition of NF-κB signaling with conditional knockout alleles or chemical inhibitors of IκB kinases is a logical next step.
to confirm the relevance of this pathway in PanIN/PDAC. Finally, 5-lipoxygenase (5-LOX) may also be a target akin to COX-2, as it is present in pancreatic preneoplasms (30). Indeed, 5-LOX inhibition can suppress proliferation (31) and induce apoptosis (32) in cell culture, and augment the efficacy of gemcitabine in vivo (33).

**Diet/environment.** Obesity, fat and sugar intake, and tobacco exposure have all been implicated in PDAC development and should be evaluated in these neoplastic models. The size and metastatic spread of transplanted Pan02-derived cancers were enhanced in obese Lep(Ob) and Lep(db/db) mice, leading to a significant reduction in survival compared with wild-type mice (34, 35). These findings imply that the obese state may establish an environment conducive to cancer cell proliferation and dissemination, which may also hold true for preneoplastic and neoplastic lesions of the pancreas. Another study maintained p48Cre/LSL-Kras mice on a high-fat diet for up to 10 weeks and found greatly increased mPanIN formation that was associated with tumor necrosis factor receptor 1–dependent inflammation (36). A similar approach was used in the related EL-Kras model, where a diet rich in α-3 fatty acid significantly reduced the incidence and frequency of pancreatic neoplasms (37). Additional reports propose that high caloric consumption (e.g., high-fructose corn syrup) and/or an increase in glycemic load can have a different means of promoting pancreatic cancer growth (38–40), although not without controversy (41). New avenues of research need to determine the molecular and signaling pathways that are affected by these dietary components (particularly fatty acids and sugars) and may serve as chemoprevention targets. Finally, rather than merely denoting a diseased organ, new onset diabetes may also play a role in stimulating PDAC in patients (42).

An environmental factor that markedly increases the incidence of PDAC is cigarette smoking. In rodents, cigarette smoke and its constituents cause chronic pancreatic inflammation and exocrine damage (43), serving as a precursor of pancreatitis and pancreatic cancer. The contribution of cigarette carcinogens to the development of pancreatic preneoplasms and cancer has been highlighted in vivo, including in EL-IL-1β mice (44) and in a 7,12-dimethylbenz(a)anthracene–induced mouse model, where nicotine provided robust progression of mPanIN to PDAC (45). Identifying the specific molecular and cellular effects of these carcinogens and their accompanying genetic lesions with respect to pancreatic cancer development has not been fully addressed in vivo.

**Developmental pathways.** Developmental pathways may also promote pancreatic cancer and thereby serve as targets for chemoprevention. Indeed, the coexpression of an active Notch allele (Rosa26NIC; ref. 11) cooperated with KrasG12D to promote PanIN formation; conversely, the attenuation of the Notch pathway with a γ-secretase inhibitor mitigated mPanIN formation and PDAC formation in Kras; p53flac/+ mice (46). Also, deficiency of notch-2 prevents the development of mPanINs in p48Cre/LSL-Kras mice (47). Despite evidence that Notch is oncogenic, conditional loss of Notch in the pancreas of Pdx1-Cre/LSL-Kras mice showed that Notch may act as a tumor suppressor gene (48), which might be related to the context and/or timing of Notch expression. Likewise, TGFβ signaling can have a similar dichotomous nature by serving tumor-promoting and tumor-suppressing functions (49, 50), which also may be dependent on context and/or timing. Loss of either smad4 or Tgfb2 in p48Cre or Pdx1-Cre/LSL-Kras mice led to more aggressive disease (8–10), yet EL-Kras mice with haploinsufficient Tgfb1 generated reduced incidence and frequency but greater size of preinvasive lesions (51). Therefore, inhibition of the Notch or TGFβ signaling pathway needs to be approached cautiously with regard to the cell type and disease stage being targeted.

