Animal Models of Gastrointestinal and Liver Diseases. The difficulty of animal modeling of pancreatic cancer for preclinical evaluation of therapeutics

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Submitted 28 May 2015; accepted in final form 25 June 2015

Logsdon CD, Arumugam T, Ramachandran V. Animal Models of Gastrointestinal and Liver Diseases. The difficulty of animal modeling of pancreatic cancer for preclinical evaluation of therapeutics. Am J Physiol Gastrointest Liver Physiol 309: G283–G291, 2015. First published July 9, 2015; doi:10.1152/ajpgi.00169.2015.—Pancreatic ductal adenocarcinoma (PDAC) is relatively rare but extremely lethal. Standard cytotoxic therapeutics provide little benefit. To date, newer targeted therapeutics have also not been highly successful. Often novel therapeutics that have appeared to perform well in preclinical models have failed in the clinic. Many factors contribute to these failures, but the one most often attributed is the shortcomings of the preclinical models. A plethora of animal models now exist for PDAC, including cell line xenografts, patient-derived xenografts, a wide variety of genetic mouse models, and syngeneic xenografts. These models have generated a tremendous amount of information useful for the understanding of PDAC. Yet none seems to well predict clinical outcomes of new treatments. This review will discuss how genetic instability and cellular heterogeneity make this disease so difficult to model accurately. We will also discuss the strengths and weaknesses of many of the popular models. Ultimately we will argue that there is no perfect model and that the best approach to understanding clinical performance is the use of multiple preclinical models with an understanding of their salient features.

Pancreatic ductal adenocarcinoma; genetic mouse models; xenografts

Currently a wide assortment of animal models exists for pancreatic cancer, including xenografts derived from available cell lines or patient-derived tumor tissue, many different genetic mouse models, and mouse syngeneic xenografts. Each of these models has contributed greatly to our understanding of the initiation, development, and metastatic spread of PDAC. However, as of yet, no models have accurately predicted clinical outcomes of treatments. This suggests that each of the existing models lacks one or more critical attributes of the disease. The shortcomings of established models are often emphasized by those promoting new models. However, until a model is developed that can successfully predict clinical outcomes, it remains unclear which parameters are the most important.

This review focuses on the specific difficulties of modeling PDAC for preclinical evaluation of therapeutics. To that end, we discuss properties of human PDAC that are likely to be central to the disease and that are highly difficult to reproduce in animal models. Our discussion argues that, because there is no “perfect” model, the best approach is to use several different models with an understanding of their strengths and weaknesses. These strengths and weaknesses are discussed for several of the most popular models.

CRITICAL PARAMETERS THAT ARE DIFFICULT TO MODEL

Cancer Heterogeneity

A major difference between modeling cancer and modeling other diseases is the high level of heterogeneity in cancer. There are large variations in the properties of cancer cells isolated from different patients, from primary tumors and metastases in the same patient, and even within different regions of primary tumors (1, 45). Interpatient heterogeneity explains the existence of subsets of patients whose cancer responds well to a particular therapy whereas others’ cancers do not. Intrapatient heterogeneity also explains the often observed phenomenon of early success of a cancer therapy followed by relapse through the selection of a subset of resistant cells within the heterogeneous cancer cell population (18). Although heterogeneity is a central characteristic of cancer, it is difficult to model and is not fully replicated in any of the currently available animal models of PDAC.

The heterogeneity of PDAC is largely the result of differences in gene expression due to genetic and epigenetic alterations. Several recent studies using a variety of approaches to genomic analysis have led to similar conclusions concerning the genetics of PDAC (29, 43, 72). PDAC tumors nearly always have oncogenic mutations in K-RAS and frequently
(>50%) possess alterations that limit the functioning of the tumor suppressors TP53, CDKN2A, and SMAD4. However, beyond these four major genes, the prevalence of recurrently mutated genes drops sharply. Most of the genetic alterations in an individual patient [estimated to average ~63 (72)] are observed in less than 10% of the patient population. These multiple genetic alterations can be parsed into 12 major biological processes (Table 1) (43). However, there is a high level of heterogeneity in the specific genes altered within the biological processes in any individual. Therefore, there is no single genetic pattern that can mimic all PDACs.

