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TOPIC HIGHLIGHT

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Mouse models of pancreatic cancer

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Abstract

Pancreatic cancer is one of the most lethal of human malignancies ranking 4th among cancer-related death in the western world and in the United States, and potent therapeutic options are lacking. Although during the last few years there have been important advances in the understanding of the molecular events responsible for the development of pancreatic cancer, currently specific mechanisms of treatment resistance remain poorly understood and new effective systemic drugs need to be developed and probed. *In vivo* models to study pancreatic cancer and approach this issue remain limited and present different molecular features that must be considered in the studies depending on the purpose to fit special research themes. In the last few years, several genetically engineered mouse models of pancreatic exocrine neoplasia have been developed. These models mimic the disease as they reproduce genetic alterations implicated in the progression of pancreatic cancer. Genetic alterations such as activating mutations in KRas, or TGFb and/or inactivation of tumoral suppressors such as p53, INK4A/ARF BRCA2 and Smad4 are the most common drivers to pancreatic carcinogenesis and have been used to create transgenic mice. These mouse models have a spectrum of pathologic changes, from pancreatic intraepithelial neoplasia to lesions that progress histologically culminating in fully invasive and metastatic disease and represent the most useful preclinical model system. These models can characterize the cellular and molecular pathology of pancreatic neoplasia and cancer and constitute the best tool to investigate new therapeutic approaches, chemopreventive and/or anticancer treatments. Here, we review and update the current mouse models that reproduce different stages of human pancreatic ductal adenocarcinoma and will have clinical relevance in future pancreatic cancer developments.

Key words: K-Ras; Mouse models; Transgenic; Pancreatic cancer; Xenografts

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INTRODUCTION

Infiltrating ductal adenocarcinoma of the pancreas (PDAC) accounts for over 85% of all pancreatic malignancies and has a poor prognosis as less than 5% of patients survive 5 years after diagnosis with a median survival period of 4-6 mo[1-3]. During the last few years there have been important advances to better understand the molecular mechanisms regulating the development of PDAC[4,5]. However, prog-

ress in prevention, early diagnosis and treatment needs major advances[6].

Some of the recent advances have been possible by employing mouse models which have provided an important model system to better understand the molecular mechanism underlying pancreatic cancer. However, in stark contrast to the successful murine models of most common human tumors, the generation and use of appropriate mouse models of pancreatic cancer has remained an area of significant frustration and not always well established. Currently, there are several different genetically modified mouse tumors and xenograft models available that offer the possibility of experimental and preclinical model systems to evaluate different strategies for targeting this disease, early detection, chemoprevention, treatment and finally improve the outcome for pancreatic cancer patients[7].

These models use a variety of approaches to target the expression of mutant or endogenous specific genes and as a result they develop a broad spectrum of pathologic changes, some of them mimic human disease while others are not equivalent to human pancreatic neoplasia. According to the cancer progression model postulated by Fearon and Volgestein[8] in 1990, at least 4-5 genetic events are required for the progression from normal epithelium to carcinoma. Since, the genetic basis of pancreatic ductal adenocarcinoma was revealed, with activation of Kras and inactivation of the p16INK4a, p53 and Smad4 tumor suppressors[9], several mouse models of invasive pancreatic cancer have been developed and modified. Also, regarding the role of pancreatic intraepithelial neoplasia (PanIN) as a direct noninvasive neoplastic precursor to human pancreatic cancer[10], different mouse models are currently available, some of these models reproduce only PanIN lesions and others progress to invasive pancreatic carcinoma. Most of these models were previously presented and evaluated at the International Workshop sponsored by the National Cancer Institute and the University of Pennsylvania in 2004. Twelve genetically engineered mouse models were included and have been considered models for the study of pancreatic disease including PanINs and carcinomas[11-18]. Since then, several new models have been introduced in the basic and translational research fields and previous models have been re-evaluated. Here, we will focus only on pancreatic cancer mouse models as PanIN lesions are considered preinvasive.