The hedgehog (Hh) pathway effector Gli2 cooperates with KrasG12D in a cell-autonomous fashion to promote PanIN/PDAC (52), and Hh pathway inhibitors alone (53) or in combination with gemcitabine (54) were shown to increase survival and decrease metastasis in two models of advanced PDAC. The deletion of Smoothered, however, disrupts the recognition of extracellular Hh ligands without affecting PanIN/PDAC formation in mouse models, showing that Gli signaling is ligand independent in PanIN/PDAC, with a direct influence on the desmoplastic stroma (55). Terpenoids may also serve to inhibit the Hh (specifically sonic Hh) pathway (56). A more recent approach using triterpenoids and rexinoids alone and in combination showed strong efficacy, leading to greatly improved survival in Kras; p53flac/+ mice, as reported elsewhere in this issue of the journal (57). The potential of a similar approach in Kras cohorts with wild-type p53 yielding a potent chemopreventive effect against mPanIN formation would seem reasonable.

Receptor tyrosine kinase pathways known to be relevant for normal development have also been implicated as potential targets in PanIN/PDAC, with the epidermal growth factor receptor (EGFR) inhibitor gefitinib causing reduced incidence of mPanIN1 and mPanIN2 as well as suppressing progression to invasive disease in p48Cre/LSL-Kras mice, as reported elsewhere in this issue of the journal (58). The receptor d’origine nantais (RON) receptor also seems to play several roles, particularly in motility and invasiveness (59) and vascular endothelial growth factor production in pancreatic cancer cells (60), showing itself to be a target of inhibition before cancer dissemination. Recent findings indicate that RON signaling mediates cell survival and gemcitabine resistance in a human pancreatic cancer–derived xenograft system, where short hairpin RNA–induced suppression eventually led to compensatory mechanisms via the c-met and EGFR cascades (61).

**Future targets.** Potential avenues of pursuit should be based on previous data collected from cell culture and/or xenograft models, which show significant efficacy at various targets. As mentioned above, Hh pathway inhibition altered the stromal composition and increased chemotherapy delivery and response (54). Therefore, individual and combinatorial approaches that alter other
components of the pancreatic tumor microenvironment should also be considered as preventive strategies, such as those targeting cancer-associated fibroblasts (62), myeloid-derived suppressor cells, and T cells (63).

Food-derived polyphenols, such as quercetin and trans-resveratrol, have exhibited notable proapoptotic effects on pancreatic cancer cells in vitro and implanted in nude mice (64). Perillyl alcohol (65) has significant chemopreventive effects on cultured pancreatic cancer cells and, when coupled with adenovirus-mediated mdr-1/interleukin-24 gene therapy in vivo, leads to nearly complete loss of human pancreatic cancer following xenograft implants in nude mice (66, 67). Expression of chemokine (C-X-C motif) ligand 12 (CXCL12) and CXCR receptor 4 (CXCR4) is higher in PanIN3 (compared with normal ducts) in both humans and mice, and a dose-dependent increase in cell proliferation of mPanIN cells was observed in mice treated with CXCL12 (68). Both chemokines were partially dependent on mitogen-activated protein kinase signaling. Indeed, a host of other targets are currently being delineated for potential use in these genetically modified mice (69).

Conclusions

The stage is set to deploy various in vivo models of pancreatic neoplasia for evaluation of multiple chemoprevention strategies against a host of target types including inflammatory, epigenetic, and developmental targets. Both model and target should maintain some aspects of etiology observed in human pancreatic cancer. The challenge is to expand findings from culture and/or xenograft/orthotopic systems into genetically modified models while preparing to translate these results for future clinical investigation (73).

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Received 09/27/2010; accepted 09/27/2010; published OnlineFirst 11/02/2010.

References


www.aacajournals.org
Cancer Prev Res; 3(11) November 2010

1387

Downloaded from cancerpreventionresearch.aacajournals.org on June 20, 2017. © 2010 American Association for Cancer Research.
Deploying Mouse Models of Pancreatic Cancer for Chemoprevention Studies

Paul J. Grippo and David A. Tuveson


Updated version Access the most recent version of this article at:
doi:10.1158/1940-6207.CAPR-10-0258

Cited articles This article cites 69 articles, 31 of which you can access for free at:
http://cancerpreventionresearch.aacrjournals.org/content/3/11/1382.full.html#ref-list-1

Citing articles This article has been cited by 5 HighWire-hosted articles. Access the articles at:
/content/3/11/1382.full.html#related-urls

E-mail alerts Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.