There have been suggestions that perhaps PDAC could be divided into subtypes. Combined analysis of transcriptional profiles of primary PDAC samples along with human and mouse PDAC cell lines was used to define three PDAC subtypes: classical, quasi-mesenchymal, and exocrine-like (14). More recently, patterns of variation in chromosomal structure were used to classify PDACs into four subtypes: stable, locally rearranged, scattered, and unstable (72). In both of these studies the goal was to define gene signatures that may have utility in stratifying patients for treatment. If these subtypes can be validated, then it may be possible to develop preclinical models based on these characteristics that better represent a subpopulation of PDAC patients. Clearly this would be an important advancement that could improve the ability to predict outcomes for individuals. However, these studies are based on the characteristics of bulk populations and are unable to account for small subpopulations within tumors.

Genetic instability is considered one of the central “hallmarks” of cancer (28) and the source of most of the heterogeneity. Genetic instability in cancer can be quite profound, and cancer has been suggested to represent a hypermutation state (47). Although this concept is somewhat controversial, the increasing availability of genetic information has provided further support for it (62). Cancer not only is genetically unstable, it is also epigenetically unstable (38). Moreover, cancer cells also show chromosomal and karyotypic instability. All of these sources of instability lead to random alterations that, over time, result in the high level of functional heterogeneity observed in tumors. These instabilities result in random spontaneous alterations; therefore, the extent of heterogeneity that develops depends on the length of time the instability has existed. Progression from early preneoplastic lesions to metastatic PDAC in humans has been estimated to require 15–20 years (77). Obviously, many different genotypes/phenotypes can arise within this length of time. Mimicking the full scope of heterogeneity observed in patients is nearly impossible in animal models.

### Importance of the Tumor Microenvironment

The behavior of cancer cells is determined not only by the genetics of the cancer cells, but also by the influences of interactions between the cancer cells and their microenvironment. Obvious variances in the microenvironment exist between primary tumors and tumors at metastatic sites, including differences in specific molecules in the extracellular matrix; in cytokines and other regulatory factors; and in vascularization and gradients of oxygen and nutrients. Although less obvious, such differences also exist in the primary tumors between leading-edge cells and cells at various distances from vasculature. The extreme importance of the microenvironment was elegantly shown by Dr. Robert S. Kerbel, who demonstrated critical differences between treatment responses of primary tumors and of late metastatic tumors by comparing responses in initial primary tumors and tumors that arise at distant sites long after resection of the original xenograft (22). He found that treatments that were effective on the primary tumor were ineffective on the metastatic sites. This strongly supports the concept that characteristics of the microenvironment influence the effectiveness of therapeutics. Therefore, to accurately mimic the clinical disease, PDAC models must occur within the context of the pancreas, or common metastatic sites. For this reason, subcutaneous implantation, although useful in other contexts, is not appropriate for preclinical testing. Of the many important aspects of the PDAC microenvironment, the fibrotic stroma and the immune components are likely the most critical.

### Fibrotic stroma.

One of the striking characteristics of the microenvironment of PDAC in humans is the presence of an abundant fibrotic stroma. Cancer cells make up a minority of the volume of PDAC tumors, often as little as 10–20%. The abundant stroma has come under increasing scrutiny for its contribution to the overall biology of the disease, and several recent studies have come up with conflicting interpretations of its role in PDAC (8, 37, 56, 61, 75). The stroma is a complex environment with abundant biologically active extracellular matrix molecules as well as several important cell types. Among the matrix components are a variety of structural molecules, including several specific collagens, fibronectins, and laminins. The stroma also possesses a variety of matricellular molecules that have important biological activities, including sparc, osteopontin, and osteonectin. Many of the important regulatory components of the stroma originate from a specialized type of myofibroblast, the pancreatic stellate cell (19, 75). Stellate cells interact with and influence both cancer and immune cells (37, 49). Thus models lacking stellate cells