Since an activating mutation of the Kras oncogene is the most frequent genetic alteration associated with pancreatic cancer, having been identified in up to 90% of all pancreatic adenocarcinomas[19-21], most of the genetically engineered mouse models are based on the Kras oncogene. As mice expressing mutant Kras develop early and advanced forms of the most common pancreatic cancers in humans, these Kras-based models provide preclinical model systems to analyze the molecular biology of this disease and measure the benefit of new therapies[7,22].

In these review, we update and describe the most common genetically engineered mouse and xenograft models of PDAC that could be useful for assessing the role of genes and pathways, environmental conditions, co-morbidities and response to new adjuvant, neoadjuvant and anti-metastatic therapies.

TRANSGENIC MOUSE MODELS

As Kras mutations are not sufficient to induce progression to the invasive stage of pancreatic adenocarcinoma, different transgenes have been used to generate combined models that progress to invasive PDAC and metastatic disease.

The common genetically engineered models are based on Kras mutations and also include PDX-1-Cre/Lox-Stop-Lox (LSL)-Kras or p48/LSL-Kras mice which have been modified with deletions or mutations of Ink4[23], p53[24], Mist[25], Smad4[26] or TGFβ[27] (Table 1).

These Kras-mutated models can be induced using inducible alleles of Cre recombinase, such as estrogen receptor-Cre fusion genes (*CreER* or *CreERT*) and cycline-responsive Cre expression alleles (TRE-Cre) which are temporally expressed and initiate the expression in adult pancreata reflecting the somatic mutation as it occur in humans[28,29]. Also, some models that only develop PanIN lesions are available as Ela-LSL- $Kras^{G12D}$[12], Nestin-Cre, LSL-$Kras^{G12D}$[30], PDX-1-CRE^{ERT}, LSL-$Kras^{G12D}$, R26Notch^{NIC}[31] and PDX-1-CRE, LSL-$Kras^{G12D}$, Tif1$\gamma^{flox/flox}$[32], however, these are not the purpose of our review.

PDX1-Cre, LSL-$Kras^{G12D}$ and P48$^{+/-Cre}$, LSL-$Kras^{G12D}$ transgenic model

After different studies identified PDX-1 and p48 as critical transcription factors in the developmental program of the pancreas[21,33], these genes have been used in almost all transgenic mouse models to study pancreatic cancer. It is well known that the first identifiable pancreatic progenitor cell in the pancreas arises in the dorsal and ventral endoderm at embryonic day 8 in the fetal mouse: expression of PDX-1 occurs around E8.5[34] and P48 is expressed slightly later and is required to commit cells to a pancreatic fate[35].

In addition, Ptf1a, a component of the pancreas transcription factor 1 complex (Ptf1) which plays an important role in mammalian pancreatic development has been used in some mouse models. Pdf1a determines whether cells allocated to the pancreatic buds continue towards pancreatic organogenesis or revert to duodenal fates[36,37]. To target the expression of oncogenic Kras in pancreatic progenitor cells, a conditionally expressed allele was constructed as previously described by Jackson *et al*[38].

Briefly, the targeting vector contains genetic elements inhibiting transcription and translation flanked by functional LoxP sites. This Lox-Stop-Lox (LSL) construct was inserted into the mouse genomic Kras locus upstream of locus 1 to contain G-A transition in codon 12 (G12D). This transition mutation results in a glycine to aspartic acid substitution in the expressed protein that activates constitutive downstream signaling of Ras effector pathways and is one of the most common mutations found in human pancreatic tumors.