### Table 1. Major pathways and representative genes frequently altered in PDAC

<table>
<thead>
<tr>
<th>KRas Signaling</th>
<th>DNA Damage</th>
<th>Apoptosis</th>
<th>TGF-B Signaling</th>
<th>c-Jun kinase Signaling</th>
<th>Integrin Signaling</th>
<th>Hedgehog Signaling</th>
<th>Regulation of Invasion</th>
<th>GTPase Dependent Signaling</th>
<th>Cell-Cell Adhesion</th>
<th>GI/S Phase Transition</th>
<th>Wnt/Notch Signaling</th>
</tr>
</thead>
<tbody>
<tr>
<td>KRAS, RASGRP3, MAP2K4</td>
<td>TP53, ERCC4, ERCC6, EP300, RANBP2</td>
<td>CASP10, CAD, VCP, HIP1, BMP2</td>
<td>SMAD4, SMAD3, TGFB2R1, ERCP2</td>
<td>MAPK3, MATF3, ATF2</td>
<td>ITGA4, ITGA9</td>
<td>SOX3, G11</td>
<td>DEPCD2, CDC42BPA</td>
<td>PLCB3, MEPIA</td>
<td>ADAM11, APG4</td>
<td>CDKN2A, CDH1</td>
<td>FAT, MYC, APC, GATA6, TSC2</td>
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</table>

Shown in the first row are the 12 major signaling pathways most frequently found altered in pancreatic ductal adenocarcinoma (PDAC) (see Ref. 43). The second row lists examples of members of each pathway.
cannot fully reproduce the interactions that occur in tumors in situ. However, cancer cells themselves also produce many of the molecules that make up the extracellular matrix. Furthermore, many characteristics of PDAC, such as chemoresistance, exist innately in the cancer cells, as indicated by resistance observed in vitro. These characteristics are influenced by the stroma but are not totally dependent on its presence. Our understanding of the complex role(s) of the PDAC stroma remains immature. Nonetheless, interpretation of data from various models must include a consideration of the stromal microenvironment.

The role of the stroma is currently highly controversial. Recently genetic mouse models have been developed using gene promoters active in stellate cells to drive expression of genes that can be used to eliminate the cells (57, 61). These studies indicated that elimination of stellate cells made the cancer more aggressive. This is in contrast to a number of studies that indicate that stellate cells increase chemoresistance and facilitate metastasis of PDAC (37, 76). Clearly more needs to be known about the influence of these cells on cancer at its various stages of development.

The immune environment. The immune system is a critical player in carcinogenesis. Immune cells influence responses to therapeutics, rates of metastasis, and nearly all important aspects of cancer (20). Although cancer is generally considered a disease caused by faulty genetics, an alternative characterization is that it is due to failure of the immune system. It has been very difficult to adequately model the role of the immune system in human cancer. One difficulty is that the immune system of mice has important differences from that of humans, as has been previously detailed (53). Nonetheless, immune-competent mice are more likely to recapitulate the human disease than are immune-compromised models. This is an especially important consideration when immune therapies are being investigated. The immune system also has impacts on standard cytotoxic therapeutics (24, 69). Undoubtedly, the immune environment is another key consideration when interpreting results from animal models of PDAC.

DIFFERENCES BETWEEN MICE AND MEN

Humans and mice share a common genetic heritage, but the two species diverged millions of years ago. Although it may seem obvious that there are important differences between men and mice, this is often overlooked by those modeling human disease. Many important differences between mice and humans are recognized in normal pancreatic physiology. For example, although cholecystokinin is a major stimulant of pancreatic secretion in both mouse and humans, the site of action is different. Receptors for cholecystokinin are expressed directly on acinar cells in rodents but not in humans (40). Differences in responses to treatments are also documented. Therapeutic tachykinin inhibitors designed to reduce inflammation during pancreatitis greatly ameliorated pancreatitis in rodent models but were not successful in human trials (68). Few species-specific differences in PDAC have been identified between mouse and human, and the genetic models are often described as “replicating the molecular characteristics of the human disease.” Nonetheless, it is clear that mouse and human PDAC patterns of gene expression are not identical. Although it is estimated that 76% of mouse genes have direct human orthologs, the structures and functions of the orthologs may vary greatly (13).