Table 1 Mouse models of pancreatic adenocarcinoma

Genotype (reference)	Time of expression	Time to tumor development (mo)	Pancreatic cancer phenotype	Survival (mo)
PDX-1-Cre; LSL-Kras^{G12D}[39]	E8.5	6	PDAC; penetrant PanIN; age dependent increase severity; occasionally PDAC with long latency	16
P48$^{+/-Cre}$; LSL-Kras^{G12D}[39]	E9.5	8	PDAC; penetrant PanIN; age dependent increase severity; occasionally PDAC with long latency	16
PDX-1-Cre; LSL-Kras^{G12D}; LSL-Trp53$^{R172H/-}$[24]	E8.5	2-3	PDAC Accelerated PanIN; well differentiated PDCA	5-6
Mist1$^{KrasG12D/+}$[25]	E10.5	2	Accelerated development of acinar-derived PanIN; mixed subtypes pancreatic cancer	10.8
KPCB$^{wt/wt}$[42]	E8.5	2-3	PDAC	5.6
KPCB$^{Tr/wt}$[42]	E8.5	3	PDAC	4.8
KPCB$^{Tr/\Delta 11}$[42]	E8.5	1.5	PDAC; mixed	2.8
CKB$^{wt/\Delta 11}$[41]	E8.5	6	PDAC	12
CKB$^{wt/wt}$[41]	E8.5	6	PDAC	13.5
CPB$^{\Delta 11/\Delta 11}$[41]	E8.5	3-5	PDAC; mixed	10
Pdx1-Cre; Kras^{G12D} Ink4a/Arf$^{flox/flox}$[23]	E8.5	2	PDAC; accelerated development of PanIN; poorly differentiated PDAC	2-3
Pdx1-Cre; Kras^{G12D} Smad4$^{flox/flox}$[55]	E8.5	2-3	IPMN; PDAC	2-6
Ptf1a$^{cre/+}$; LSL-Kras$^{G12D/+}$; Tgfbr2$^{flox/flox}$[27]	E9.5	1	PDAC; accelerated PanIN; PDAC development	2

PDAC: Ductal adenocarcinoma of the pancreas; PanIN: Pancreatic intraepithelial neoplasia; IPMN: Intraductal papillary mucinous neoplasia.

Hingorani *et al*[39] developed a mouse model expressing a Cre-activated Kras^{G12D} allele inserted into the endogenous Kras locus, and these mice were crossed with mice expressing Cre recombinase in pancreatic tissue, either by virtue of a PDX-1 promoter-driven transgene or by Cre knockin at the Ptf1-p48 locus. Prior lineage studies suggest that both of these lines express Cre in a common endocrine/exocrine precursor cell during development, while expression in adults is retained in mature islet cells in the case of PDX-1-Cre transgenics and in mature acinar cells in the case of the Ptf1-p48+/Cre knockin[35].

The subsequent recombination resulted in interbreeding LSL-Kras^{G12D} mice with animals that express Cre recombinase from the pancreatic-specific promoters PDX-1 or P48 is a heterozygous mutant condition (KRAS$^{+/G12D}$). Note that only genomic DNA isolated from pancreata and not from tails evidence the recombination. The mutant mice PDX-1-Cre, LSL-Kras^{G12D} and P48$^{+/-Cre}$, LSL-Kras^{G12D} have increased Kras oncogenic protein and their pancreata are larger than their wild type littermate controls.

The pancreata of compound mutant mice develop ductal lesions identical to all three stages of human PanINs. PanIN-1A lesions are observed in compound mutant mice as young as 2 wk old. As the mice age, higher-grade PanINS were observed with increasing frequency and in many of the older mice, the pancreata contained extensive ductal lesions and the acinar parenchyma was replaced by stromal or desmoplastic fibroblasts and inflammatory cells. This fibroinflammatory reaction is highly reminiscent of that seen in human pancreatic cancers. PanIN lesions show evidence of histologic progression and it has been demonstrated that these PanINs activate quiescent pathways such as Notch. These mice have increased Hes1 and Cox2, components of the prostaglandin pathway involved in the inflammatory response and increased matrix metalloproteinase-7. Finally, at low frequency these mice progress to invasive and metastatic ductal adenocarcinoma within one year. In these mice, profuse hemorrhagic ascites was noted, the pancreas was large, firm and fibrotic and nodular densities were observed in liver, diaphragm, pleural surfaces and adrenal cortex.

This model developed by Hingorani *et al*[39] shows progressive PanIN lesions and low-frequency progression to invasive and metastatic adenocarcinoma following activation of oncogenic K-Ras in mouse pancreas. The physiopathology and the sites of metastases observed in these mice are precisely found in human pancreatic ductal adenocarcinoma and further underscore the applicability of this model to study the human disease.

PDX-1-Cre, LSL-Kras^{G12D}, LSL-Trp53$^{R172H/-}$ transgenic model

This mouse model was generated based on the previously described PDX-1-Cre, LSL-Kras^{G12D} mouse. Using similar methods, Hingorani *et al*[24] generated a conditionally expressed point mutant allele of the Li-Fraumeni human ortholog, Trp53^{R175H}[40]. Activation of both the Kras^{G12D} and the Trp53^{R172H} alleles occurs in tissue progenitor cells of the developing mouse pancreas through interbreeding with PDX-1-Cre transgenic animals. The presence of each rearranged, activated allele can be detected in the pancreata but not in tails. Thus, tissues not expressing Cre recombinase (non-pancreatic tissue) remain functionally heterozygous for these loci.

Four to six weeks old mice PDX-1-Cre, LSL-Kras^{G12D}, LSL-Trp53$^{R172H/-}$ present early PanIN lesions similar to what it is observed in single PDX-1-Cre, LSL-Kras^{G12D} mice. A significant disease burden is observed in animals by ten weeks of age at the earliest and the full spectrum of preinvasive lesions is apparent. Histological analyses reveal a predominant moderately well-differentiated to well-differentiated morphology organized as is observed

in the human disease. The carcinomas express CK19 and frequently contain mucin. Metastasis to the liver and lungs are similar to the pancreatic primaries. Finally, PDX-1-Cre, LSL-Kras^{G12D}, LSL-Trp53$^{R172H/-}$ mice have dramatically shortened median survival of approximately 5 mo, significantly less than wild type, PDX-1-Cre, LSL-Trp53$^{R172H/-}$ and PDX-1-Cre, LSL-Kras^{G12D}.

The triple mutant mice succumb earlier than PDX-1-Cre, LSL-Kras^{G12D} animals which spontaneously develop PDA with a proscribed latency after manifesting preinvasive neoplasia. These triple mutant animals develop cachexia, abdominal distension, and hemorrhagic ascites. They also present metastasis in the liver, diaphragm and adrenals and all of them die before 12 mo.

PDX-1-Cre, Brca2^{F11}, LSL-Kras^{G12D}, Trp53 F2-10 transgenic model

This transgenic mouse is a conditional Brca2^{F11}, LSL-Kras^{G12D}, Trp53 F2-10 and PDX-1-Cre and has been used as a model of pancreatic cancer, although the role of Brca2 in pancreatic cancer development is still unclear[41,42]. Brca2 plays a key role in the maintenance of genomic integrity, particularly through regulation of DNA repair by homologous recombination repair[43], a process that is also controlled by another tumor suppressor protein, Brca1[44]. However, the significance of Brca2 in pancreatic cancer is not clear[45].

While Rowley *et al*[41] demonstrated that the inactivation of Brca2 promotes Trp53-associated but inhibits Kras^{G12D}-dependent pancreatic cancer development in mice, Skoulidis *et al*[42] showed that Brca2 heterozygosity promotes Kras^{G12D}-driven carcinogenesis in the murine model of familial pancreatic cancer. In this model, the mouse expressed a functional wild type *Brca2* gene, in which exon 11 of Brca2 is flanked by loxP sites (B2^{F11}). Conditional rearrangement of this allele in the developing pancreas in response to PDX-1-Cre expression results in the deletion of Brca2 exon 11, and the generation of a functionally null Brca2 allele (B2$^{\Delta 11}$). These authors crossed CB2$^{\Delta 11/\Delta 11}$ mice with conditional Trp53F2-10/F2-10 (P) mice, in which exons 2 and 10 are flanked by loxP sites to generate Trp52 null CPB2$^{\Delta 11/\Delta 11}$, CPB2$^{wt/\Delta 11}$ and CPB2$^{wt/wt}$ mice.

CPB2$^{\Delta 11/\Delta 11}$ mice develop pancreatic cancer at high frequency and their median survival is 300 d, showing substantially reduced pancreatic cancer-free survival relative to CB2$^{wt/\Delta 11}$. However, in contrast, CB2$^{\Delta 11/\Delta 11}$, CB2$^{wt/\Delta 11}$ and CB2$^{wt/\Delta 11}$ mice expressing wild type Trp53 alleles failed to develop pancreatic cancer.