Other important species differences between mouse and human cells include differences in telomere biology (52) and requirements for transformation (60). Several examples of differences in gene expression are found when comparing human and mouse PDAC. For example, S100P is a molecule that is highly expressed in human PDAC, and its expression is highly specific, easily discriminating between PDAC and chronic pancreatitis, such that it makes an excellent histological biomarker for the disease (4, 55). Furthermore, S100P has been observed to trigger biological actions in PDAC, including increased cell proliferation, tumor growth, metastasis, and chemoresistance (6). However, there is no homolog to S100P in mice. Likewise, mouse PDAC expresses high levels of several members of the prolactin-like family of genes, which arose in mice but not humans, including proliferin (Prl2c2) (unpublished observation, C. D. Logsdon). In the mouse, proliferin can activate the insulin-like growth factor 2 receptor and stimulate angiogenesis and increases Notch and Wnt pathway activation (73). Which molecules fulfill these roles in human PDAC is unknown. The full importance of differences in PDAC due to species-specific genes has yet to be elucidated. Nevertheless, collectively these differences may have significant consequences for the effects of engineered genetic alterations on complex processes such as metastasis and resistance to therapies, as has previously been reviewed (63). The potential for species differences to be relevant is greatest in models that use nonhuman PDACs, such as genetically engineered mouse models (GEMMs) and syngeneic xenografts.

CLASSES OF ANIMAL MODELS OF PDAC

A variety of animal models of PDAC have been developed. Each type of model has advantages and disadvantages. Here we will briefly describe different types of models and some of the relevant issues related to their translational usage.

Immunocompromised Mouse Models

The laboratory mouse has long been the model of choice for investigations into the development and treatment of human disease. However, transplantation of human cells into wild-type mice leads to immune rejection. To overcome this obstacle, a variety of different mice with compromised immune systems have been developed (66). The first useful mouse model for the purpose of studying human cells was athymic nude mice (21). These mice are deficient in T cells because of a mutation in the forkhead box N1 (Foxn1) gene. They are able to support human cancer cell engraftment and tumor growth and remain widely used. However, they maintain intact B cell activity and a functioning innate immunity including significant numbers of natural killer (NK) cells, which reduces their ability to support some human cells (23). Subsequent to the discovery of athymic nude mice, severe combined immune deficient (SCID) mice were identified that possess a spontaneous autosomal recessive mutation in the protein kinase, DNA-activated catalytic polypeptide (Prkdcscid) gene, which severely impairs lymphopoiesis and results in the lack of both T and B cells (10). However, these mice retain an intact innate immunity as well as natural killer cells and tend to produce functional T and B cells during aging (65). A breakthrough
occurred when these SCID mice were crossed with a line of nonobese diabetic mice (NOD) that have impaired innate immunity (51). The resulting NOD/SCID mice have defects in both innate and adaptive immunity. Further levels of immune suppression have been achieved by genetic manipulation of specific genes, and a number of other types of immune-compromised mice are available (see Jackson Laboratories). Because of the importance of the various immune components, differences in behavior of individual cell lines xenografted into different immune-compromised mice are likely.

Cancer cell xenografts. Transplantation of human PDAC into mice (xenografts) is a common and highly useful approach. Cell lines derived from a human tumor clearly represent relevant aspects of the disease, including genetics and the correct species. However, there are issues with xenografts that limit their usefulness as representations of the disease. One major issue is heterogeneity. Important variations exist among tumors from different patients, such that no individual tumor sample can represent the whole of the disease. Furthermore, as mentioned previously, individual tumors are highly heterogeneous at the cellular level, and any specific cell selected from the large population of cells forming the tumor can represent only a subset of the cells of that tumor.

An additional difficulty is that, because of their inherent genetic instability, cancer cell populations have the capacity to adapt to changes in conditions via selection of cells with the best fit. Thus, if a tumor is placed into in vitro culture, the cells that are best able to proliferate under the specific conditions of growing on plastic, nutrient availability, oxygenation, and other parameters are likely to outgrow other tumor cells. The result is that cell lines are not exact replicas of the majority of tumor cells from which they are obtained. This issue becomes more prominent over time, as the cells continue to respond to selective pressure. Thus the longer the cell lines have been in culture, the less likely they are to accurately resemble cancer cells within patient tumors. This genetic instability and adaptability can be used to obtain lines with specific properties. For example, cycling of cells in vivo by multiple rounds of orthotopic implantation and collection of metastasized cells leads to highly metastatic variants (12). Similarly, cells with greater levels of chemoresistance can be selected. These types of studies can be used to learn about the mechanisms involved in the specific processes. However, the artificially selected variants are not faithful replications of the majority of cells that occur in patient tumors.