This mouse model shows that the inactivation of Brca2 alone does not promote pancreatic cancer, but the disruption of Trp53 signaling in combination with the inactivation of Brca2 promotes pancreatic cancer formation. CPB2$^{\Delta 11/\Delta 11}$ mice display severe acinar cell dysplasia and a reduced number of islets. The pancreas is atrophic with acini replaced by mature adipose tissue, inflammatory infiltrates and little evidence of fibrosis. In contrast, in CPB2$^{wt/\Delta 11}$ and CPB2$^{wt/wt}$ mice the dysplasia, atrophy and chronic inflammatory infiltrate is less severe and frequent[41]. The mouse model combining Brca2^{F11} and LSL-Ras^{G12D} (K) shows that CKB2$^{\Delta 11/\Delta 11}$, CKB2$^{\Delta 11/\Delta 11}$ and CKB2$^{wt/wt}$ mice display normal development although CKB2$^{wt/\Delta 11}$ and CKB2$^{wt/wt}$ present PanINs and metaplastic lesions at 8 mo but not CKB2$^{\Delta 11/\Delta 11}$. This mouse model showed that the loss of Brca2 tumor suppressor inhibits the development of premalignant lesions and pancreatic tumors that are induced by activated Kras. Only 13% of CKB2$^{\Delta 11/\Delta 11}$ mice develop tumors, whereas 66% of CKB2$^{wt/\Delta 11}$ and 61% of CKB2$^{wt/wt}$ develop pancreatic tumors with an average latency of 366 and 406 d, respectively[41].

Skoulidis *et al*[42] described a mouse model PDX-1-Cre-Kras^{G12D} with two distinct mutant alleles of Brca2. The first encodes a germline truncating allele Brca2^{Tr} (Tr), that mimics Brca2 human mutations in pancreatic cancer, and the second is a conditional deletion (F11) in which LoxP sites flank Brca2 exon 11 and emulates the loss of heterozygosity observed in human cancers.

Homozygous Brca2 inactivation in KPCB2$^{Tr/\Delta 11}$ mice displays pancreatic cancer in high penetrance with rapid and predictable clinical decline. The median survival was 84 d compared with the KPCB cohort whose median survival was 168 d. Mice with germline heterozygosity for Brca2^{Tr} display pancreatic carcinogenesis, as even KCB$^{Tr/wt}$ mice with wild type Trp53 and mutant Kras-G12D in which pancreatic cancer is reported to develop less readily[39]. There is a reduction in PDAC-free survival of KCB$^{Tr/wt}$ mice in comparison with KCB controls with wild type Brca2. The pancreatic tumors observed in these mice display histological features similar to human pancreatic cancers with desmoplastic stroma. These tumors evolved with pancreatic intraepithelial neoplasia and metastatic behavior.

Interestingly, the KPCB$^{Tr/\Delta 11}$ mice which carry biallelic Brca2 mutations uniquely develop an acinar cell carcinoma component in 18% of cases, not observed in the other cohorts with Brca2 heterozygosity. This model shows that Brca2 inactivation promotes Kras-driven pancreatic malignancies[42].

Mist1$^{KrasG12D/+}$ transgenic model

To generate this transgenic model, Tuveson *et al*[25] used homologous recombination to target the expression of Kras^{G12D} to the Mist1 locus, a gene known to be expressed at earlier stages of pancreatic exocrine development. Mist1 is a basic helix-loop-helix transcription factor that is expressed at low levels in the embryonic pancreas at day 10.5[43,46,47] and in the adult, Mist protein is restricted to mature pancreatic acinar cell and is not found in ductal or islet cells[48,49]. Mist1$^{KrasG12D/+}$ mice have a diminished median survival of 10.8 mo compared with 24.2 mo in control wild type mice. Newborn mice show acinar hyperplasia with an increased proliferative index and acinar adenomas at 2 mo known as "acinar-ductal metaplasia". Metaplastic ductal structures with mucinous cytoplasm that resemble murine PanIN-IA are found in the pancreas in close association with metaplastic acini. These metaplastic ducts are

characterized by the presence of CK19 and acidic mucin staining with alcian blue. At three months of age they become cachectic with pancreatic tumors and metastasis. Most of these tumors are acinar although some of them are cystic papillary neoplasms with acinar differentiation. Surprisingly, these mice also develop early and advanced hepatocellular carcinoma and some of them succumb before invasive pancreatic carcinoma. Mist1$^{KrasG12D/+}$ mice die of advanced pancreatic exocrine carcinoma.