Cell line models reduce heterogeneity, as each cell line represents only one of many possibilities. This is an advantage or disadvantage depending on the goals of the study. Often, for investigation of a specific function or mechanism, it is valuable to have relatively homogeneous cell models with differences in the property of interest. Multiple cell lines have been developed for the investigation of PDAC. These cell lines typically harbor the critical and nearly universal mutation in K-ras, though there are exceptions (e.g., Bxpc3 cells). However, there is considerable variation in the other genetic hallmarks of PDAC, such as mutations on p53, p16, SMAD4, and others. Many of the widely utilized PDAC cell lines have been extensively characterized (7). Not surprisingly, along with large variations in genetics, there are also large variations in the phenotypes of PDAC cell lines (5, 48). For example, analysis of gemcitabine resistance in different PDAC cell lines revealed a spectrum of sensitivities (5). The generally available cell lines also possess significant differences in their in their growth rates as well as their abilities to metastasize, to develop a fibrotic stroma, and to resist the cytotoxic effects of gemcitabine (Table 2).

Phenotypic differences among PDAC cell lines can be exploited to investigate the mechanisms involved in the different characteristics. Unfortunately, these differences can also lead to interpretations of experiments conducted in a limited set of cell lines that cannot be generalized and are therefore not useful for predicting clinical results. It is not helpful to repeat analysis of a potential therapeutic on multiple cell lines with similar characteristics. Rather, it is important to test treatments on cell lines that are phenotypically different. In particular, it is important to note that responsiveness to standard therapy varies greatly among cell models, for example, going from cells that are highly sensitive to gemcitabine to those that are totally resistant (5). Therefore, it is important to be cautious about applying data obtained with one or even a few different cells to the disease as a whole. Because of the tremendous level of heterogeneity in PDAC, the existence of PDAC cell lines that are resistant to a specific therapeutic suggests that similar resistance may be found in some subpopulation of cancer cells within the tumor of virtually any patient. Thus treatments that cannot inhibit all available PDAC cell lines are likely to select resistant cells in patients. This would be expected to manifest itself by initial clinical success followed by relapse, as is frequently observed.

Another weakness of xenografts formed with most available PDAC cell lines is the lack of the correct microenvironment, since most do not reform significant stroma when transplanted into mice. This seems to be particularly true of cell lines that have been in culture for long periods of time. However, many primary cell lines do induce stroma formation to nearly the same extent observed in human tumors (44) (Fig. 1). The stroma found in these tumors is of mouse origin. Currently there are no animal models that include human stroma.

Cotransplantation of human stroma-producing stellate cells with PDAC cell lines has revealed that the interactions between stromal and neoplastic cells can significantly influence the outcome of therapy, and that the tumor microenvironment can play a crucial role in mediating resistance to cytotoxic agents. This has important implications for the development of new therapeutic strategies. For example, it has been shown that the tumor microenvironment can promote the development of resistance to chemotherapy by facilitating the survival of drug-resistant tumor cells (5). This suggests that targeting the tumor microenvironment may be an effective strategy for overcoming drug resistance.

Table 2. Heterogeneous characteristics among PDAC cell lines as xenografts in Nude mice

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>In vivo Tumor Growth Rate</th>
<th>Metastasis</th>
<th>Stroma Formation</th>
<th>Gemcitabine Resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPAC</td>
<td>High</td>
<td>High</td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>L3.6pl</td>
<td>High</td>
<td>High</td>
<td>Low</td>
<td>Low</td>
</tr>
<tr>
<td>ASPC1</td>
<td>High</td>
<td>High</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>MIAPACA-2</td>
<td>High</td>
<td>High</td>
<td>Medium Low</td>
<td>Medium Low</td>
</tr>
<tr>
<td>PANC-1</td>
<td>Medium</td>
<td>Medium</td>
<td>Low</td>
<td>Medium Low</td>
</tr>
<tr>
<td>PANC-48</td>
<td>Medium</td>
<td>Medium</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>PANC-28</td>
<td>Medium</td>
<td>Medium</td>
<td>Low</td>
<td>Medium Low</td>
</tr>
<tr>
<td>HPAF-2</td>
<td>Medium</td>
<td>Medium</td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>MOH</td>
<td>Low</td>
<td>Low</td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>CFPCA-1</td>
<td>Low</td>
<td>Low</td>
<td>Low</td>
<td>Medium</td>
</tr>
<tr>
<td>BxPC3</td>
<td>Low</td>
<td>Low</td>
<td>Medium Low</td>
<td>Low</td>
</tr>
<tr>
<td>SU6656</td>
<td>Low</td>
<td>Low</td>
<td>Low</td>
<td>Medium Low</td>
</tr>
<tr>
<td>PATC1</td>
<td>Low</td>
<td>Low</td>
<td>High</td>
<td>High</td>
</tr>
<tr>
<td>CAPAN-2</td>
<td>Low</td>
<td>Low</td>
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</table>

For analysis of in vivo tumor growth rate, metastasis, and stroma formation, cells (1 × 10^6) were implanted into the head of the pancreas and allowed to grow for 6 wk. For analysis of gemcitabine responses, cells were cultured in vitro at 70% confluence, and gemcitabine (1 μM) was added for 3 h, then removed. Apoptosis was estimated by TUNEL assay 24 h later.
Thus, in many ways, patient-derived tumor grafts are the model closest to the human disease. However, a major weakness of tumor grafts is that they can exist only in immune-compromised animals, limiting their usefulness for some studies. Potentially, with improvements in humanized mice, these tumor grafts will become even more useful.

**Immunocompetent mouse models: syngeneic mouse models.** Transplantation of cancer cells from mice of identical genetic background does not result in immune rejection and therefore does not require immune compromise. Such xenografts are referred to as syngeneic mouse models (SMMs). Syngeneic models are particularly valuable when the immune contribution to cancer is considered. Unfortunately, the availability of mouse PDAC cell lines for study was very limited until recently. The Panc02 cell line was developed in 1984 to study pancreatic cancer genetics and the influence of immunity; it was derived from a tumor formed after implantation of 3-methylcholanthrene into the pancreas of C57BL/6 mice (15). In vivo cycling of highly metastatic variants of Panc02 resulted in a more aggressive variant, the Panc02-H7 line. These cell lines have been widely utilized in investigations of PDAC. However, recent genetic characterization showed that neither cell line expresses the common genetic alterations found in human PDAC, including mutations in K-ras, p53, or p16, whereas they do have a deletion of SMAD4 (74). The usefulness of these cells is limited because of these important differences in genetics. Fortunately, the development of GEMMs for PDAC has made available many more mouse cancer cell lines (71). Several of these cell lines have been described and can be utilized for the development of syngeneic models.

**Humanized mouse models.** A humanized mouse is an immunocompromised mouse that has missing components of the immune system replaced by transplantation of human immune cells. The purpose of these models is to more closely recapitulate clinical conditions and to model the human antitumor immune response (reviewed in Ref. 32). Efforts to develop humanized mice have provided important insights into the immune system. However, currently available models have important limitations.

Humanized mice can be developed with various sources of human immune components. Transplantation of isolated human peripheral blood mononuclear cells is relatively easy and leads to the stable engraftment of activated human T cells. However, this results in a rapid onset of graft-vs.-host disease. Another approach to humanization is the transplantation of human hematopoietic CD34+ stem cells into the murine host (70). This approach has multiple variations based on how the stem cells are isolated and delivered to the host, but it does yield mice with human B, T, and myeloid cells, thus more completely recapitulating the human immune system. Unfortunately, the distribution of B, T, and myeloid cells is not similar to that in humans (70). Also, the adaptive immune system that is reconstituted through this approach is naive (65), and human T cells cannot be educated through human leukocyte antigen restriction within the thymus of these mice. It has also been observed that human B cells that develop within these models are immature and lack appropriate antibody switching from IgM to IgG after immunization (9). Efforts are ongoing to use genetic engineering to supply needed cytokines for maturation of the human lymphocytes in mice. No doubt better models will be developed using these approaches. At this
time, however, the humanized models have not gained wide usage.