PDX1-Cre, Kras^{G12D}, Ink4a/Arf$^{flox/flox}$ transgenic model

As the loss of function of the G1 cyclin-dependent kinase inhibitor, INK4A, appears to be a near universal event in pancreatic adenocarcinoma when there is an alternate reading frame or distinct first exon in the INK4A/ARF locus[50-52], transgenic mice with this modification have been studied.

It was shown that mice with a constitutive deletion of both or either component of the Ink4a/Arf locus do not develop spontaneous pancreatic cancer[53]. Aguirre *et al*[23] demonstrated the cooperative interaction between Ink4 and Kras using mice engineered with Cre-mediated activation of mutant Kras (Kras^{G12D}) and the deletion of a conditional Ink4/Arf tumoral suppressor allele.

In this model, the LSL-Kras^{G12D} allele is expressed at the endogenous level after Cre mediates the expression of a transcriptional stopped element. The conditional Ink4a/Arf allele (Ink4/Arf^{flox}) was engineered to sustain Cre-mediated excision of exon 2 and 3, thereby eliminating p16^{Ink4} and p19^{Arf} proteins. The double engineered mouse expressed the Kras^{G12D} allele and lack of both copies of the conditional Ink4/Arf allele specifically in the pancreas after using the PDX-1-Cre transgene. Between 7 and 11 wk of age, PDX-1-Cre, Kras^{G12D} Ink4a/Arf$^{flox/flox}$ mice show weight loss, ascites, jaundice and pancreatic tumors ranging in diameter from 4 to 20 mm. These pancreatic tumors are highly invasive, frequently involving the duodenum, stomach and spleen but no liver or lung metastasis. Furthermore, invasion of the lymphatic and vascular system is detected, an observation suggestive of metastatic potential of these neoplasms.

Consistent with a ductal phenotype, the tumors are positive for CK-19, DBA lectin and show stromal collagen deposition. In contrast, they do not show reactivity for amylase and insulin.

In conclusion, KrasG12D expression in combination with Ink4a/Arf deficiency resulted in an earlier appearance of PanIN lesions and these neoplasms progressed rapidly to highly invasive and metastatic cancers, resulting in death in all cases by 11 wk.

PDX1-Cre, Kras^{G12D}, Smad4$^{flox/flox}$ transgenic model

Although selective SMAD4 has no discernable impact on pancreatic development or physiology, when combined with the activated KRAS^{G12D} allele, SMAD4 deficiency enabled rapid progression of Kras^{G12D}-initiated neoplasms including pancreatic tumors. The combination of Kras^{G12D} and SMAD4 deficiency resulted in the rapid development of tumors resembling intraductal papillary mucinous neoplasia (IPMN), a precursor to PDAC in humans. The SMAD4 tumor suppressor gene encodes a transcription factor that is a central effector of transforming growth factor-β (TGF-β)[30] and inactivating mutations in this gene are common in PDAC[54]. Bardeesy *et al*[55] generated a conditional knockout allele of Smad4 (Smad4^{lox}) harbouring loxP sites flanking exons 8 and 9 in the mouse germline. They crossed Smad4^{lox} homozygous mice to either the PDX1-Cre or Ptf1a-Cre transgenic mice. Mice with a homozygous deletion of Smad4 in the pancreas showed no evidence of any gross anatomic or physiological abnormalities, and exhibited normal pancreatic cytoarchitecture and differentiation.