Genetic mouse models. Genetic mouse models are extremely useful tools, particularly for addressing some questions that cannot be addressed with transplantation models. There have been several recent reviews of PDAC GEMMS (25, 54, 64). Cancer is primarily a genetic disease. Therefore, clinically relevant PDAC models must mimic as closely as possible the genetic alterations found in the human disease. Although it is relatively simple to alter two or three genes in a mouse model, the full genetics of PDAC are far too complex to allow complete engineering. Fortunately, expression of oncogenic mutant K-Ras alone is sufficient to achieve PDAC tumors in mice that are histologically correct, develop through appropriate preneoplastic stages, and ultimately will metastasize similar to the human disease (31). These tumors develop in a correct microenvironment with inherent fibrosis and a fully functioning immune system. Furthermore, GEMMs allow access to PDAC at early stages that are not available in transplantation models. Thus GEMMs are ideally suited to studies on the influence of specific genetic alterations on PDAC initiation and development. However, GEMMs have limitations for translational studies partially due to differences between mice and humans, but more importantly because of the unknown importance of slow, sporadic stepwise development of human PDAC and the accompanying heterogeneity of the tumors, which are generally not present in these models. Despite their initial promise, GEMMs have not so far been better at predicting clinical outcomes than other PDAC animal models. Nevertheless, there are specific considerations that can be applied to GEMMs and influence their clinical relevance.

Correct targeted cell of origin for genetic models. In GEMMs, it is important to target genetic manipulations to the correct cell. The cell of origin of human PDAC is unclear and impossible to study directly in humans. GEMMs are, therefore, the only available tool for this line of study, but developing an appropriate model without knowledge of the correct cell of origin is problematic. PDAC has ductal characteristics, and the precursor preneoplastic lesions, pancreatic intrapithelial neoplasms (PanINs), are found in ductlike structures (35, 50). Therefore, for many years the pancreatic duct cell was considered the most likely candidate cell of origin for this disease. However, acinar cells were observed to undergo metaplastic alterations to a ductlike phenotype after stress (acinar-ductal metaplasia), leading some to suggest that acinar cells might be the true cell of origin for PDAC (17, 27). More recently, studies in mice have shown that adult pancreatic ductal cells are resistant to K-ras-induced neoplastic transformation (11, 34), whereas acinar cells readily give rise to PanINs (26, 41). Lineage tracing has supported acinar cells as the cells of origin of PDAC in GEMMs (33, 46). It has also been demonstrated that inactivation of genes necessary for acinar-to-ductal metaplasia inhibits PanIN formation (3, 46). However, pancreatic duct cells can be transformed into PDAC in vitro (39). These observations have led to an increasing consensus that acinar cells are the most likely cell of origin for PDAC. Therefore, there may be several possible cells of origin, at least in mouse models, and the actual cells involved in the human disease may never be fully known.

Importance of K-ras activity. PDAC is unusual in that there is a single oncogene of major importance, K-ras. Thus expression of mutant K-ras is generally considered requisite for an accurate model of PDAC tumorigenesis. It is most reasonable to assume that, in humans, K-ras mutation occurs initially at a single allele. This is best modeled by using a “knock-in” strategy to express a mutated version of K-ras from its endogenous locus. Such a model was developed by Hingorani et al. (31); that model uses Cre-mediated activation of the mutant K-ras. This was the first model to develop histologically accurate PDAC through the predicted series of preneoplastic stages. This model was a major breakthrough and has led to a large number of important discoveries. Some of the most interesting discoveries have come about in attempts to address issues concerning when, where, and at which level mutant K-ras should be expressed.

In particular, it is likely that the developmental stage of the cells in which oncogenic K-ras is expressed is important. It is well known that PDAC occurs in older adults and not in infants. However, many widely used GEMMs of PDAC utilize developmental promoters such as PDX1 or P48 to express Cre and activate expression of oncogenic K-ras during pancreas development. Therefore, these genetic alterations do not occur at a time that is most relevant to the human disease. Nonetheless, the comparison of developmental and adult models has yielded important information. Knock-in expression of mutant K-ras generates PanINs and progresses to PDAC in developmental models (31). In contrast, no phenotype, or a very modest phenotype, is observed when mutant K-ras is expressed at physiological levels in adult acinar cells (26, 41). This has led to the suggestion that an “embryonic” phenotype is more sensitive to mutant K-ras. It has also been observed that addition of an inflammatory stimulus to adult pancreatic acinar cells expressing mutant K-ras rapidly generates PanINs and leads to PDAC. One explanation for this observation that has been offered is that inflammation leads to acinar cell damage and repair, which produces cells with an embryonic phenotype that is sensitive to oncogenic K-ras (26).