In contrast, LSL-Kras^{G12D}-Smad4$^{lox/lox}$ mice showed low-grade PanINs and acinar-ductal metaplasia from 4 wk of age, an abdominal mass between 7 and 12 wk and reached terminal morbidity between 8 and 24 wk of age and a tumor-free survival of 13-15 wk. The pancreatic tumors were positive for cytokeratin 19, Shh, Hes1, phospho-stat3, mucin, Muc1, Muc4 and Muc5AC, but lacked acinar (amylase) and islet (insulin) marker expression. Mice showed palpable abdominal masses between 7 and 12 wk of age, and reached terminal morbidity between 8 and 24 wk of age.

Since the combination of Kras^{G12D} expression and Smad4 deletion showed a rapid onset of IPMN and advanced PanIN lesions, but exhibited only moderate pancreatic malignant progression, and since SMAD4 loss occurs with concurrent INK4A loss and Kras activation in human PDAC, the authors developed a transgenic mouse PDX1-Cre, Kras^{G12D} Ink4a/Arf$^{lox/lox}$ Smad4$^{lox/lox}$. These mice have significantly reduced survival, around 8 wk associated with PDAC and a small number of them also have IPMN and liver metastasis.

Ptf1a$^{cre/+}$, LSL-Kras$^{G12D/+}$, Tgfbr2$^{flox/flox}$ transgenic model

TGF-β signaling plays an important role in PDAC progression, as indicated by the fact that Smad4, which encodes a central signal mediator downstream from TGF-β, is deleted or mutated in 55% of human PDAC[54,56-58]. Pancreas-specific Tgfbr2 knockout mice have also been generated, alone or in the context of active Kras^{G12D} expression. Ijichi *et al*[27] crossed the LSL-Kras$^{G12D/+}$ mice with Tgfbr2 knockout mice[59] (previously developed) and generated mice of the genotype Ptf1a$^{cre/+}$, LSL-Kras$^{G12D/+}$, Tgfbr2$^{flox/flox}$. These mice had active KrasG12D expression plus Tgfbr2 knockout both in a pancreas epithelium-specific manner.

Ptf1a$^{cre/+}$, Tgfbr2$^{flox/flox}$ mice did not have pancreas development effects or discernable pancreatic cancer phenotype during 1.5 years.

In contrast, $^{Ptf1acre/+}$, LSL-Kras$^{G12D/+}$, Tgfbr2$^{flox/flox}$ mice had abdominal distension due to ascites, weight loss, and jaundice at 6-7 wk of age. Finally, these mice developed well-differentiated PDAC with 100% penetrance and a median survival of 59 d. Tumors are always accompanied by a whole panel of mPanINs and acinar-ductal metapla-

sia lesions from 3.5 wk and mice frequently have liver and lung metastases, direct invasion to the duodenum, and peritoneal dissemination.

While Ptf1a$^{cre/+}$, LSL-Kras$^{G12D/+}$, Tgfbr2$^{flox/+}$ mice show normal pancreas histology, tumors from Ptf1$^{acre/+}$, LSL-Kras$^{G12D/+}$, Tgfbr2$^{flox/flox}$ mice exhibited uniformly well-differentiated glandular architecture, which occupied the entire pancreas, resulting in almost complete loss of normal pancreatic tissue. Tumoral cells show positive ductal markers, CK19 and mucin, and are negative for the acinar and islet markers, amylase and insulin, indicating ductal adenocarcinoma. In addition, these tumors are rich in stromal component, positive for vimentin and smooth muscle actin staining.

In conclusion, Tgfbr2 knockout mice combined with Kras^{G12D} expression developed well-differentiated PDAC with 100% penetrance and a median survival of 59 d. Moreover, a distinct and important feature of this mouse model is that the Ptf1a$^{cre/+}$, LSL-Kras$^{G12D/+}$, Tgfbr2$^{flox/flox}$ tumors did not show sarcomatoid architecture, which was seen in one-third of the KrasG12D, Ink4a/Arf knockout model[23].

XENOGRAFT MOUSE MODELS

Tumor xenograft mouse models have been commonly used in preclinical studies for the last few years[60-62]. Human tumor xenograft models are created by the injection of human tumor cells grown from culture into a mouse or by the transplantation of a human tumor mass into a mouse. The xenograft may be readily accepted by immunocompromised mice such as athymic nude mice or severely compromised immunodeficient mice[63]. Xenografts show different advantages as they mimic genetic and epigenetic abnormalities that exist in tumors, can be used in the development of individualized molecular therapeutic approaches and can be implanted into the same organ to reproduce the organ microenvironment or the tumor[63].

There are two main types of human xenograft mouse models used for pancreatic cancer research, heterotopic and orthotopic, defined by the location of the implanted xenograft.

Heterotopic xenograft model

For heterotopic subcutaneous models, the xenograft is implanted between the dermis and underlying muscle and is typically located on the flank, on the back or the footpad of the mice. For many years, the subcutaneous xenograft model has been the most widely used preclinical mouse model for cancer research because it is rapid, inexpensive, reproducible, and has been considered sufficiently preclinical to test anti-cancer drugs. The subcutaneous model also has the advantages of providing visual confirmation that mice used in an experiment have tumors prior to therapy; and provides a means of assessing tumor response or growth over time, compared to intracavitary models where animal survival is the sole measure of response[64].

Different studies have used tumor engraftment in nude mice to study the possible response to chemotherapy treatment such as gemcitabine[65] or new pharmacological blocking agents[66] obtaining good results and suggesting new potential treatment options for pancreatic cancer.

One of the disadvantages of the heterotopic model is that it was observed that drug regimens that are curative in these models often do not have a significant effect on human disease as the subcutaneous microenvironment is not relevant to that of the organ site of primary or metastatic disease. Additionally, subcutaneous tumor models rarely form metastases. These observations suggest that heterotopic tumor models that do not represent appropriate sites for human tumors are not predictive when used to test responses to anti-cancer drugs[60,67,68].

Orthotopic xenograft model

Orthotopic tumors are transplanted to the appropriate organ in the mouse. For example, human pancreatic cancer cells are injected into the mouse pancreas and not into the skin on the mouse's back. Advantages of orthotopic models include use of the relevant site for tumor-host interactions, the development of metastases, the ability to study site-specific dependence of therapy, organ-specific expression of genes and the clinical scenario can be replicated. Major disadvantages are that orthotopic tumor xenograft generation is labor intensive, technically challenging, expensive, requires longer healing and recovery time and that monitoring tumor volume requires relatively lower throughput imaging methods[67]. Nonetheless, orthotopic tumor models are emerging as the preferred model for cancer research due to the increased clinical relevance.

To study pancreatic cancer, the standard procedure uses anesthetized mice 6-8 wk old. The abdominal skin and muscle are incised just off the midline and directly above the pancreas to allow visualization of the pancreatic lobes; the pancreas is gently retracted and positioned to allow direct injection of tumoral cells. The pancreas is replaced within the abdominal cavity; and both the muscle and skin layers are closed with surgical glue. Following recovery from surgery, mice are monitored and weighed daily to evaluate the tumor or response to treatment[61].

These models have been employed to study gene expression profiling of liver metastases and tumour invasion in pancreatic cancer[69] in basic research. In translational medicine, ortothopic models have been used to evaluate the antitumor efficacy of gemcitabine plus emodin[70].

In conclusion, different *in vivo* models of pancreatic cancer have been developed for the evaluation of multiple chemotherapeutic drugs and to study the molecular mechanisms implicated in resistance to different treatments.

These models are now available to investigate basic and translational aspects, but multiple considerations should be kept on mind for model selection depending on the purpose. The optimal model system should investigate

Table 2 Comparison of mouse models for the clinical approach in pancreatic cancer

Mouse model	Cost	Time consuming	Clinical approach	Clinical reproducibility (human disease)
Transgenic engineered	++++	++++	+	++++
Xenograft heterotopic	+	+	++++	+
Xenograft orthotopic	++	++	+++	++

+: Low; ++: Medium; +++: High; ++++: Very high.

invasiveness or metastasis, the criteria for assessing response and altered molecular pathways, expression of markers and time expression and tumor development are some of the most important factors (Table 2).

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