Contrary to the suggested need for an embryonic phenotype, at high levels of mutant K-ras expression, adult acinar cells are readily transformed without the need for inflammatory stimulus (41). The level of K-ras activity in these “overexpression” transgenic models actually matches that observed in human and mouse PDAC but exceeds that found in pancreases of mice expressing endogenous levels of mutant K-ras (41). Therefore, it is apparent that K-ras activity of a sufficient level is able to transform adult acinar cells without the need for prior dedifferentiation. Furthermore, Ras activity itself causes acinar ductal metaplasia. Therefore, it has been suggested that the effects of inflammation are to increase K-ras activity to a sufficient level for cellular transformation. This concept sounds heretical in the setting of the generally held belief that oncogenic K-ras is constitutively fully active. However, it was shown recently that oncogenic K-ras is not constitutively active (36) and that K-ras activity can be increased in cells expressing mutant K-ras by a variety of stimuli (16).

Together these observations have generated an alternative explanation based on K-ras activity for the capacity of inflammatory stimuli to generate PDAC. In this model, K-ras activity level, rather than expression per se, is the key to cellular transformation, and external stimuli are required to generate sufficient ras activity. This model could explain a large number of previous observations made in oncogenic K-ras knock-in
models: for example, that EGFR, a Ras activator, is required for $K$-*ras* activity sufficient for PDAC development (3); that elevation of $K$-*ras* activity by feeding a high-fat diet induces PDAC formation in adult acinar cells (which may suggest an explanation for obesity as a risk factor for PDAC) (58); that treatment with lipopolysaccharide elevates oncogenic $K$-*ras* activity and induces PDAC in adult acinar cells (which may suggest an explanation for infection as a risk factor for PDAC) (41); and that loss of *Pten* leads to activation of the NF-κB pathway, generates inflammatory cytokines that are able to activate K-ras, and accelerates PDAC formation (78).

**IMPACT OF TUMOR SUPPRESSOR ALTERATION.** PDAC generally shows the loss of at least one tumor suppressor, and loss of p16 and/or p53, or mutations in p53, account for nearly all PDAC tumors. GEMMs based on the expression of oncogenic Ras spontaneously lose either p16 or p53 (59). The engineered simultaneous deletion of either of these tumor suppressors accelerates tumor development but also is liable to reduce heterogeneity. Significant differences in the characteristics of PDAC developed by using models with alterations in different tumor suppressors have been described (25, 59).

**IMPACT OF THE TIMING SEQUENCE OF GENETIC ALTERATIONS.** PDAC in patients occurs after the sequential development of multiple genetic alterations. Typically, $K$-*ras* is mutated first, followed by the loss of a tumor suppressor (p16 and/or mutation of p53) and subsequent events that affect aggressiveness, such as loss of SMAD4. In patients, the development of fully metastatic PDAC has been estimated to require many years from initiation (77). Furthermore, most individuals who die of other causes are found to harbor early PanIN lesions (2) that follow by the loss of a tumor suppressor (p16 and/or mutation of $p53$) and subsequent events that affect aggressiveness.

The rapid development of tumors would be expected to reduce heterogeneity such that these rapidly developing cancer models are unlikely to fully mimic the human disease with its high level of inherent heterogeneity.

**SUMMARY AND CONCLUSIONS**

Great progress and important insights have been achieved by using animal models of PDAC. Many new models have been developed in the past few years, providing useful tools for investigating mechanisms, origins, and specific properties of PDAC. Unfortunately, no models have so far accurately predicted clinical outcomes. This underscores the difficulties of truly replicating PDAC in animal models. However, although no individual model can provide the necessary insights to predict clinical outcome, the use of multiple models may allow better predictions. Future models based on a better understanding of the molecular basis for the existence of subsets tumors with shared characteristics may provide promise for the future. Understanding the strengths and weaknesses of the available models should be useful for designing studies whose results can be generalized and that may be able to better predict clinical outcomes.


19. Erkan M, Adler G, Apte MV, Bachem MG, Buchholz M, Detlefsen S, Diaz LA Jr, Guerra C, Barbacid M.


